BAX and BAK mediated apoptosis.

MCMV lacking m38.5 or m41.1 prevent viral infected cells from further spread of the virus to the uninfected cells may be reduced and further retinal damage potentially eliminated.

Results: When compared with mock-infected eyes, MCMV-infected eyes of MAIDS mice that showed retinitis exhibited expression of SOCS1 and SOCS3 in infiltrating macrophages, infiltrating granulocytes, resident Muller cells, and resident microglial cells of the retina at 10 days postinfection. Quantification of SOCS1 and SOCS3 production by these cells suggested infiltrating granulocytes ≈ Muller cells > microglial cells > infiltrating macrophages.

Conclusions: The sources of SOCS1 or SOCS3 production in the retina during development of MAIDS-related MCMV retinitis includes infiltrating granulocytes and infiltrating macrophages as well as resident Muller cells and resident microglial cells. Of these cell types, however, major sources for SOCS1 and SOCS3 production are infiltrating granulocytes and resident Muller cells.

Commercial Relationships: None; Christine I. Alston, None; Emily L. Blalock, None; Jessica Fleming, None; Hsin Chien, None

Support: NIH Grant EY010568, NIH/NEI Core Grant P30/EY006360, Research to Prevent Blindness, and Fight for Sight

Program Number: 127 Poster Board Number: C0132

Presentation Time: 8:30 AM - 10:15 AM

Murine Cytomegalovirus (MCMV) Lacking m38.5 or m41.1 Increases Apoptosis of Viral Infected Retinal Cells

Jason Covar, Juan Mo, Brendan Marshall, Sally S. Atherton, Ming Zhang. Georgia Health Sciences University, Augusta, GA.

Purpose: Previous results in our laboratory showed that during MCMV retinitis, uninfected retinal cells become apoptotic while infected cells do not. The purpose of this study was to determine if MCMV lacking m38.5 or m41.1 prevent viral infected cells from BAX and BAK mediated apoptosis.

Methods: Immunosuppressed female Balb/c mice were injected via the supraciliary route with m38.5 and m41.1 mutant murine cytomegaloviruses (MCMV) and K181 parent MCMV virus. Eyes were collected at days 4 and 7 post infection (p.i.) and sectioned for immunohistochemistry or homogenized for plaque assay. Double staining for MCMV Early Antigen (EA) and TUNEL were performed. Virus titers were performed by plaque assay on monolayers of mouse embryo fibroblast (MEF) cells and in-vitro studies were performed using an organotypic retinal culture model.

Results: Staining for MCMV EA showed more cells to be positive in the m38.5 and m41.1 mutant viruses than in the K181 parent virus. Late stage apoptosis activity was observed by TUNEL staining and in both m38.5 and m41.1 mutant viruses more DNA fragmentation was seen than in the K181 virus. Virus titers were lower in the injected eyes of the m38.5 and m41.1 mutants compared to the K181 virus after day 7 p.i.. Likewise, in retinal cultures at both days 4 and 7 p.i., less virus was recovered from cultures infected with mutant viruses than from cultures infected with the parent virus.

Conclusions: Our results indicate that MCMV lacking m38.5 or m41.1 has a reduced capacity to prevent viral infected cells, of immunosuppressed mice, from undergoing BAX and BAK mediated apoptosis. In altering the apoptotic process of the viral infected cells, further spread of the virus to the uninfected cells may be reduced and further retinal damage potentially eliminated.

Commercial Relationships: Jason Covar, None; Juan Mo, None; Brendan Marshall, None; Sally S. Atherton, None; Ming Zhang, None

Support: NIH RO1 EY009169

Program Number: 128 Poster Board Number: C0133

Presentation Time: 8:30 AM - 10:15 AM

Suppressor of cytokine signaling (SOCS1) and SOCS3 expression is upregulated following intraocular, but not systemic, murine cytomegalovirus (MCMV) infection of mice with retrovirus-induced immunosuppression (MAIDS)

Christine I. Alston1, Hsin Chien1, Moon K. Han1, Richard D. Dix1,2. 1Biology, Viral Immunol Ctr, Georgia State Univ, Atlanta, GA; 2Ophthalmology, Emory University School of Medicine, Atlanta, GA.

Purpose: SOCS family proteins govern the regulation of immune responses to pathogen invasion by a negative feedback regulatory mechanism to prevent cytokine over-expression. SOCS1 is an inhibitor of interferon signaling, whereas SOCS3 regulates the divergent actions of IL-6 and IL-10. We have shown previously that SOCS1 and SOCS3 mRNAs and proteins are upregulated in MCMV-infected eyes with retinitis during MAIDS. These findings prompted us to define the cell types that serve as sources for SOCS1 and SOCS3 production.

Methods: Groups of C57BL/6 mice with MAIDS were injected subretinally with MCMV or mock injected (control). At 6 and 10 days postinfection, whole eyes were collected, cryostat sectioned, and subjected to immunostaining for detection and localization of SOCS1 or SOCS3 production to specific cells types within the retina. These included infiltrating macrophages (F4/80), infiltrating granulocytes (neutrophils) (Ly-6G), resident Muller cells (GFAP), and resident microglial cells (Iba-1).

Results: Infiltrating granulocytes and resident Muller cells are major sources for suppressor of cytokine signaling (SOCS1) and SOCS3 production during murine cytomegalovirus (MCMV) retinitis in immunosuppressed mice, from undergoing BAX and BAK mediated apoptosis. In altering the apoptotic process of the viral infected cells, further spread of the virus to the uninfected cells may be reduced and further retinal damage potentially eliminated.

Commercial Relationships: Jason Covar, None; Juan Mo, None; Brendan Marshall, None; Sally S. Atherton, None; Ming Zhang, None

Support: NIH RO1 EY009169

Program Number: 128 Poster Board Number: C0133

Presentation Time: 8:30 AM - 10:15 AM

Suppressor of cytokine signaling (SOCS1) and SOCS3 expression is upregulated following intraocular, but not systemic, murine cytomegalovirus (MCMV) infection of mice with retrovirus-induced immunosuppression (MAIDS)

Christine I. Alston1, Hsin Chien1, Moon K. Han1, Richard D. Dix1,2. 1Biology, Viral Immunol Ctr, Georgia State Univ, Atlanta, GA; 2Ophthalmology, Emory University School of Medicine, Atlanta, GA.

Purpose: SOCS family proteins govern the regulation of immune responses to pathogen invasion by a negative feedback regulatory mechanism to prevent cytokine over-expression. SOCS1 is an inhibitor of interferon (IFN) signaling, whereas SOCS3 regulates the divergent actions of IL-6 and IL-10. We have shown previously that SOCS1 and SOCS3 mRNAs and protein are robustly upregulated in MCMV-infected eyes with retinitis during MAIDS. We therefore sought to determine the patterns of SOCS1 and SOCS3 expression as well as SOCS-regulated cytokines during systemic MCMV infection of mice with MAIDS that fail to develop retinitis.

Methods: Groups of C57BL/6 mice with MAIDS or healthy mice were inoculated systemically with MCMV or mock infected (control). At 2, 4, 7, and 10 days postinfection, eyes and spleens were collected from all groups and quantified for SOCS1 and SOCS3 mRNA expression as well as mRNAs for IFNs and IL-6 by real-time RT-PCR assay.

Results: Systemic MCMV infection of MAIDS mice resulted in no statistically significant upregulation of SOCS1, SOCS3, Type I IFNs, or IL-6 mRNAs in whole eyes or whole splenic cells when compared with controls. In comparison, Type II IFN mRNA expression was upregulated significantly in whole eyes, but not in whole splenic cells, of MAIDS mice following systemic MCMV infection.

Conclusions: Whereas direct intraocular MCMV infection and development of retinitis during MAIDS resulted in robust
upregulation of SOCS1 and SOCS3 mRNA and protein within the ocular compartment, systemic MCMV infection without retinitis during MAIDS did not lead to significant upregulation of SOCS1, SOCS3, Type I IFNs, or IL-6 mRNAs within the ocular compartment or spleen, although there was a surprising upregulation of Type II IFN in the ocular compartment without retinitis. We conclude that upregulation of SOCS1 and SOCS3 expression takes place within the ocular compartment only during MCMV retinitis development in mice with MAIDS. The precise role in SOCS1 and SOCS3 in the pathogenesis of MAIDS-related MCMV retinitis requires further investigation.

Commercial Relationships: Christine I. Alston. None; Hsin Chien. None; Moon K. Han. None; Richard D. Dix. None

Support: NIH Grant EY010568, NIH/NEI Core Grant P30/EY006360, Research to Prevent Blindness, Fight for Sight

Program Number: 129 Poster Board Number: C0134

Presentation Time: 8:30 AM - 10:15 AM

The M33 gene of murine cytomegalovirus (MCMV) is involved in the stimulation of VEGF-A production by macrophages

Moon K. Han, Hsin Chien, Hamed Laroui, Didier Merlin, Scott W. Cousins, Richard D. Dix. 1Viral Immunology Center, Georgia State University, Atlanta, GA; 2Center for Inflammation, Immunity, and Infection, Georgia State University, Atlanta, GA; 3Ophthalmology, Emory University School of Medicine, Atlanta, GA; 4Ophthalmology, Duke University, Durham, NC.

Purpose: Our laboratory recently demonstrated that chronic MCMV infection of healthy C57BL/6 mice results in a more severe experimental choroidal neovascularization (CNV) (PLoS Pathogens 8:e1002671, 2012). Because the human cytomegalovirus (HCMV)-encoded chemokine receptor US28 has been shown to play a multifunctional role in angiogenesis, we hypothesized that the M33 gene of MCMV (the MCMV homologue to HCMV US28) plays a similar role in experimental CNV. The purpose of this study is to test the hypothesis that the M33 gene of MCMV is involved in the stimulation of VEGF production by mouse macrophages.

Methods: Monolayers of IC-21 mouse macrophages were treated for 24 hours with either M33 siRNAs (single or combined M33-1 siRNA and M33-2 siRNA) or scrambled siRNAs (control) using either HiPerFect transfection reagent (Qiagen) or Lipofectamine RNAiMAX transfection reagent (Invitrogen) and inoculated with MCMV (moi=3 pfu/cell). All siRNA-treated and control MCMV-infected monolayers were harvested at 24 and 48 hours post-infection and subjected to western blot analysis for the comparison of VEGF-A protein production.

Results: Although no significant differences were observed when comparing relative band densities of VEGF-A production by siRNA-treated MCMV-infected macrophages at 24-hour post-infection, treatment using a combination of M33-1/M33-2 siRNAs resulted in a decrease in VEGF-A protein production by ~51%. Use of Lipofectamine RNAiMAX transfection reagent was superior to HiPerFect transfection reagent in reducing VEGF-A protein production by the M33 siRNAs.

Conclusions: The M33 gene of MCMV is involved in the stimulation of VEGF-A production by IC-21 mouse macrophages, but the downregulation of M33 gene by siRNAs did not result in a greater reduction in VEGF-A protein production as expected. We therefore postulate that one or more genes in addition to the M33 gene of MCMV contribute to the stimulation of VEGF-A production by mouse macrophages.

Commercial Relationships: Moon K. Han. None; Hsin Chien. None; Hamed Laroui. None; Didier Merlin. None; Scott W. Cousins. Alcon (F), Alcon (C), Heidelberg Engineering (C), Narrow

River (C), Nordic Biotech (C), PanOptica (C), Pfizer (C), Salutaris Medical Devices (C), Sanofi-Fovea (C), Valeant Ophthalmics (C), Imageon Biotech (I), Richard D. Dix. None

Support: NIH Grant EY010568, NIH/NEI Core Grant P30/EY006360, Research to Prevent Blindness, Fight for Sight

Program Number: 130 Poster Board Number: C0135

Presentation Time: 8:30 AM - 10:15 AM

Autophagy Is Anti-apoptotic during Murine Cytomegalovirus (MCMV) Infection of the Retina or RPE Cells

Juan Mo, Ming Zhang, Brendan Marshall, Jason Covar, Sally S. Atherton. Cellular Biology & Anatomy, Georgia Health Sciences University, Augusta, GA.

Purpose: The contribution of apoptosis and autophagy to the pathogenesis of cytomegalovirus infection has not been explored. The purpose of this study was to determine if MCMV infection affects apoptosis and autophagy during MCMV retinitis, and if so, how these processes influence the pathogenesis of MCMV infection.

Methods: In vitro, RPE cells were mock-infected or infected with MCMV at an MOI of 1 PFU/cell and treated with 10-6M of rapamycin or 10-5M of chloroquine. Mock-infected and MCMV-infected RPE cells were collected and examined by western blot, electron microscopy, plaque assay and trypan blue exclusion assay. In vivo, immunosuppressed (IS) Atg5lox/+ , nestin-Cre mice or Atg5+/+, nestin-Cre mice were inoculated with 1x104 PFU of MCMV K181 via a supraciliary injection. Inoculated eyes were collected at several times p.i., examined by MCMV staining, H&E staining, TUNEL assay and western blotting.

Results: In vitro, the levels of LC3B-II were increased in MCMV infected RPE cells, which was consistent with EM results showing increased accumulation of autophagic vacuoles in MCMV infected cells compared to uninfected control cells. Autophagy was increased while caspase 3-mediated apoptosis was decreased by rapamycin treatment (blocks mTOR signaling pathway). In contrast, autophagy was decreased and caspase 3-mediated apoptosis was increased by chloroquine treatment. In vivo, when the number of MCMV and TUNEL-positive cells was compared in the retina of Atg5lox/+ , nestin-Cre infected mice with that in Atg5+/+, nestin-Cre infected mice. Atg5lox/+ , nestin-Cre infected mice had more MCMV and TUNEL-positive cells in the retina than Atg5+/+, nestin-Cre infected mice at early stage of infection. Disruption of the architecture of the retinas of infected eyes in Atg5lox/+ , nestin-Cre mice was more severe than that observed in Atg5+/+, nestin-Cre mice.

Conclusions: Rapamycin induced autophagy through blocking mTOR signaling pathway and decreased caspase 3-mediated apoptosis while chloroquine treatment had the opposite effects. The retinas of infected eyes in Atg5lox/+ , nestin-Cre mice had more severe disruption than that of Atg5+/+, nestin-Cre mice. Taken together, these results suggest that autophagy during MCMV infection is anti-apoptotic and contributes to maintenance of the retinal structure.

Commercial Relationships: Juan Mo. None; Ming Zhang. None; Brendan Marshall. None; Jason Covar. None; Sally S. Atherton. None

Support: NIH grant RO1 EY009169

Program Number: 131 Poster Board Number: C0136

Presentation Time: 8:30 AM - 10:15 AM

Cell death pathway inhibitors do not significantly reduce the frequency or severity of murine cytomegalovirus (MCMV) retinitis in mice with retrovirus-induced Immunosuppression (MAIDS)
Hsin Chien¹, Emily L. Blalock¹, Christine I. Alston¹, Richard D. Dix²
¹Biology, Viral Immunology Center, Georgia State Univ, Atlanta, GA; ²Ophthalmology, Emory University School of Medicine, Atlanta, GA.

Purpose: The relative roles of apoptosis, pyroptosis, and necroptosis during AIDS-related HCMV retinitis development remain unclear. We recently provided evidence for simultaneous operation of all three cell death pathways during experimental MAIDS-related HCMV retinitis (J Virol 86:10961, 2012). We therefore hypothesized that selective inhibition of individual cell death pathway(s) will significantly reduce the frequency and/or severity of HCMV retinal disease during MAIDS. This hypothesis was tested using a pan-caspase inhibitor (Z-VAD-FMK), a caspase-1 inhibitor (Z-YVAD-FMK), or necrostatin-1 ( nec-1) to abolish apoptosis/pyroptosis, pyroptosis, or necroptosis, respectively.

Methods: Groups of C57BL/6 mice with MAIDS were injected subretinally with MCMV and systemically treated daily with Z-VAD-FMK, Z-YVAD-FMK, nec-1, or vehicle (control). Eyes from all groups were collected 10 days later, analyzed histopathologically, and scored for frequency and severity of retinitis. Parallel MCMV-infected eye sections were subjected to TUNEL assay.

Results: MCMV-infected eyes from MAIDS mice treated with vehicle showed a high frequency (100%) and severity (3.67) of retinitis. Surprisingly, MCMV-infected eyes from all MAIDS mice treated with each inhibitor continued to show a dampened yet high and equivalent retinitis frequency (80%). Differences in retinitis severity, however, were observed among drug-treated groups. MAIDS mice treated with the necroptosis inhibitor showed a severity score of 2.67, but MAIDS mice treated with the apoptosis/pyroptosis or pyroptosis inhibitors showed a severity score of 2.33. When compared with control MAIDS mice by TUNEL staining, MCMV-infected eyes of MAIDS mice treated with each inhibitor showed reduced TUNEL staining, the least observed following treatment with the apoptosis/pyroptosis or pyroptosis inhibitors. Decreased TUNEL staining was observed in the retinal pigment epithelium.

Conclusions: Inhibitors of apoptosis/pyroptosis, pyroptosis, or necroptosis dampened retinitis frequency and severity during MAIDS, but failed to reduce significantly overall retinal disease development as hypothesized. It is possible that loss of one cell death pathway is replaced by others to yield consistently high levels of retinal tissue damage during MAIDS-related MCMV retinitis.

Commercial Relationships: Hsin Chien, None; Emily L. Blalock, None; Christine I. Alston, None; Richard D. Dix, None

Support: NIH Grant EY010568, NIH/NEI Core Grant P30/EY006360, Research to Prevent Blindness, and Fight for Sight

Program Number: 132 Poster Board Number: C0137
Presentation Time: 8:30 AM - 10:15 AM

Inhibition of Autophagy by HSV-1 Beclin-Binding Domain (BBD) Induces Apoptosis of Viral Infected Retinas

Ming Zhang, Jason Covar, Juan Mo, Sally S. Atherton, Brendan Marshall. Cellular Biology & Anatomy, Georgia Health Sciences University, Augusta, GA.

Purpose: The autophagy response induced by HSV-1 is antagonized by the neurovirulence gene product, ICP34.5, which binds to the essential autophagy protein Beclin1 (Atg6) via Beclin-Binding Domain (BBD). The purpose of this study was to determine if inhibition of autophagy by HSV-1 BBD induces apoptosis of virus infected retinas.

Methods: Female BALB/c mice were injected with 1 x 10⁶ PFU of BBD-deficient HSV-1 (Δ68H) or its marker-resistant counterpart (Δ68HR) via the AC route. Neural retinas were removed from C57 mice, Atg5flo/flo, nestin-Cre mice and control Atg5+/+ flo/flo, nestin-Cre mice, cultured at 37°C and infected with 5 x 10⁵ PFU of Δ68H or Δ68HR. At different intervals, the injected eyes and the retinal cultures were harvested and analysed by plaque assay, HSV-1 staining, western blot for protein light chain 3-1 (LC3-I) and LC3-II and cleaved caspase 3.

Results: More replication virus was recovered from Δ68HR injected eyes than from Δ68H injected eyes starting from day 3 p.i. Less cleaved caspase 3 was detected in Δ68H infected eyes, compared to the eyes infected with the rescued strain Δ68HR. HSV-1 infected and spread in the cultured retina with a similar pattern as that observed in vivo and in a similar manner to in vivo infection, less cleaved caspase 3 was detected in Δ68H infected retinal cultures than in Δ68HR infected retinal cultures. However, in contrast to in vivo infection, similar amounts of virus were recovered from both Δ68H and Δ68HR infected retinas. Our results also showed that more cleaved caspase 3 was observed in Δ68H infected Atg5+/+ flo/flo, nestin-Cre retina than in Δ68H infected Atg5−/− flo/flo, nestin-Cre retina.

Conclusions: More apoptosis were induced by wild type HSV-1 than by BBD-deficient HSV-1 and absence of autophagy increased apoptosis of retinas infected by BBD-deficient HSV-1. The results suggest that inhibition of autophagy by HSV-1 BBD constitutes one mechanism of HSV-1 induced apoptosis of infected retinas.

Commercial Relationships: Ming Zhang, None; Jason Covar, None; Juan Mo, None; Sally S. Atherton, None; Brendan Marshall, None

Support: RO1 EY06012

Program Number: 133 Poster Board Number: C0138
Presentation Time: 8:30 AM - 10:15 AM

Role of Immunomodulatory Therapy for Herpes Simplex Virus Associated Ocular Inflammation

Asima Bajwa1,2, Sandra Hu-Torres1,2, C. Stephen Foster1,3
1Massachusetts Eye Research and Surgery Institution, Cambridge, MA; 2Ocular Immunology & Uveitis Foundation, Cambridge, MA; 3Department of Clinical Ophthalmology, Harvard Medical School, Boston, MA.

Purpose: To assess the impact of immunomodulators in patients diagnosed with Herpes Simplex virus associated ocular inflammation, uncontrolled with chronic antiviral treatment.

Methods: Retrospective case series. Patients with Herpes virus associated ocular inflammation between October 2006 and October 2011 at Massachusetts Eye Research and Surgery Institute in whom inflammation was not controlled with chronic antiviral medication and who have at least a 6 months follow up after the initiation of immunomodulatory therapy (IMT). Patients taking IMT for other ocular inflammatory conditions and those who started IMT at the same time as antivirals were excluded.

Results: Eight patients being treated with antiviral medication and IMT, age 27 to 80 years (mean 46.3). Clinical diagnosis was scleritis in 7 patients, panuveitis, and anterior uveitis in 1 each. Diagnostic methods employed included scleral biopsy in 6, vitreous PCR in 1 and, conjunctival biopsy in 1. All patients underwent antiviral treatment with either Acyclovir, Famvir or Valtrex . IMT was added for suspected autoimmune component. 7 out of 8 patients were on single drug therapy and 1 on combination of 2 drugs. At 6 months follow up after initiation of IMT all 8 were in remission.

Conclusions: Some patients diagnosed with Herpetic ocular inflammation may not fully respond to systemic antiviral medication in spite of freedom from viral replication. The immune response of the body to damaged tissues may require IMT to control inflammation in such patients.

Commercial Relationships: Asima Bajwa, None; Sandra Hu-Torres, None; C. Stephen Foster, Abbott Medical Optics (C)

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Abbott Medical Optics (F), Alcon Laboratories, Inc. (C), Alcon Laboratories, Inc. (F), Allergan, Inc. (C), Allergan, Inc. (F), Eyegate Pharmaceuticals, Inc. (F), Eyegate Pharmaceuticals, Inc. (F), IOP Ophthalmics (C), Ista Pharmaceuticals (C), Lux Biosciences, Inc. (C), Lux Biosciences, Inc. (F), Novartis Pharmaceuticals Corporation (C), Novartis Pharmaceuticals Corporation (F), XOMA Ltd (C)

Program Number: 134 Poster Board Number: C0139
Presentation Time: 8:30 AM - 10:15 AM

The effects of systemic LPS and CpG-ODN on retinal microglia in the Ins2Akita mouse
Jelena M. Kezic1,2, Paul G. McMenamin1. 1Anatomy & Developmental Biol, Monash University, Melbourne, VIC, Australia; 2Centre for Eye Research Australia, Melbourne, VIC, Australia.

Purpose: We have previously demonstrated morphological changes to retinal microglia and the disruption of microglial networks in response to hyperglycemia in Ins2Akita mice. Here we sought to determine whether exposure to toll-like receptor (TLR) 4 ligand lipopolysaccharide (LPS) or TLR 9 ligand CpG-ODN enhances microglial responses to hyperglycemia and further, drives the development of retinopathy in the Ins2Akita mouse.

Methods: Ins2Akita mice bear a point mutation in the insulin2 gene, resulting in spontaneous development of hyperglycemia by 4 weeks of age. For this study, Ins2Akita mice were crossed with C57BL/6J Cx3cr1+/gfp mice, allowing for the clinical (Micron III Fundus Camera) and microscopic visualization of Cx3cr1+ microglia. Non-diabetic Cx3cr1+/gfp and diabetic Cx3cr1+/gfp Ins2Akita mice were injected with either 9 μg/μl Ultrapure LPS (or PBS control) or 40 μg CpG-ODN (or control Oligo GpC-ODN) and were clinically examined after 24 hours and 1 week respectively. Analysis of changes to microglia and macroglia were performed using a range of monocytic and glial cell markers.

Results: Clinical evaluation at 24 hours after LPS and 1 week after CpG-ODN treatment revealed no overt changes to the retinal fundus or vasculature in non-diabetic Cx3cr1+/gfp mice or diabetic Cx3cr1+/gfp Ins2Akita mice. Whilst LPS and CpG-ODN treatment of non-diabetic mice resulted in characteristic changes to microglial morphology indicative of activation, microglial reactivity was not further enhanced in Ins2Akita mice. The upregulation of MHC Class II expression was observed on the inner retinal vasculature of non-diabetic Cx3cr1+/gfp mice following CpG-ODN treatment, a phenomenon that was absent in Ins2Akita mice. Muller cell activation was evident in both non-diabetic and diabetic mice 1 week after CpG-ODN treatment, but not 24 hours after treatment with LPS.

Conclusions: Systemic treatment with LPS or CpG-ODN does not significantly alter microglial reactivity or drive the development of retinopathy in the Ins2Akita mouse.

Commercial Relationships: Jelena M. Kezic, None; Paul G. McMenamin, None
Support: NHMRC #634469, Rebecca L. Cooper Foundation

Program Number: 136 Poster Board Number: C0141
Presentation Time: 8:30 AM - 10:15 AM
Role of Toll like Receptor 5 (TLR5) in Experimental Bacillus cereus Endophthalmitis
Salai Madhumathi Arasi Parkunan1, Nanette R. Wheatley1, Michelle C. Callegan2, 1Microbiology & Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2Ophthalmology, Dean McGee Eye Institute, Oklahoma City, OK; 3Dean McGee Eye Institute, Oklahoma City, OK.

Purpose: To test the hypothesis that TLR5 contributes to bacterial recognition and inflammation during Bacillus cereus endophthalmitis. B. cereus possesses flagella which allow the organism to migrate within the eye during endophthalmitis. Flagella is a TLR5 ligand.

Methods: Experimental endophthalmitis was induced in B6.129S1-Tlr5tm1Flv/J TLR5-/- mice and wild type C57BL/6J mice by intravitreally injecting 100CFU of B. cereus ATCC 14579. At various time points after infection, eyes were analyzed for intraocular bacterial growth, retinal function, and acute intraocular inflammation by published methods. Values represented N=4 eyes/time point, mean ± SEM.

Results: The intraocular growth rates of B. cereus in infected eyes were determined following 4, 8 and 12 hours of infection. B. cereus grew rapidly and at similar rates in infected eyes of wild type mice and TLR5-/- mice. A greater loss in retinal function in both groups of mice was observed at 8 and 12 hours following infection. Retinal architecture disruption and acute inflammation (neutrophil infiltration and proinflammatory cytokine concentrations) increased during the course of infection and were significant at 8 and 12 hours postinfection. Acute inflammation was comparable in TLR5-/- and control mice.

Conclusions: The absence of TLR5 did not have a significant effect on limiting the intraocular growth rates of B. cereus during endophthalmitis.

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endophthalmitis. TLR5−/− mice had retinal function loss and retinal architecture disruption similar to wild type mice, suggesting that TLR5 deficiency does not alter vision loss in the endophthalmitis model. Moreover, deficiency of TLR5 did not lead to a significant change in the intraocular inflammatory process to infection. These results suggest that the inflammatory cascade in endophthalmitis is initiated by TLRs other than TLR5 following intraocular recognition of B. cereus.  

Commercial Relationships: Salai Madhumathi Arasi Parkunan, None; Nanette R. Wheatley, None; Michelle C. Callegan, None  

Support: National Institutes of Health Grant R01EY012985 (MCC). Research program supported in part by NIH CORE Grant P30EY12191 (Robert E. Anderson, OUHSC), National Center for Research Resources COBRE Grant P20RR017703 (Robert E. Anderson, OUHSC), and an unrestricted grant to the Dean A. McGee Eye Institute from Research to Prevent Blindness.  

Program Number: 137 Poster Board Number: C0142  

Presentation Time: 8:30 AM - 10:15 AM  

The pathogenicity island-encoded AraC-type transcriptional regulator, PerA, contributes to the course and severity of Enterococcus faecalis endophthalmitis  

Phillip Coburn1, Frederick C. Miller2, Michelle C. Callegan1.  
1Ophthalmology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2Biology, Oklahoma Christian University, Edmond, OK.  

Purpose: To evaluate the contribution of the AraC-type virulence gene regulator, PerA, in the development of Enterococcus faecalis endophthalmitis.  

Methods: To evaluate the incidence of perA among endophthalmitis isolates, genomic DNA was prepared from 39 endophthalmitis isolates and PCR performed using perA-specific primers. To directly test whether PerA is involved in infection, we utilized a new mouse model of E. faecalis endophthalmitis to compare the course and severity of endophthalmitis caused by the clinical E. faecalis isolate E99 with that of an isogenic perA mutant, DBS01, and perA complemented strain GT101. 50 cfu of strains E99, DBS01, or GT101 were injected directly into the vitreous of C57BL/6J mice. At 12 and 24 h postinfection, mice were subjected to electroretinography (ERG) and the percent ERG retention was calculated relative to the control, uninfected eye for each animal. Following ERGs, mice were euthanized and infected eyes harvested for quantitative determination of E. faecalis per eye.  

Results: PCR analysis of 39 isolates revealed that 23 were PCR positive for perA, suggesting an enrichment of this gene among endophthalmitis isolates, and consistent with our previous findings of enrichment among E. faecalis clinical isolates. During E. faecalis endophthalmitis, at 12 h postinfection, a statistically significant decrease in the %ERG retained for both A- and B-waves was observed in eyes infected with the perA mutant strain DBS01 compared to the wildtype E99 and perA complemented strain GT101. This suggested that inactivation of the perA gene might result in greater virulence in this model. At 24 h postinfection, there were no differences in %ERG retained between the strains. Quantitation of bacterial concentrations in infected eyes did not reveal a difference in growth rate, suggesting that PerA does not influence growth in the vitreous.  

Conclusions: The results of this study demonstrate an enrichment of perA among endophthalmitis isolates, and suggest that PerA contributes to the course and severity of E. faecalis endophthalmitis. Further, our results suggest that PerA might serve as a direct or indirect repressor of virulence genes, and future studies will aim to identify and directly test these factors in the mouse endophthalmitis model.  

Commercial Relationships: Phillip Coburn, None; Frederick C. Miller, None; Michelle C. Callegan, None  

Support: Funded by National Center for Research Resources COBRE Grant P20RR017703 (Robert E. Anderson, OUHSC, COBRE Pilot Project for PC). Research program supported in part by National Institutes of Health Grant R01EY012985 (MCC), NIH CORE Grant P30EY12191 (Robert E. Anderson, OUHSC), and an unrestricted grant to the Dean A. McGee Eye Institute from Research to Prevent Blindness.  

Program Number: 138 Poster Board Number: C0143  

Presentation Time: 8:30 AM - 10:15 AM  

Visual outcome of ocular toxoplasmosis in an University Center in Argentina  

Bernardo A. Schlaen1,2, Mariana Ingolotti1, Cristina E. Wong Pang1,3, Jorge Mancini1, Maria Noel Gabin1, Cristobal A. Couto1, Mario J. Saravia1. 1Ophthalmology, Hospital Universitario Austral, Capital Federal, Argentina; 2Ophthalmology, University of Buenos Aires, Buenos Aires, Argentina; 3Ophthalmology, University of Panama, Panama, Panama.  

Purpose: To evaluate the visual outcome of patients with diagnosis of ocular toxoplasmosis in an University Center in Argentina.  

Methods: This is a retrospective case series study. Clinical records of patients who have diagnosis of active retinitis due to ocular toxoplasmosis at Hospital Universitario Austral were included. Every episode of each patient was included in the study. Collected data from patients included age, gender, visual acuity at baseline, visual acuity at the end of the episode, anterior inflammation, 2+ or more vitreous haze, ocular inflammatory associated signs, acute or chronic infection, origin of the infection, primary or recurrent active retinitis, type of lesion, location, complications, and number of recurrences.  

Results: 52 patients with diagnosis of ocular toxoplasmosis were found (13 females and 22 males). Active retinitis was observed in 39 patients (22 females and 17 males) with a total of 47 active retinitis events. The average age of occurrence of active retinitis events was 34.76 ± 17.26 years. Primary active retinitis was present in 22 patients whereas 25 patients have recurrent disease. Visual acuity at presentation was better than 20/40 in 22 episodes out of 47 (46.80%), whereas visual acuity less than 20/200 was observed in 10 patients out of 47 (21.28%). A total number of 24 complications were detected in 15 patients. Exudative detachment occurs in 6 events with active retinitis in a central location out of 13 and in 2 events out of 26 where the lesion was peripheral (Fisher exact test 0.006). Final visual acuity was better than 20/40 in 33 patients (84.61%) and less than 20/200 in 5 patients (12.82%). Only 1 patient out of 10 with 1 complication had a final visual acuity less than 20/200. Four out of 5 patients with 2 or more complications had a final visual acuity less than 20/200. The difference of the final visual acuity in these groups was statistically significant (Fisher exact test: 0.022). Recurrences were observed during the studied period in 11 patients out of 29. Seven patients had 1 recurrence and 4 patients had two or more.  

Conclusions: Visual acuity at presentation was less than 20/200 in 22% of the patients. Final visual acuity was less than 20/200 in 12.82% of the patients. Greater number of complications was associated with worse final visual acuity. During the period studied 20% of patients developed at least one recurrence.  

Commercial Relationships: Bernardo A. Schlaen, None; Mariana Ingolotti, None; Cristina E. Wong Pang, None; Jorge Mancini, None.
Purpose: Ocular toxoplasmosis (OT) is the leading cause of infectious posterior uveitis. It can relapse and cause function loss after each episode. Contradicting results of previous studies exist regarding frequency of relapses. The aim of this study was to identify temporal dynamics of relapses.

Methods: We contacted 156 patients with OT treated in our hospital since January 2000 and requested to complete a questionnaire addressing course, activity and possible risk factors of their disease. Results were compared and completed with data from our uveitis data base and treating ophthalmologists. Moreover we invited every patient for a clinical follow up examination. Finally, data of 84 patients could be included in this study.

Results: The mean follow-up time was 11.4 years (range 1.3 - 55.6, SD: 9.5) with a total of 953.9 observational years. 57 (67.5%) patients were female, 27 (32.5%) patients were male. The mean age of the cohort was 40.4 years (range 7.9 - 74.3). The mean reported age at the time of clinical diagnosis was 27.2 years (range 6.7 - 69.7). A total of 280 relapses were documented, with a mean 3.2 relapses per patient (range 1-27). The mean annual relapse frequency was 0.31, median relapse free survival was 2.52 years. Relapse risk was inversely related to duration of infection and patients age.

Conclusions: Relapse rate is highest in the first year after an episode. The risk of relapse is influenced by duration of infection and patients age.

Commercial Relationships: Mira Ruppenstein, None; Michael Reich, None; Matthias D. Becker, Novartis (F); Bayer (F), Allergan (F); Friederike Mackensen, Abbott (F), Heidelberg Engineering (F), Heidelberg Engineering (R)
a function of serum IL-17, (F (1) =10, p-value<0.05). As well, using linear regression, it was a significant predictor (β=-0.888, p-value < 0.05). Also it showed a significant regression pattern in relation to retinal pigment epithelium detachment (β=0.037, p-value <0.05).

Conclusions: Detection of high level of some of inflammatory cytokines supports a role for inflammation in AMD pathogenesis. IL-17 was the most significantly associated cytokine with the disease activity in term of exudation and retinal thickening.

Commercial Relationships: Khaled Nassar, None; Elshaymaa Elfar, None; Julia Lüke, None; Matthias Lüke, Novartis Germany (F); Salvatore Grisanti, Novartis (C), Allergan (C), Bayer (C), Pfizer (C), Thrombogenics (C)

Support: The work was supported by The Novartis Pharma GmbH Research Grant

Program Number: 142 Poster Board Number: C0147
Presentation Time: 8:30 AM - 10:15 AM

Inflammatory cytokines in the vitreous of postmortem eyes: Relationship to age and drusen load

Purpose: Recent studies provide evidence that chronic local inflammation plays an important role in the pathogenesis of age-related macular degeneration (AMD). While aging, drusen load and complement factor H (CFH) polymorphism increase AMD risk, their relationships to the local inflammatory processes in the eye require further investigation. We examined the level of inflammatory cytokines (ICs) and growth factors (GFs) in the vitreous of postmortem donor eyes and their relationship to age, drusen load and single nucleotide polymorphisms for the CFH (Y402H) at risk variant (T>C).

Methods: Sixteen pairs of post-mortem human donor eyes, with average time from death to tissue processing of 15.14 hr (SD±4.7 hr), were used in this study. To assess the effect of postmortem time on cytokine degradation, vitreous samples from Long Evans rats were collected at 0, 10 and 20 hrs after sacrifice and examined with multiplex suspension arrays (BioplexTM). In processing the human postmortem eyes, the right eyes were embedded in paraffin to assess for drusen load; the left eyes were dissected circumferentially at the pars plana for collection of vitreous. Retinal tissues were used to express or knockdown with lentivirus system to RPE cells.

Results: In vitro study, the POMC gene (precursor of a-MSH) will be over-expressed or knockdown with lenti-virus infection system to RPE cells, then to study the immune privilege function of the RPE cells. In vivo study, RPE cells were treated with different doses of IL-17A, the expression of POMC was detected by real-time PCR and western blot. Secretion levels of TGF-β, IFN-γ and IL-17 were detected by ELISA.

Commercial Relationships: Shujie Zhang, None; Jihong Wu, None

Support: NSFC81200675

Program Number: 144 Poster Board Number: C0149
Presentation Time: 8:30 AM - 10:15 AM

Retinal pigment epithelial (RPE) cells convert bone marrow derived macrophages into myeloid-derived suppressor cells with a novel phenotype
Heping Xu, Chang Luo, Mei Chen. Centre for Vision and Vascular Science, Queen’s University Belfast, Belfast, United Kingdom.

Purpose: We have shown previously that macrophages/microglia accumulate in the subretinal space in the aging eye. The phenotype and function of subretinal macrophages/microglia remain elusive. Subretinal macrophages are in close contact with retinal pigment epithelium (RPE) cells, which may affect / regulate macrophage functions. The aim of this study was to investigate the effect of RPE cells on the phenotype and function of bone marrow-derived macrophages (BM-DMs).

Methods: Bone marrow cells from C57BL/6 mice were cultured in DMEM supplemented with GM-CSF for 5 days to generate BM-DMs. The phenotype of BM-DMs was confirmed by flow cytometry as C11b+/F4/80+/MHC-II+/Gr1+. Primary RPE cells were cultured from C57BL/6 mice and confirmed by RPE65 and cytokeratin staining. BM-DMs were co-cultured with sub-confluent RPE cells for different times. Macrophages were then collected for phenotypic and
functional assays.

**Results:** Co-culture of BM-DMs with RPE cells results in a time-dependent down-regulation of MHC-II expression and the generation of CD11b+F4/80+Gr1+ myeloid-derived suppressor cells (MDSC). Real-time RT-PCR analysis showed that RPE-induced MDSCs expressed high levels of IL-6, IL-1β, and Arginase-1, but lower levels of IL-12p40 and TNF-α compared to naïve BM-DMs. The expression levels of iNOS, TGF-β and Ym1 did not differ between native BM-DMs and RPE-induced MDSCs. Furthermore, a functional study showed that these MDSCs could suppress anti-CD3 antibody mediated T cell activation and proliferation.

**Conclusions:** Our results suggest that RPE cells can convert bone-marrow derived macrophages into myeloid-derived suppressor cells under in vitro culture conditions. RPE-induced myeloid-derived suppressor cells are CD11b+F4/80+Gr-1+MHC-II+IL-6-IL-1β-Arg1+ and can suppress T cell activation.

**Commercial Relationships:** Heping Xu, None; Chang Luo, None; Mei Chen, None

**Support:** Age UK (322)

**Program Number:** 145 Poster Board Number: C0150

**Presentation Time:** 8:30 AM - 10:15 AM

**Retinal pigment epithelial cells (RPE) induced from induced pluripotent stem (iPS) cells inhibit activation of T cells in vitro**

Sunao Sugita, Masayo Takahashi. Laboratory for Retinal Regeneration, Center for Developmental Biology, RIKEN, Kobe, Japan.

**Purpose:** To determine whether human retinal pigment epithelial cells (RPE) from induced pluripotent stem (iPS) cells can inhibit T-cell activation in vitro.

**Methods:** Cultured iPS-derived RPE cells (iPS-RPE) were established from fresh skin tissues (skin fibroblasts) obtained from healthy donors after informed consent was obtained. Target activated T cells were established from autologous or allogeneic T cells (CD4 or CD8) from peripheral blood mononuclear cells. As another targets, T cell clones of patients with active uveitis were established from healthy donors after informed consent was obtained. Target activated T cells were starved for 24 hours under in vitro culture conditions. RPE-induced myeloid-derived suppressor cells are CD11b+F4/80+Gr-1+MHC-II+IL-6-IL-1β-Arg1+ and can suppress T cell activation.

**Commercial Relationships:** None

**Support:** None in the Support

**Program Number:** 146 Poster Board Number: C0151

**Presentation Time:** 8:30 AM - 10:15 AM

**Effects of bisphosphonates on cytotoxicity and cytokine expression in hRPE cells in vitro**

Chris Or1, Jing Z. Cui1, Joanne A. Matsubara1, Farzin Foroozghan1,2

1 Ophthalmology & Visual Sciences, University of British Columbia, Vancouver, BC, Canada; 2 Ophthalmology, St Paul's Hospital, Vancouver, BC, Canada.

**Purpose:** Bisphosphonates are the most commonly prescribed drugs used to treat osteoporosis. In addition to a recent report supporting an association between bisphosphonate use and ocular inflammation (Elminan et al., 2012), preliminary data also support an association between bisphosphonate use and age-related macular degeneration (AMD). Therefore, we studied the effects of bisphosphonates on primary culture of human retinal pigment epithelium (hRPE), a cell type known to secrete pro-inflammatory cytokines in the outer retina. Alendronate, an amino-bisphosphonate, and etidronate, a non-amino-bisphosphonate, were selected for this experiment as they are members of the two structurally different classes of bisphosphonates.

**Methods:** Primary cultures of hRPE were serum-starved for 24 hours and then treated for 24 hours with alendronate (0.0001, 0.1, 100 µM) and etidronate (0.01, 1 µM). Cell viability was measured using the MTT assay. Investigation of cytokines induced by bisphosphonates was performed using a human cytokine 29-Plex Panel (Bio-Plex) array and the results were analyzed with an ANOVA.

**Results:** Etidronate, at the lower concentration, significantly increased the expression of IL-6 (p=0.03) and IL-8 (p=0.04). At the higher concentration, etidronate significantly decreased the expression of GM-CSF (p=0.02) and bFGF (p=0.02). Alendronate, at the highest concentration, significantly increased the expression of IL-8 (p=0.02) and decreased the expression of eotaxin (p=0.02). Alendronate also significantly decreased the expression of bFGF at all concentrations (p<0.05). Alendronate and etidronate did not significantly alter the expression of the other cytokines measured. Cell viability was not significantly affected by either alendronate or etidronate treatment for 24h.

**Conclusions:** Alendronate and etidronate display dose dependent effects in hRPE cells. Etidronate at lower concentration and alendronate at higher concentration appear to induce pro-inflammatory effects. In addition, anti-angiogenic effects of both drugs were demonstrated by the reduction in bFGF and eotaxin expression observed with the highest concentration of alendronate. The anti-angiogenic effects observed are consistent with a previous study (Nagai et al., 2007). Further studies are required to elucidate the association between bisphosphonate use and AMD.

**Commercial Relationships:** Chris Or, None; Jing Z. Cui, None; Joanne A. Matsubara, None; Farzin Foroozghan, None

**Support:** Canadian Institutes of Health Research Grant (CIHR: MOP 97806)

**Program Number:** 147 Poster Board Number: C0152

**Presentation Time:** 8:30 AM - 10:15 AM

**TNF-α and Aβ promote activation of metalloproteinases in retinal pigment epithelial cells and is mediated by the NF-κB pathway: implications for AMD pathogenesis**


**Purpose:** Inflammation and oxidative stress play important roles in the pathogenesis of age-related macular degeneration (AMD). Unfortunately, the interactions between these processes have yet to be elucidated. Drusen components, such as Aβ, may trigger pro-inflammatory events in the outer retina via activation of the NF-κB pathway - causing injury to retinal pigment epithelial (RPE) cells, increased cytokine production, and matrix metalloproteinase (MMP) activation. Inflammation-induced MMP activation causes extracellular matrix (ECM) degradation and Bruch's membrane malfunction. To investigate the role of inflammation and drusen on the activation of MMPs in RPE cells, we carried out a series of
experiments to study the link between TNF-α or Aβ induced MMP activation and the activation of NF-kB.

Methods: Long Evans rats (N=6) were intravitreally injected with a 5 μL solution containing 0.2 ng of TNF-α, 7 μg of Aβ(1-40), or 7 μg of Aβ(40-1). Eyes were enucleated after 24 hours, cryosections were prepared, and in-situ gel zymography using DQ-Gelatin (LifeTechnologies Inc.) was used to detect MMP activity. Immunofluorescence was imaged with a confocal microscope (Zeiss) at 40x. In vitro studies were undertaken with an ARPE19/ NF-kB stable cell line with luciferase as the reporter protein. Reporter cells were seeded and treated with Aβ(1-40) or Aβ(40-1) at a concentration of 0.1, 0.3, or 1 μM. Recombinant hTNF-α at a concentration of 20 ng/mL for 8 hrs was also carried out. All experiments were performed in quadruplicate.

Results: Intravitreal injections of TNF-α and Aβ resulted in RPE MMP activation as demonstrated by in-situ gel zymography. Compared with untreated reporter cells, recombinant hTNF-α significantly up-regulated NF-kB activity up to 12.64 fold, indicating a robust reporter system was established. No differences were seen in NF-kB activity using stimulation dosages of Aβ(1-40) at 0.1 and 0.3 μM, but NF-kB was 1.31 fold higher at 1 μM.

Conclusions: Intravitreal injections with TNF-α or Aβ resulted with RPE MMP activation. A drusen component, Aβ, up-regulated NF-kB activity. Further assessment of MMP activation by drusen components is needed to gain further insight into the pathological changes in RPE, possibly leading to strategies that can limit abnormal MMP activation associated with AMD pathogenesis.

Commercial Relationships: Brett Poulis, None; Aikun Wang, None; Jing Z. Cui, None; Luba Kojic, None; Joanne A. Matsubara, None

Support: Canadian Institutes of Health Research Grant (CIHR: MOP 97806)

Program Number: 148 Poster Board Number: C0153
Presentation Time: 8:30 AM - 10:15 AM

Targeting the Inflammasome with the Caspase Activation Recruitment Domain (CARD) in an In Vitro Model of RPE Inflammation

Cristhian J. Idefonso1, Henrique Jaime2, Alfred S. Lewin1,3
1Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, FL; 2Department of Biology, University of Florida College of Liberal Arts and Sciences, Gainesville, FL.

Purpose: Dry Age-related macular degeneration (AMD) has been associated with an increase in oxidative stress and inflammatory processes within the retina. Oxidized molecules like 4-hydroxynonenal (4-HNE) have been detected in the eyes of dry AMD patients, supporting the role of these processes in the diseases. The purpose of this work is to develop an AAV vector that delivers a secretable and cell penetrating CARD to study the role of the proinflammatory cytokine IL-1β in AMD.

Methods: The CARD domain was amplified from the human apoptosis-associated speck-like protein containing a CARD (ASC) gene and fused to the cell-penetrating peptide sequence derived from the HIV Tat protein using PCR. The product was fused to either a puromycin resistance gene in a lentiviral vector plasmid or to a secretable form of GFP through a furin cleavage site. The sequence of the TatCARD insert was verified by DNA sequencing and cloned in an AAV plasmid. The monocytic cell line THP-1 and the retina pigmented epithelium like cell line ARPE-19 were transduced with lentiviral vectors to generate stable cell lines by selecting for puromycin resistant cells. Expression of TatCARD was determined by western blot, and its biological effect on LPS or 4-HNE induced secretion of IL-1β was measured by ELISA. Cellular localization of the secretable TatCARD was determined by fluorescence microscopy. Detection of secreted TatCARD was inferred western blot using cell conditioned media.

Results: The expression of the TatCARD significantly inhibited the LPS induced secretion of IL-1β from THP-1 cells. The cellular distribution of sGFP-TatCARD in transfected cells was punctate in contrast to the cytoplasmic distribution of GFP. The fused sGFP-TatCARD construct was detected in transfected cell lysates, whereas the cleaved GFP was detected in the corresponding cell conditioned media. Stable ARPE-19 cells transduced with either empty lentivector control or TatCARD lentivector were stimulated with 4-HNE at 30μM for 24 hours. The levels of secreted IL-1β in the conditioned media were lower in cells expressing the TatCARD construct than in the empty lentivector control cells.

Conclusions: The expression of TatCARD inhibits the LPS and the 4-HNE induced secretion of IL-1β. We have successfully constructed a secretable form of the TatCARD protein that may be useful in blocking retinal and RPE inflammation.

Commercial Relationships: Cristhian J. Idefonso, None; Henrique Jaime, None; Alfred S. Lewin, University of Florida (P) Support: Florida Biomedical Research Foundation grant e10KG-08; NIH grants R01 EY02025, and EY021721; and a grant from the Macula Vision Research Foundation

Program Number: 149 Poster Board Number: C0154
Presentation Time: 8:30 AM - 10:15 AM

NLRP3 inflammasome activation in human retinal pigment epithelium under inflammation and oxidative stress

Yujuan Wang1,2,3, Mones S. Abu-Asab1, DeFen Shen4, Xi K. Chu4, Alexander J. Ogilvy5, Jingsheng Tuo6, Chi-Chao Chan4
1Immunopathology Section, Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD; 2Zhongshan Ophthalmic Center, Sun Yat-sun University, Guangzhou, China; 3Histopathology Core, National Eye Institute, National Institutes of Health, Bethesda, MD.

Purpose: Inflammation and oxidative stress are closely involved in age-related macular degeneration (AMD). The NLRP3 inflammasome, a key mediator of neuroinflammation, is linked to AMD pathogenesis. Inflammasomes promote the maturation of certain pro-inflammatory cytokines such as IL-1β and IL-18. In this study, we aimed to evaluate the NLRP3 inflammasome-mediated effects on human retinal pigment epithelium (RPE) under inflammation and oxidative stress.

Methods: A human RPE cell line (ARPE-19) and cultured primary adult human RPE (hRPE) were starved for 24 h and stimulated with 10 ng/ml tumor necrosis factor-alpha (TNFα), 10 μg/ml lipopolysaccharide (LPS)+10 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), or 200 μM H2O2 for 24 h to mimic inflammatory and oxidative stress. Expression of NOD-like receptor family, pyrin domain containing 3 (NLRP3), interleukin-1 beta (IL-1β), and IL-18 transcripts was measured by quantitative reverse transcript polymerase chain reaction (qRT-PCR); and expression of NLRP3 and cleaved caspase-1 proteins was evaluated by immunohistochemistry. In ARPE-19 cells, ultrastructural changes under the stimuli were evaluated by transmission electron microscopy (TEM). The effects of NLRP3 inflammasome on IL-1β, IL-18 and cleaved caspase-1 expressions under inflammatory and oxidative stress status were also assessed by transfecting NLRP3-targeting siRNA in ARPE-19 cells.

Results: In both ARPE-19 and hRPE, stimulation with TNFα, LPS+TCDD, or H2O2 significantly enhanced NLRP3, IL-1β and IL-18 transcripts (all p<0.05); ARPE-19 produced a higher level of IL-18 mRNA than hRPE under all stimulation. Immunohistochemistry illustrated elevated NLRP3 protein expression and caspase-1

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cleavage in ARPE-19 cells under inflammatory or oxidative stress. Conversely, knockdown of NLRP3 expression in ARPE-19 showed less caspase-1 cleavage under all stimulation and reduced IL-1β and IL-18 transcripts with TNFα stimulation (all p<0.05). TEM illustrated presence of autophagosomes, cytoplasmic vesicles, mitochondrial damage, cytoskeleton disruption, and nuclear membrane separation in all stimulated ARPE-19 cells as compared with controls.

Conclusions: Inflammation and oxidative stress activates NLRP3 inflammasome in human RPE cells. The pro-inflammatory cytokine production and cellular damage of RPE caused by NLRP3 inflammasome activation could play an important role in AMD pathogenesis.

Commercial Relationships: Yujuan Wang, None; Mones S. Abu-Asab, None; DeFen Shen, None; Xi K. Chu, None; Alexander J. Ogilvy, None; Jingsheng Tou, None; Chi-Chao Chan, None
Support: The National Eye Institute Intramural Research Program supported the study.

Program Number: 150 Poster Board Number: C0155
Presentation Time: 8:30 AM - 10:15 AM
7-Ketocholesterol Induces Interleukin(IL)-1 beta and IL-18 Production in RPE Cells via Activation of the Inflammasome
Purpose: 7-Ketocholesterol is a form of oxidized cholesterol. It has been found to be associated with lipoprotein deposits in Bruch’s membrane, choriocapillaris, and RPE cells in the primate retina as well as a component of drusen in human eyes affected by AMD. The purpose of this study was to assess whether 7-Ketocholesterol could activate inflammasomes in RPE cells to induce IL-1 beta and IL-18 production.
Methods: Primary cultured RPE cells from an eighty-year-old human donor, or ARPE-19 cells, were primed using LPS plus IL-1 alpha for 6 hours, followed by incubation with 7-Ketocholesterol for 16 hours. The production of IL-1 beta and IL-18 was assessed in cell culture supernatant using enzyme linked immunosorbant assay. Total protein extracted from treated ARPE-19 cells was subjected to western blot assay to detect cleaved caspase-1 p20, using ATP treatment as a positive control. Glyburide, an inflammasome inhibitor, was added into cell culture together with 7-Ketocholesterol to confirm signaling pathway involvement.
Results: RPE cells from an eighty-year-old human donor incubated with 10 µM 7-Ketocholesterol produced a considerable amount of IL-1 beta, but a lower amount of IL-18. Treatment with glyburide reduced the release of the IL-1 beta by about 60%, but had no effect on IL-18 production. Incubation of 7-Ketocholesterol with ARPE19 cells produced similar amounts of IL-1 beta and IL-18. Western blot of 7-Ketocholesterol-treated ARPE-19 cells showed elevated caspase-1 p20 production to levels similar to those stimulated by ATP-treatment.
Conclusions: 7-Ketocholesterol induces IL-1 beta and IL-18 production from RPE cells. Accumulation of 7-Ketocholesterol in drusen of AMD patients may therefore have a pathogenic role in the disease process through the release of the proinflammatory cytokine IL-1 beta and IL-18.
Commercial Relationships: Guangpu Shi, None; Osato J. Ogbeifun, None; Lindsey F. Nugent, None; Ignacio R. Rodriguez, None; Igal Gery, None
Support: NEI intramural research program

Program Number: 151 Poster Board Number: C0156
Presentation Time: 8:30 AM - 10:15 AM
Modulation of Laser-Induced Choroidal Neovascularization by Differentiated Macrophages from Patients with Age Related Macular Degeneration
Shira Levi1, Tareq Z. Jaouni1, Michelle Grunin1, Tal Burstyn-Cohen2, Itay Chowers3. 1Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; 2Institute of Dental Sciences, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.
Purpose: Monocytes and macrophages were implicated in the pathogenesis of age-related macular degeneration (AMD). We aimed to evaluate if M1 and M2 polarized macrophages from AMD patients can modulate the course of laser-induced choroidal neovascularization (CNV) in rodents.
Methods: Monocytes were isolated from peripheral blood of neovascular AMD (NVAMD) patients (n=12), cultured and matured to an M0 macrophage phenotype. Macrophages were then polarized to either M1 or M2 phenotypes using LPS and IFN-g for M1, and IL-13 and IL-4 for M2, respectively. Generation of polarized macrophages was validated by QPCR for CCL22, CCL17, TNF-α and IL-12. Fluorescently-labeled polarized macrophages and M0 macrophages or PBS were injected into the vitreous of rat eyes 7 days following the formation of laser-induced CNV. Presence of CNV was verified with fluorescein angiogram (FA). Masked observers graded CNV size based on isolectin staining of retinal pigment epithelium (RPE)-choroid flat mounts. Macrophage survival and migration pattern were assessed in retinal and RPE flat mounts.
Results: QPCR validated macrophage polarization to the M1 and M2 phenotypes. Intravitreal delivery of polarized human macrophages did not cause inflammation according to clinical examination and histology. Injected cells survived more than 7 days in rat eyes and were detected throughout the retinal layers and the vicinity of CNV lesions. Masked assessment of isolectin staining of RPE-choroid flat mounts demonstrated increased CNV size 7 days following injection of M1 (1.3-fold, Mann-Whitney P=0.0001), and M2 (1.4-fold, P=0.012) macrophages, but not following M0 macrophage injection (1.05-fold, P=0.98).
Conclusions: Intravitreal delivery of polarized macrophages from NVAMD patients is associated with a pro-angiogenic effect in the rat model of laser-induced CNV. This data supports the putative pathogenic role of macrophages in NVAMD and suggests that macrophages may potentially serve as therapeutic target for the disease.
Commercial Relationships: Shira Levi, None; Tareq Z. Jaouni, None; Michelle Grunin, None; Tal Burstyn-Cohen, None; Itay Chowers, Novartis (C), Teva (C)
Support: Israeli Ministry of Health, Israel Science Foundation

Program Number: 152 Poster Board Number: C0157
Presentation Time: 8:30 AM - 10:15 AM
Gene Expression Signature in the Monocyte Population of Patients with Age-Related Macular Degeneration
Michelle Grunin1, Shira Levi2, Tal Burstyn-Cohen2, Itay Chowers3. 1Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; 2Institute of Dental Sciences, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.
Purpose: Further evidence in recent years has linked monocytes/macrophages and their respective populations with the pathogenesis of age-related macular degeneration (AMD). We speculate that monocyte involvement in AMD is reflected in the peripheral blood circulation. To evaluate this hypothesis, we have characterized the gene expression signature of monocytes from patients with AMD.
Methods: Peripheral blood was taken from treatment-naive neovascular AMD patients (n=14), and age-matched controls (n=15).
Peripheral blood mononuclear cells (PBMC) were separated using a Histopaque gradient, and total blood monocytes, including the CD14+ and CD14+/CD16+ subgroups, were isolated via negative selection with magnetic beads. Total mRNA was extracted and gene expression was evaluated using the Affymetrix Gene 1.0 ST microarrays. Analysis was performed using the open source software programs R, DAVID, Expander, TANGO, ISMARA and other genomics tools. Age, ethnicity, response to treatment, and severity of disease were taken into account for analysis. Quantitative PCR (QPCR) was performed to validate microarray data.

**Results:** Using RMA normalization and ANOVA, 1,522 genes were associated with AMD (P<0.05), of which 77 genes had a fold change>1.5. Alternative algorithm (Expander program with RMA) validated the existence of an altered expression pattern in AMD patients. QPCR analysis of 3 genes on AMD and control samples not previously used for microarray (AMD: n=6; control: n=6), supported the microarray results. DAVID functional analysis of the 1,522 differentially expressed genes generated 6 main annotation clusters that were upregulated (FDR-corrected P< 0.05) in AMD. Alternative algorithm (TANGO) detected 27 differentially expressed clusters (FDR-corrected P< 0.05). Both algorithms identified “immune system process” as the highest ranked cluster. Other clusters involved cytokine/chemokine activity, defense mechanisms, activation of cellular response, and apoptotic response. ISMARA motif analysis identified motifs of known transcription factor families involved in immune system regulation.

**Conclusions:** Microarray analysis revealed an altered gene expression signature in peripheral blood monocytes from neovascular AMD patients. The data supports the activation of the systemic immune response in monocytes from AMD patients.

**Commercial Relationships:** Michelle Grunin, None; Shira Levi, None; Tal Burstyn-Cohen, Novartis (C); Teva (C)

**Support:** Israeli Ministry of Health, Israel Science Foundation

**Program Number:** 153 Poster Board Number: C0158
**Presentation Time:** 8:30 AM - 10:15 AM

**Recombinant VEGF165b inhibits TNF-α-induced ICAM-1 expression and monocyte adhesion in primary human retinal pigment epithelial cells**

**Peeradech Thichanpiang**1, 2, David O. Bates3, Kanokpan Wongprasert1. 1Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand; 2Microvascular Research Laboratories, School of Physiology and Pharmacology, University of Bristol, Bristol, United Kingdom.

**Purpose:** Local inflammation at the retinal pigment epithelial (RPE) cell layer is associated with inflammatory cell migration and secretion of pro-inflammatory cytokines such as Tumor necrosis factor (TNF)-α. TNF-α up-regulates Intercellular Cell Adhesion Molecule (ICAM)-1 expression on RPE that allows binding of lymphocyte function-associated antigen-1 (LFA-1) on leukocytes contributing to leukocyte adhesion at sites of inflammation. VEGF165b is generated by alternative splicing of VEGF-A in the terminal exon 8. VEGF165b is cytoprotective and anti-angiogenic but its effects on inflammation have not been elucidated. Therefore, our aim was to investigate the effects of VEGF165b on TNF-α-induced ICAM-1 expression and monocyte adhesion in RPE cells.

**Methods:** Primary RPE cells were pretreated with 20 ng/ml TNF-α alone, 100 ng/ml VEGF165b alone, 100 ng/ml VEGF165b with 100 ng/ml anti-VEGF165b, 100 ng/ml VEGF165b or 10 nM ZM323881 (VEGFR2 inhibitor) prior to exposure to 20 ng/ml TNF-α for 24 h. After the experiment, cells were lysed for Western blotting and monocyte adhesion assays were performed.

**Results:** VEGF165b and ZM323881 inhibited TNF-α-induced upregulation of ICAM-1 in RPE cells. VEGF165b was neutralized by antibody to VEGF165b (Figure 1). VEGF165b ameliorated the TNF-α-induced monocyte-RPE adhesion (Figure 2).

**Conclusions:** These findings indicate that VEGF165b inhibits the TNF-α-mediated upregulation of ICAM-1 expression and increases monocyte-RPE cell adhesion, suggesting an anti-inflammatory property of VEGF165b in the eye.

![Figure 1. Western blotting showing effects of VEGF165b and ZM323881 on TNF-α-induced upregulation of ICAM-1 in RPE cells.](Image)

![Figure 2. Decreased adhesion of monocytes to RPE cells after inhibition of ICAM-1 expression by VEGF165b.](Image)

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importantly prior to any further exaggerated immune activation as evident by massive deposition of membrane attack complex. Following acute events in the laser model, macrophage infiltration and complement activation both recede 7-14 days post-laser when the angiogenesis has established. The novelty of the early events, occurring prior to endothelial proliferation, angiogenesis formation or complement deposition include: transient Arg-1+VEGF+ macrophage infiltration and such early recruited macrophages displayed high phagocytic and endocytic activity, engulfing fragments of damaged RPE at laser sites. In vitro data demonstrates that the Arg-1+VEGF+ macrophages are induced specifically by the uptake of damaged RPE.

**Conclusions:** The results demonstrate that early myeloid cell recruitment, by either microglia or monocytes, initiates the local proangiogenic response before complement activation or endothelial proliferation, which leads to the development of CNV. Furthermore, macrophages acquire an Arg-1+VEGF+ phenotype that drives early angiogenic events, which results from phagocytosis of damaged RPE. The data supports current therapeutic attempts aimed at preventing apoptotic or necrotic RPE cell death, as this may suppress the early trigger that drives the proangiogenic macrophage phenotype.

**Commercial Relationships:** 
Shintaro Horie, None; David A. Copland, None; Jian Liu, None; Wei-Kang Wu, None; Mi Chen, None; Yunhe Xu, None; Heping Xu, None; Lindsay B. Nicholson, None; Andrew D. Dick, Novartis (C), Novartis (F), GSK (F), Abbott (F)

**Support:** Dunhill Medical Trust (UK) and National Eye Research Centre (UK)

**Program Number:** 155 Poster Board Number: C0160
**Presentation Time:** 8:30 AM - 10:15 AM

**CD200 Receptor signaling subverts pro-angiogenic macrophage phenotype generation and experimental choroidretinal neovascularisation**

Shintaro Horie1,2, David A. Copland3, Jian Liu4, Wei-Kang Wu4, Lindsay B. Nicholson5, Manabu Mochizuki6, Andrew D. Dick7.

1Department of Academic Unit of Ophthalmology, School of Clinical Sciences, University of Bristol, Bristol, United Kingdom;
2Department of Ophthalmology and Visual Science, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan.

**Purpose:** To investigate how CD200R signaling regulates PGE2-mediated modulation of macrophage phenotype and function in the suppression of angiogenesis.

**Methods:** Bone marrow derived macrophages (BMMφ) were generated from wild-type C57BL/6 (WT) or CD200R1/-/- mice. For in vitro assays, BMMφ were stimulated with purified Prostaglandin E2 (PGE2) or supernatant from conditioned murine RPE cell line, and cell phenotype and pro-angiogenic function assessed by quantitative RT-PCR, ELISA and immuno-cytochemistry. Assessment of angiogenic potential was performed in vitro, using the endothelial tube formation assay from human umbilical vein endothelial cells (HUVECs) in co-culture with conditioned BMMφ, and in vivo using a laser-induced choroidal neovascularization (CNV) model. Operative PGE2-signaling pathways (including MAPK) were analyzed by flow cytometry.

**Results:** BMMφ from CD200R1/-/- mice expressed increased VEGf-a mRNA and secreted higher amounts of VEGF protein following PGE2-stimulation when compared to WT cells. Similar levels of expression were observed when BMMφ were cultured with conditioned RPE cell supernatant. The formation of endothelial tubes by HUVECs was promoted when co-cultured with PGE2 or PGE2-conditioned CD200R1/-/- BMMφ. In vivo assessment demonstrated increased CNV area in CD200R1/-/- as compared to WT mice. Furthermore, PGE2-stimulation lead to up-regulation of phosphorlated MAPK in CD200R1/-/- BMMφ.

**Conclusions:** In the absence of inhibitory CD200R signaling, VEGF expression from macrophages is elevated, which results in an increased pro-angiogenic potential in HUVECs co-culture, and increased area of laser-induced CNV. Furthermore, this data shows that CD200R suppression of PGE2-mediated myeloid-induced VEGF expression, in part is due to inhibition of MAPK pathway, and demonstrates the anti-angiogenic function of myeloid CD200R signaling.

**Commercial Relationships:** Shintaro Horie, None; David A. Copland, None; Jian Liu, None; Wei-Kang Wu, None; Lindsay B. Nicholson, None; Manabu Mochizuki, Santen (F), Senju (F), Ohtsuka (F), Daiichi-Sankyo (F), AMO Japan (F), Alcon Japan (F); Andrew D. Dick, Novartis (C), Novartis (F), GSK (F), Abbott (F)

**Support:** National Eye Research Center, UK

**Program Number:** 156 Poster Board Number: C0161
**Presentation Time:** 8:30 AM - 10:15 AM

**The role of Fcy receptors in immune-complex-mediated inflammation in the retina: implications for early age related macular degeneration**

Salome Mariniello1, Andrew J. Lotery2, V. Hugh Perry1, Robert F. Mullins3, Jessica L. Teeling1. 1Center for Biological Sciences, University of Southampton, Southampton, United Kingdom; 2Clinical and Experimental Sciences, University of Southampton, Southampton, United Kingdom; 3Department of Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA.

**Purpose:** The association of immunoglobulin G (IgG), complement and activated macrophages/microglia with drusen implicates immune complex (IC) inflammation in age-related macular degeneration (AMD), but IC responses in the eye have been poorly characterised. This study assessed the role of complement and IgG Fcy Receptors (FcyRs) using a localized IC reaction (Arthus) in the retina of wild-type, C1q and Fcy chain deficient mice and evaluated the inflammatory response. We then investigated the IC mediated inflammation in AMD by looking for the presence of IgG, complement activation and Fcy receptor expression in donor eye tissue from AMD patients.

**Methods:** Wild-type, C1q/-/- and Fcy chain/-/- BALB/c mice were immunized against ovalbumin (OVA), followed by intravitreal challenge of OVA or saline. Eye tissue was collected three days following OVA challenge and analysed for the presence of IC and microglial activation by immunohistochemical staining of the retina. Human donor eyes were used for immunohistochemical detection of IgG, membrane attack complex (MAC, C5b-9), FcyRIIb and FcyRIIa, and the leukocyte marker CD45.

**Results:** IC formed throughout the retina of all mice, following intravitreal injection of OVA, including large deposits in the subretinal space. In wild-type and C1q/-/- these deposits were associated with inflammation and increased expression of MHC II and all four murine FcyRs. These changes were not observed in Fcy chain/-/- or OVA immunized wild type mice challenged with saline. IgG and MAC deposition was increased in the choroid of AMD donor eyes, when compared to aged matched controls. This was accompanied by increased numbers of CD45+ leukocytes and upregulation of FcyRIIb and FcR on these cells.

**Conclusions:** Our results suggest that IC formed in the retina can induce a potent inflammatory response with up-regulation of FcyRs and MHCII on microglia and recruitment of macrophages. We show FcyRs, but not C1q, are crucial for the generation of this
inflammatory response. We confirmed the presence of IgG and MAC in the eyes of AMD patients, as well as increased numbers of leukocytes and upregulation of the FcγRIIb and FcγRIa. Our results suggest that immune-complexes in the eye could mediate local inflammation and contribute to AMD through the interaction of IgG with FcγRs. These findings may have implications for antibody therapies for AMD.

Commercial Relationships: Salome Murinello, None; Andrew J. Lotery, Novartis (F), Bayer (R); V. Hugh Perry, None; Robert F. Mullins, Alcon Research Ltd (F); Jessica L. Teeling, None

Support: Fight for Sight UK

Program Number: 157 Poster Board Number: C0162
Presentation Time: 8:30 AM - 10:15 AM

The Correlation of Plasma Cytokines with Complement Factor H Polymorphism Y402H, Choroidal Thickness and Drusen Load in Dry Age-Related Macular Degeneration

Sijja Cao, Ashley Ko, Aikun Wang, Marita Partanen, Andrew Merkur, David A. Albiani, Andrew W. Kirker, Jing Z. Cui, Farzin Forooghian, Joanne A. Matsubara

Purpose: Several key components of the complement cascade are associated with the pathogenesis of age-related macular degeneration (AMD), including the Y402H variation in the gene encoding complement factor H (CFH), which confers a several-fold increased risk for developing AMD. Earlier studies, including ours, demonstrated the presence of complement proteins, including C5b-9, in the outer retina of postmortem eyes with drusen, the hallmark deposit of early stage AMD. It is hypothesized that early treatment to inhibit the complement cascade may prevent the progression to late stage AMD. This study investigates the effect of ATA, on C5b-9 accumulation and apoptosis in the rodent retina.

Methods: Twelve C57B/6 mice, aged 3.5 months, were divided into a control group (Group 1) and two experimental groups receiving a subcutaneous injection of 200mg/kg (Group 2) or 500mg/kg (Group 3) of ATA dissolved in PBS. After 24 hrs, animals were sacrificed, eyes enucleated and fixed in 4% paraformaldehyde. Tissue was embedded in paraffin and 4 µm thick sections subjected to TUNEL assay following manufacturer’s protocol (Millipore) or immunohistochemistry using a 1:500 dilution of rabbit polyclonal antibody against C5b-9 (Bloss). Under 40X, TUNEL+ cells were counted, C5b-9 immunoreactivity was scored semiquantitatively and retinal thicknesses measured.

Results: ATA treatment reduced retinal C5b-9 immunoreactivity from 3.0 ± 0.00 (no treatment), to 1.4 ± 0.83 (200mg/kg) and 1.1 ± 0.68 (500mg/kg), reaching significance at p<0.05 between Group 1 vs 2 and 3. ATA treatment reduced numbers of TUNEL+ cells in the ONL from 93.0±30.47 (no treatment) to 90.7 ± 65.69 (200mg/kg, p=0.05) and to 10.5 ± 3.69 (500mg/kg, p<0.05) per 8379 µm² area of ONL, indicating significant anti-apoptotic effects at the higher dosage of 500 mg/kg ATA. Significant differences in retinal thickness measurements were not observed.

Conclusions: Systematic administration of ATA (MW=422) has been shown to block the addition of C9 to C5b-8 in several tissues (Lee et al 2012). This study demonstrates significant lowering of C5b-9 immunoreactivity and apoptosis in rodent retina. Future studies are needed to assess whether inhibitors of C5b-9 formation, such as ATA, may be useful in combination treatment for multifactorial diseases such as AMD.

Commercial Relationships: Jenifer Van, None; Jing Z. Cui, None; Eleanor To, None; Patrick McGeer, Patent Application (P); Joanne A. Matsubara, None

Support: Canadian Institutes of Health Research Grant (CIHR: MOP 97806)

Program Number: 158 Poster Board Number: C0163
Presentation Time: 8:30 AM - 10:15 AM

Systemic administration of aurin tricarboxylic acid (ATA) reduces C5b-9 immunoreactivity and TUNEL+ cells in the rodent eye: A potential treatment for suppressing complement activation and apoptosis associated with AMD

Jenifer Van, Jing Z. Cui, Eleanor To, Patrick McGeer, Joanne A. Matsubara

Purpose: Several key components of the complement cascade are associated with the pathogenesis of age-related macular degeneration (AMD), including the Y402H variation in the gene encoding complement factor H (CFH), which confers a several-fold increased risk for developing AMD. Earlier studies, including ours, demonstrated the presence of complement proteins, including C5b-9, in the outer retina of postmortem eyes with drusen, the hallmark deposit of early stage AMD. It is hypothesized that early treatment to inhibit the complement cascade may prevent the progression to late stage AMD. This study investigates the effect of ATA, on C5b-9 accumulation and apoptosis in the rodent retina.

Methods: Twelve C57B/6 mice, aged 3.5 months, were divided into a control group (Group 1) and two experimental groups receiving a subcutaneous injection of 200mg/kg (Group 2) or 500mg/kg (Group 3) of ATA dissolved in PBS. After 24 hrs, animals were sacrificed, eyes enucleated and fixed in 4% paraformaldehyde. Tissue was embedded in paraffin and 4 µm thick sections subjected to TUNEL assay following manufacturer’s protocol (Millipore) or immunohistochemistry using a 1:500 dilution of rabbit polyclonal antibody against C5b-9 (Bloss). Under 40X, TUNEL+ cells were counted, C5b-9 immunoreactivity was scored semiquantitatively and retinal thicknesses measured.

Results: ATA treatment reduced retinal C5b-9 immunoreactivity from 3.0 ± 0.00 (no treatment), to 1.4 ± 0.83 (200mg/kg) and 1.1 ± 0.68 (500mg/kg), reaching significance at p<0.05 between Group 1 vs 2 and 3. ATA treatment reduced numbers of TUNEL+ cells in the ONL from 93.0±30.47 (no treatment) to 90.7 ± 65.69 (200mg/kg, p=0.05) and to 10.5 ± 3.69 (500mg/kg, p<0.05) per 8379 µm² area of ONL, indicating significant anti-apoptotic effects at the higher dosage of 500 mg/kg ATA. Significant differences in retinal thickness measurements were not observed.

Conclusions: Systematic administration of ATA (MW=422) has been shown to block the addition of C9 to C5b-8 in several tissues (Lee et al 2012). This study demonstrates significant lowering of C5b-9 immunoreactivity and apoptosis in rodent retina. Future studies are needed to assess whether inhibitors of C5b-9 formation, such as ATA, may be useful in combination treatment for multifactorial diseases such as AMD.
Curcumin, Luteolin and DHA Supplementation Abates Microglia Activation and Retinal Degeneration in the CLN6null Neuronal Ceroid Lipofuscinosis Mouse Model

Myriam Mirza1,2, Cornelia Volz1, Herbert Jägle1, Thomas Langmann1,3, 1Institute of Human Genetics, University of Regensburg, Regensburg, Germany; 2Department of Ophthalmology, Experimental Immunology of the Eye, Cologne, Germany; 3Department of Ophthalmology, University Eye Clinic Regensburg, Regensburg, Germany.

Purpose: Neuronal ceroid lipofuscinoses (NCL) are early onset lysosomal storage disorders characterized by vision loss, mental and motor deficits, and spontaneous seizures. Notably, massive accumulations of autofluorescent material in neurons lead to progressive neuronal degeneration and cell loss. Our previous studies on CLN6null mice revealed that progressive retinal degeneration starts before one month of age and is accompanied by microglia activation. The aim of our study was to examine the effect curcumin, luteolin and DHA supplementation would have on microglia activation and retinal/brain degeneration in these mice.

Methods: CLN6null mice were supplemented with 0.6% curcumin, luteolin and 5% DHA for 30 weeks starting at 4 weeks of age. Visual acuity and retinal function was determined by measuring the optokinetic response in an Optomotry system and by use of electroretinography, respectively. Microglial morphology and migration was analyzed in retinal, optic nerve and brain tissues by immunohistochemistry. Retinal and brain neurodegenerative gene expression markers were compared to see if there was an overall beneficial effect.

Results: Our data shows that supplemented CLN6null mice have a significant improvement in optokinetic and ERG response compared to control CLN6null mice which were given a standard diet. Histological analyses of the retina reveal a mixed population of active and resting microglia in the supplemented mice compared to mostly active microglia found in the control mice. The same can be seen in the brain tissues. Interestingly, there is a greater preservation of photoreceptor in DHA supplemented mice compared to the other groups. A comparison of gene expression markers in the retina and cortex/cerebellum revealed that the tissues were affected differently by the supplementation.

Conclusions: Our results show that curcumin, luteolin and DHA have beneficial effects on supplemented mouse retina and brain. The mixed populations of microglia seen in the retina suggest that these supplements can to some extent attenuate microglia activation coinciding with a more functional retina. Furthermore, these anti-inflammatory substances could have further beneficial effects when paired with different therapies.

Commercial Relationships: Myriam Mirza. None; Cornelia Volz. None; Herbert Jägle. None; Thomas Langmann. None

Support: NCL - Gruppe Deutschland e. V.

Program Number: 160 Poster Board Number: C0165
Presentation Time: 8:30 AM - 10:15 AM

Increased Fundus Autofluorescence and Retinal Cell Infiltration in Middle-Aged Mice Fed with High Fat, Cholesterol-Rich Diet

Zhen Yang Zhao1, Yiqin Zuo1, Bo Yu1, Massoud Motamedi2, 1, Adam Boretsky1, Yan Chen1, Jiayang Cai1, 1Ophthalmology, University of Texas Medical Branch, Galveston, TX; 2Center for Biomedical Engineering, University of Texas Medical Branch, Galveston, TX.

Purpose: Oxidative stress and high plasma cholesterol level have been postulated as contributing to the pathogenesis of various retinal diseases such as age-related macular degeneration and diabetic retinopathy. The purpose of this study is to characterize the retinal phenotype of mice on high fat, cholesterol-rich (HF-C) diet and study the underlying mechanisms that accelerate retinal aging and inflammation.

Methods: B6.129 mice at 12 months of age were fed with either standard rodent chow or HF-C diet, with 5% and 20% (w/w) fat, respectively. The HF-C diet also contained 0.15% cholesterol. Fundus phenotype of both groups was characterized using optical coherence tomography (OCT) and scanning laser ophthalmoscope (SLO) both before and after the HF-C diet for 2 months. Immunohistochemistry and immunofluorescence staining of paraffin-induced 43% cell loss in cultured RPE cells after 24 hour incubation. Since RPE cell death is the direct cause of geographic atrophy, it is crucial to find agents that can rescue RPE cells when threatened by endogenous pathological stimuli, for instance, oAβ40. The purpose of this study is to elucidate the role of X-linked inhibitor of apoptosis (XIAP), previously used to rescue photoreceptor cells, as a RPE cell survival factor when challenged by oAβ40 in vitro.

Methods: The full length, open-reading frame of human XIAP was cloned into a pcDNA3.1 vector. ARPE-19 cells transfected with either the XIAP or the control construct were subsequently stressed with oligomer solutions. The transfection efficiency was assured by qRT-PCR and western blot. The ARPE-19 cell viability at several oligomer concentrations and timepoints was measured by MTT assays. Paraffin sections from AMD and normal post-mortem donor eyes were probed with an XIAP antibody to evaluate changes of XIAP expression in AMD progression.

Results: Preliminary findings suggest XIAP immunoreactivity was observed in RPE of AMD eyes and in young normal eyes without drusen deposits. The old normal eyes demonstrated less XIAP immunoreactivity than the other groups. In vitro, qRT-PCR showed a 371 fold increase of XIAP mRNA in XIAP-transfected cells, compared to cells transfected with the control plasmid and cells without transfection (p<0.001). Protein levels of XIAP were confirmed by western blot. Next, we challenged the three groups of cells with 0.03μM oAβ40, and demonstrated that the XIAP transfected ARPE-19 cells exhibited higher cell viability than control plasmid transfected cells at both 6 hours and 24 hours.

Conclusions: The current study reveals an intriguing facet of XIAP’s capacity against oAβ40’s cellular stressor effects on RPE cells. The increased level of XIAP renders the XIAP-transfected cells more resistant to oAβ40. Given oAβ40’s presence in drusen, it is possible that an anti-apoptotic mechanism, such as known for XIAP, may protect RPE from cellular insults in vivo. The merit of using XIAP as a therapeutic agent for AMD warrants further investigation.

Commercial Relationships: Jiangyuan Gao. None; Aikun Wang. None; Jing Z. Cui. None; Catherine Tsifidis. US patent 60/648,304 (not yet issued) (P); Joanne A. Matsubara. None

Support: Canadian Institutes of Health Research Grant (CIHR: MOP 979806)

Program Number: 161 Poster Board Number: C0166
Presentation Time: 8:30 AM - 10:15 AM

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or cryosections were performed to further validate findings from the in vivo imaging.

**Results:** At beginning of the study all animals showed healthy fundus photographs and marginal autofluorescence. Two months after diet change, mice fed with HF-C food showed dome-shaped dotted retinal lesions that were absent in mice on normal diet. SLO revealed significant increase of fundus autofluorescence in HF-C group. Most of the fluorescent spots were in the focal plane of the neuroretina, usually adjacent to retinal vessels. This was also confirmed by OCT scanning. Histology sections of HF-C mice showed increased isolecitin- and CD68-positive cells, which were mainly present in the inner plexiform layer as well as the subretinal space.

**Conclusions:** Feeding high HF-C diet to 12-month old mice could accelerate the retinal aging process, which was presented as elevated fundus autofluorescence. The infiltration of CD68 positive cells indicated microglia and/or macrophages activation that can lead to a pro-inflammatory milieu in the retina and contribute to age-related retinal pathology.

**Commercial Relationships:** Zhen-Yang Zhao, None; Yiqin Zuo, None; Pei Xu, None; Bo Yu, None; Massoud Motamedi, None; Adam Boretsky, None; Yan Chen, None; Jiyan Cai, None

**Support:** NIH grants EY 021937, EY 019706, American Health Association Foundation and Research to Prevent Blindness, Inc.

**Program Number:** 162 Poster Board Number: C0167
**Presentation Time:** 8:30 AM - 10:15 AM
**Change in the distribution and phenotype of subretinal macrophages with aging in C57BL/6 mice**

Bogale Aredo, xiao chen, Kaiyan Zhang, Rafael Ufret-Vincenty, Ophthalmology, UT Southwestern Medical Center, Dallas, TX.

**Purpose:** There is significant debate surrounding the role of macrophages-microglia in age-related macular degeneration (AMD) and also in retinal dystrophies. In order to better understand the behavior of subretinal microglia, we explored the natural history of their distribution and phenotype in wild type B6 mice vs. RD8 mutant mice.

**Methods:** Naïve C57BL/6 mice wild type (WT), heterozygous or homozygous for the RD8 mutation were used. Mice were divided into two age groups: young/adult (1-9 months) and old (14-21 months). Retinal images were taken using a Micron III rodent fundus camera (Phoenix Research Labs). Yellow spots in the central fundus (5 disc radius from the center of optic disc) were counted. Eyes were enucleated and fixed in 4% paraformaldehyde. Posterior segment flat mounts (sclera-choroid-RPE complex) were single or triple stained for ionized calcium binding adaptor molecule 1 (Iba-1), mouse Macrophage Mannose Receptor (MMR) or mouse FcyII/II/Receptor (CD16/CD32). Stained microglia on flat mounts were counted for each of the markers, for both the central region (within a 5 disc diameter radius from the optic nerve), and for the total RPE. RPE cells were isolated from either the central or peripheral retina, using a new technique we developed, and the expression of inflammatory and chemotactic genes was analyzed by RT-PCR.

**Results:** The number of yellow spots in the central fundus of RD8 mutant mouse was higher than in WT and highly correlated with Iba-1+ cells. The differences increased with aging; in the young/adult mice, mutants had more central Iba-1+ cells than WT (p=0.002) and this difference was more dramatic in the old group (p=0.001). MMR+ and CD16/32+ staining cells were significantly increased in the RD8 mutant mouse vs. WT and the ratio of CD16+/MMR+CD16- increased with age in RD8 mutants. The total and peripheral microglia counts in the whole RPE FM also increased in the RD8 mutant mouse vs. WT. We will also present gene expression levels of microglia activation markers and chemotacticants in central vs. peripheral RPE.

**Conclusions:** Both aging and the RD8 mutation affect the distribution and phenotype of subretinal microglia.

**Commercial Relationships:** Bogale Aredo, None; xiao chen, None; Kaiyan Zhang, None; Rafael Ufret-Vincenty, None

**Support:** Research to Prevent Blindness (RPB) Unrestricted Grant, Disease Oriented Clinical Scholars Program, Visual Science Core Grant EY020799

**Program Number:** 163 Poster Board Number: C0168
**Presentation Time:** 8:30 AM - 10:15 AM
**Effect of Posterior Segment Components on BV-2 Microglial cell M1/M2 polarization**

Bongsu Kim1, Rania Kusibati1, Elaine M. Binkley1, Jonathan P. Godbout2, Andrew J. Fischer2, Colleen M. Cebulla1, 1Department of Ophthalmology and Visual Science, The Ohio State University, Columbus, OH; 2Department of Neuroscience, The Ohio State University, Columbus, OH.

**Purpose:** Retinal detachment (RD) activates microglia/macrophages which acquire amoeboid morphology and accumulate in the subretinal space. There is up-regulation of select M1 and M2 macrophage genes in murine hyaluronic acid model of retinal detachment (HA RD). The purpose of this study was to test the hypothesis that hyaluronic acid (HA), Vitreous (VIT), or photoreceptor outer segments (PROS) induce preferential macrophage polarization and morphology changes.

**Methods:** Murine microglial BV-2 cells were treated with HA, VIT, or PROS using high and low doses in serum free conditions. As controls, BV-2 cells were differentiated into an M1 or M2 phenotype with 6 hour LPS (10ng/mL) or IL-4 (20ng/mL) incubation, respectively. Total RNA was extracted at 12 hours and real time PCR was performed for M1 (Ccl2, Nos2, Cxcl10, Tnfa, Ifib) and M2 genes (Mrc1, Arg1). Cell morphology was determined from phase contrast photographs of cultured cells at 6 and 12 hours.

**Results:** At 12 hours, VIT induced Arg1 M2 gene expression, 2.3-fold in low-dose and 29.9 fold in high-dose conditions. High-dose PROS also increased Arg1 expression by 4-fold. HA did not induce Arg1 gene expression. Ifib was induced by PROS exposure (4.7-9.1-fold), with lower levels induced by VIT and HA. Ccl2, Nos2, Cxcl10, Tnfa, and Ifib and M2 genes (Mrc1, Arg1). Cell morphology was determined from phase contrast photographs of cultured cells at 6 and 12 hours.

**Conclusions:** Select M1/M2 gene expression and morphology changes were induced in BV-2 cells by critical components of RD, including vitreous and photoreceptor outer segments.

**Commercial Relationships:** Bongsu Kim, None; Rania Kusibati, None; Elaine M. Binkley, None; Jonathan P. Godbout, None; Andrew J. Fischer, None; Colleen M. Cebulla, None

**Support:** Award number KL2RR025754 from the National Center for Research Resources, OSU Department of Ophthalmology start-up funds and the Patti Blow Fund. We thank Debra Thompson for gift of PROS.

**Program Number:** 164 Poster Board Number: C0169
**Presentation Time:** 8:30 AM - 10:15 AM
**IgG Autoimmunity Analysis In Aqueous Humor Of Patients With Pseudoexfoliation Syndrome, Pseudoexfoliation Glaucoma and Cataract Controls**

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Phenotypical characterization of T cells in allergic conjunctivitis

Phenotypical characterization of T cells in allergic conjunctivitis

Presentation Time: 8:30 AM - 10:15 AM

An enhanced inflammatory response in the aged choroid is associated with the upregulation of CCL2 and neutrophil markers following the induction of choroidal neovascularisation Scott J. Robbie1, 2, Ulrich F. Lahmann1, Anastasios Georgiadis1, Susie E. Barker1, Yanai Duran1, Alexander J. Smith1, Robin R. Ali1, James W. Bainbridge1, 2, 3Genetics, UCL Institute of Ophthalmology, London, United Kingdom; 2NIHR BRC, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom.

Purpose: CNV severity increases with age but the kinetics and mechanism for this are not fully understood. We hypothesised that age-related chronic inflammatory changes in the chorioretinal complex of the eye drive the recruitment of myeloid cells that modulate CNV severity.

Methods: We determined the kinetics of CNV growth in young (<4 months) and old (>18 months) wild-type mice using serial fundus fluorescein angiography. Based on the results we induced choroidal neovascularisation (CNV) at 10 locations in the fundus of young and old wild-type animals and harvested retinal pigment epithelium/choroid for analysis by quantitative reverse-transcriptase polymerase chain reaction of VEGF, CD11b, CCL2, CCR2, CXCR1, CXCR2, CX3CR1, TNFa, IFNg, II-4, II-6, II-8 and II-10.

Results: We found the myeloid cell chemokine CCL2 to be undetectable in young choroid but highly upregulated in tissue derived from aged mice. We also found the myeloid cell marker CD11b and the chemokine receptors CXCR1, CXCR2 and CX3CR1 to be upregulated in the aged unlesioned choroid, indicating that myeloid cell numbers in this tissue increase with age. Analysis of choroid at 3 days post-induction of CNV revealed 40-fold higher levels of expression of CCL2 in aged compared with young animals.

Program Number: C0171
Presentation Time: 8:30 AM - 10:15 AM

An enhanced inflammatory response in the aged choroid is associated with the upregulation of CCL2 and neutrophil markers following the induction of choroidal neovascularisation Scott J. Robbie, 1, 2 Ulrich F. Lahmann, 1 Anastasios Georgiadis, 1 Susie E. Barker, 1 Yanai Duran, 1 Alexander J. Smith, 1 Robin R. Ali, 1 James W. Bainbridge, 1, 2, 3 Genetics, UCL Institute of Ophthalmology, London, United Kingdom; 2NIHR BRC, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom.

Purpose: CNV severity increases with age but the kinetics and mechanism for this are not fully understood. We hypothesised that age-related chronic inflammatory changes in the chorioretinal complex of the eye drive the recruitment of myeloid cells that modulate CNV severity.

Methods: We determined the kinetics of CNV growth in young (<4 months) and old (>18 months) wild-type mice using serial fundus fluorescein angiography. Based on the results we induced choroidal neovascularisation (CNV) at 10 locations in the fundus of young and old wild-type animals and harvested retinal pigment epithelium/choroid for analysis by quantitative reverse-transcriptase polymerase chain reaction of VEGF, CD11b, CCL2, CCR2, CXCR1, CXCR2, CX3CR1, TNFa, IFNg, II-4, II-6, II-8 and II-10.

Results: We found the myeloid cell chemokine CCL2 to be undetectable in young choroid but highly upregulated in tissue derived from aged mice. We also found the myeloid cell marker CD11b and the chemokine receptors CXCR1, CXCR2 and CX3CR1 to be upregulated in the aged unlesioned choroid, indicating that myeloid cell numbers in this tissue increase with age. Analysis of choroid at 3 days post-induction of CNV revealed 40-fold higher levels of expression of CCL2 in aged compared with young animals.
This was associated with a further increase in CXCR2 together with elevated CCR2 in lasered, aged tissue. CXCR2 is highly expressed on neutrophils while CCR2 is expressed on both ‘inflammatory’ monocytes and neutrophils entering an inflamed microenvironment. These results therefore suggest an enhanced recruitment of both CCR2+ myeloid cells populations to sites of CNV in aged animals. We also observed higher levels of expression of the pro-inflammatory cytokine TNFa and the pro-angiogenic cytokines II-6 and II-10 in aged choroid compared with young at 3 days post-induction of CNV.

Conclusions: Levels of CCL2 in the aged choroid are elevated and the tissue is therefore primed for myeloid cell recruitment. The induction of CNV in aged eyes results in further upregulation of CCL2 and its cognate receptor CCR2 at 3 days post laser. The strong upregulation of chemokine receptors (CXCR2 and CCR1) most highly expressed on neutrophils suggests that these cells may contribute to the increased inflammatory response, and therefore CNV severity, in aged animals.

Commercial Relationships: Scott J. Robbie. None; Ulrich F. Luhmann. None; Anastasios Georgiadis. None; Susie E. Barker. None; Yanai Duran. None; Alexander J. Smith. None; Robin R. Ali. None; James W. Bainbridge. Novartis (F), Alimera (C), Gene Signal (C), Advanced Cell Technology (F), Targeted Genetics (P), Oxford Biomedica (C), GSK (F)

Support: National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology

Program Number: 167 Poster Board Number: C0172
Presentation Time: 8:30 AM - 10:15 AM

Differential effects of a MyD88 knockout on ocular inflammation induced by the TLR2/3/4 ligands (PAM3CSK4, PolyI:C, and LPS)


Purpose: To determine the effect of MyD88 deficiency on ocular inflammation in a murine model of TLR ligand induced inflammation.

Methods: Inflammation was induced in seven-week-old MyD88 knockout and wild type littermates by intraperitoneal injection of 50 µg LPS in 0.1 ml PBS, 25 µg of PAM3CSK4 in 0.1 ml water, or 100 µg polyI:C in 0.1 ml PBS. Mice were euthanized 3, 8, or 24 hours after injection. Plasma and right eyes were collected and protein extracts prepared for cytokine and chemokine analysis using a multiplex assay (Aushon Biosystems). The contralateral eyes were fixed in 4% paraformaldehyde and the retinas and posterior eye cups were dissected and prepared for whole flatmount immunostaining of neutrophils (Gr-1), macrophages (F4/80), and microglia (Iba1). Fluorescent images of five regions of each retina and two regions of each posterior eye cup were captured using an Axiocam MR3 camera on an Axio. ImageM1 microscope. The numbers of neutrophils, macrophages, and microglia were counted with Axiovision software.

Results: In comparison with wild-type littermates, MyD88 knockout mice had a reduced number of neutrophils and macrophages in the retina after intraperitoneal injection of the TLR2 & TLR4 ligands (PAM3CSK4 and LPS, respectively). Injection of the TLR3 ligand - poly I:C - had no effect on neutrophils and macrophages in the retina. Microglia cells in the posterior eye cup in response to the TLR2, TLR4, and TLR3 ligands in MyD88 knock mice were reduced, in comparison with wild-type littermates, by 100%, 50%, and 0%, respectively. Cytokines in the plasma and eye induced by the TLR2 ligand (PAM3CSK4) was severely reduced, partially inhibited with TLR4 ligand (LPS), and mostly unaffected with TLR3 ligand (poly I:C) in MyD88 deficient mice compared to wild-type littermates.

Conclusions: MyD88 deficiency impaired TLR2 & TLR4 responses, but not TLR3 responses, as measured by retinal inflammation and cytokine production. These in vivo data support the hypothesis that TLR2 signaling is MyD88 dependent, TLR3 signaling is MyD88 independent, and TLR4 signaling is partially MyD88 dependent.

Commercial Relationships: Maura Crowley, Novartis (E); Omar Delgado, Novartis (E); Steve Louie, Novartis (E); Michael Stefanidakis, Novartis (E); Bruce D. Jaffee, Novartis (E); Sha-Mei Liao, Novartis (E)

Program Number: 168 Poster Board Number: C0173
Presentation Time: 8:30 AM - 10:15 AM

Critical role of TLR2 and MyD88 signaling in controlling bacterial burden in mouse model of S. aureus endophthalmitis

Ashok Kumar, Pawan Kumar Singh, Deepa Talreja. Ophthalmal & Anatomy/Cell Biology, Wayne State Univ/Kresge Eye Inst, Detroit, MI.

Purpose: Recent studies have implicated an important role of Toll like receptor 2 (TLR 2) in providing retinal innate defense in bacterial endophthalmitis. TLR2 signals via MyD88-dependent pathway to initiate inflammatory cascades, which are critical for recruitment of immune cells, which in turn limit bacterial growth. In this study we investigated the role of TLR2 and MyD88 in pathogenic clearance using knockout mice.

Methods: Endophthalmitis was induced in wild-type (WT), TLR2-/- and MyD88-/- mice by intravitreal injections of S. aureus. Eyes were examined for corneal opacity and vitreous haze 1, 2, and 3 days post infection (dpi). Bacterial burden was determined by colony forming units (CFUs) of eye lysates. Flowcytometry was used for PMN infiltration and cytometric bead array (CBA) detection of inflammatory mediators. Eyes were also fixed and embedded in paraffin for histological analysis.

Results: Both TLR2-/- and MyD88-/- exhibited increased bacterial burden with MyD88-/- being the highest as compared to WT mice. Similar trend was observed for clinical scores, with MyD88-/- mice showing increased and TLR2-/- showing intermediate clinical scores. At 3 dpi, eyes were completely damaged in MyD88-/- mice as supported by histological data. At early stages the PMN infiltration and levels of inflammatory cytokines was less in TLR2-/- and MyD88-/- mice. However, their levels dramatically increased at later stages (3 dpi) of infection.

Conclusions: Our data suggests that TLR2 and MyD88 deficiencies results in increased bacterial proliferation in the eye leading to severe intraocular inflammation and retinal damage during endophthalmitis.

Commercial Relationships: Ashok Kumar, None; Pawan Kumar Singh, None; Deepa Talreja, None

Support: EY19888, Research to Prevent Blindness

Program Number: 169 Poster Board Number: C0174
Presentation Time: 8:30 AM - 10:15 AM

Significance of measuring immune mediators for differentiating malignant from benign pigmented intraocular tumors

Yoshihiko Usui, Shunichiro Ueda, Yoko Okunuki, Kinya Tsubota, Kazuki Tajima, Takeshi Kezuka, Yoshihiro Wakabayashi, Hiroshi Goto. Ophthalmology, Tokyo Medical Univ Hospital, Shinjuku-ku, Japan.

Purpose: Currently, malignant and benign pigmented intraocular tumors are differentiated by comprehensive judgment of clinical findings based on ophthalmoscopic examination and imaging findings. However, the differentiation is often difficult. The aim of this study was to examine the usefulness of measuring immune
mediators in aqueous humor samples to differentiate malignant from benign pigmented intraocular tumors.

**Methods:** In 29 eyes (13 with benign pigmented tumor, 16 with malignant melanoma), undiluted aqueous humor samples were collected, and cytometric bead array was used to determine the aqueous humor concentrations of 35 immune mediators including 14 interleukins (IL), interferon (IFN)-γ, interferon-γ-inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated on activation, normal T cell expressed and secreted (RANTES), monokine induced by interferon-γ (Mig), basic fibroblast growth factor (bFGF), Fas ligand, granzyme A, granzyme B, eotaxin, interferon-inducible T-cell chemokine and TNF-α, LT-α, and CD40L.

**Results:** Aqueous humor levels of angiogenin and MCP-1 were significantly higher in eyes with malignant melanoma than in those with benign tumor (p<0.05).

**Conclusions:** The results of our study demonstrate the applicability of MCP-1 and angiogenin in aqueous humors as useful markers in distinguishing malignant and benign pigmented intraocular tumors and may be helpful as an adjunct to histomorphology and other markers in the diagnosis and appropriate clinical management.

**Commercial Relationships:** Yoshikito Usui, None; Shunichiro Ueda, None; Yoko Okunuki, None; Kinya Tsubota, Tokyo Medical University (E); Kazuki Tajima, None; Takeshi Kezuka, None; Yoshihiro Wakabayashi, None; Hiroshi Goto, None

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**Commercial Number:** 170 Poster Board Number: C0175
**Presentation Time:** 8:30 AM - 10:15 AM
**Transcriptional Regulatory Network Analysis of Uveal Melanoma**

Ronald Tongbai1, Jennifer Vargas1, Gintien Huang1, Bradley R. Straatsma2.
1New York Eye and Ear Infirmary, New York, NY; 2Jules Stein Eye Institute, Los Angeles, CA.

**Purpose:** Uveal melanoma is the most common form of intraocular melanoma in adults and metastasis to the liver and lungs is associated with a worse prognosis. A study by Onken et al utilized microarrays to characterize the gene expression profiles of tumors which metastasized and compared them with the gene expression profiles of tumors which did not metastasize. The study identified specific genetic signatures which discriminated between tumors that do and do not metastasize. The study provides an important insight into the molecular pathways and biological processes that govern the establishment and growth of these metastases.

**Methods:** Promoter region sequences from a 600 bp region (500 bp upstream to +100 bp downstream) were obtained for each gene in each of the gene lists using the ProSpector free web-based promoter annotation tool (4). The promoter regions of each of the gene signatures were analyzed for matches to approximately 300 position weight matrices (TFBS) using the MatInspector module of the GEMS Launcher 4.1 (Genomatix, Munich, Germany).

**Results:** A regulatory profile was generated based on the methods described above. Each of the gene lists were analyzed to generate p-values representing the degree of statistical enrichment for each of the approximately 300 TFBS in the promoter regions of these genes as described in the methods.

**Conclusions:** These results provide an important insight into the cell biology that governs the regulatory networks involved in uveal melanoma. Transcription factors implicated in the cell biology of uveal melanoma include AP2, ATF, and MapK/ERK.

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**Commercial Relationships:** Ronald Tongbai, None; Jennifer Vargas, None; Gintien Huang, None; Bradley R. Straatsma, None

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**Program Number:** 171 Poster Board Number: C0176
**Presentation Time:** 8:30 AM - 10:15 AM
**Efficacy of Systemic Rituximab Injection for Treating Primary Ocular Adnexal MALT Lymphoma**

Noriyasu Hashida, Kenji Matsuahita, Kohji Nishida. Department of Ophthalmology, Osaka University Graduate School of Medicine, Suita, Japan.

**Purpose:** To retrospectively review the clinical course of systemic rituximab for treating ocular adnexal mucosa-associated lymphoid tissue-type (MALT) lymphoma.

**Methods:** Twenty patients (10 men, 10 women) with stage I MALT adnexal lymphoma between 1993 and 2012 at Osaka University Hospital were included. We reviewed the clinical courses of 8 (2 men, 6 women, average age, 63.7±11.5) of the 20 cases treated with adjunctive rituximab therapy. The pathological diagnosis of all cases obtained intraoperatively was CD20-positive MALT lymphoma. Weekly rituximab infusions for 4 weeks were administered as a one-course protocol; additional injections were administered with disease recurrence. We investigated the effects and the clinical course of rituximab treatment and the relationship between serum sIL-2R concentrations and the recurrence rate.

**Results:** The mean follow-up time was 72.0±34.5 months. The mean number of rituximab infusions was 6.8±3.2 (range, 4-12). The mean follow-up time after administration was 44.3±34.9 months. Malignant lymphoma involved the conjunctiva in 2 cases and extended into the orbit in 6 cases. Tumor regression occurred immediately after the one-course protocol. Among the eight cases, three cases had relapses after the initial treatment; two of these cases initially had multiple relapses despite prior systemic monochemotherapy. Remission was achieved with several courses of rituximab. In another case, after initial rituximab treatment induced tumor regression, a long remission was obtained with adjunctive radiation therapy. The serum sIL-2R concentrations were significantly higher in cases with relapses than in those without relapses.

**Conclusions:** Systemic rituximab therapy could be a good option for treating orbital MALT lymphoma. Periodic examinations are needed until remission is achieved since single-modality therapy could cause a relapse. The sIL-2R concentration could be a good biomarker for recurrence.

**Commercial Relationships:** Noriyasu Hashida, None; Kenji Matsuahita, None; Kohji Nishida, Alcon (C), Alcon (F), HOYA (F), Senju (F), Pfizer (F), Santen (F), Osaka University (P)

**Clinical Trial:** UMIN000009512

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**Program Number:** 172 Poster Board Number: C0177
**Presentation Time:** 8:30 AM - 10:15 AM
**Inflammatory stress upregulates chemokine gene expression in uveal melanoma cell lines resulting in increased monocyte chemotaxis**

Tina Jehs1, Helene B. Juè1, Carsten Faber1, Inge H. Bronkhorst2, Martine M. Jager2, Mogens H. Nissen1. 1International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark; 2Ophthalmology, Leiden University Medical Center, Leiden, Netherlands.

**Purpose:** Uveal melanoma is the most common primary intraocular tumor in adults. In contrast with many other malignancies, the presence of infiltrating T cells and macrophages is associated with a poor prognosis. Moreover, higher concentrations of several inflammatory cytokines and chemokines in the vitreous fluid

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correlate with an increasing tumor size underlying the significance of inflammation in the uveal melanoma pathogenesis. The purpose of this study is to investigate in vitro the effect of activated T cells on uveal melanoma cell gene expression and ability to attract monocytes.

**Methods:** T cells were purified from healthy human donors, activated with anti-CD3/CD28 beads and co-cultured over three uveal melanoma cell lines (92.1, Mel 270 and Mel 290) for 64h in a membrane insert. Supernatant was collected and RNA was purified from the uveal melanoma cell lines and gene expression analysis was performed with whole-transcriptome microarrays. For the migration assay, CD14+ monocytes purified from healthy human donors were added in the upper chamber of a transwell plate. Supernatants were added to the lower chamber and plates were incubated for 2.5h. The number of migrated cells was counted with flow cytometry.

**Results:** Gene expression analysis of uveal melanoma cell lines co-cultured with activated T cells resulted in a more than two-fold upregulation of over 500 genes including several genes coding for chemokines and cytokines such as CCL2, CXCL8, CXCL9, CXCL10, CXCL11, IL6, IL14 and IL1β. This increase coincides with increased monocyte attraction towards co-culture supernatants in a migration assay.

**Conclusions:** Cytokines derived from activated T cells shift the uveal melanoma cell-transcriptome towards a more inflammatory state, including the upregulation of several cytokines and chemokines, which lead to an increased migration of monocytes. Therefore, uveal melanoma cells might actively participate in generating an inflammatory environment around the tumor which corresponds to a worse prognosis. With these findings immunotherapy may prove to be a useful strategy in treating uveal melanoma.

**Commercial Relationships:** M. Jager, None; Carsten Faber, None; Inge H. Bronkhorst, None; Martine M. Jager, None; Mogens H. Nissen, None

**Support:** ARVONED-Alcon 2012 award

**Program Number:** 173 Poster Board Number: C0178

**Presentation Time:** 8:30 AM - 10:15 AM

**The Expression and meaning of Skp2, p27, PTEN proteins in non-Hodgkin B-cell lymphoma of ocular adnexa**

Guigu Zhao, Jing Lin, Qian Wang, Qiang Xu, Ophthalmology, the Affiliated Hospital of Medical College, Qingdao University, Qingdao, China.

**Purpose:** To investigate the expression and the correlation of protein of S-phase kinase associationprotein 2(Skp2), p27 and phosphatases phosphatase and tensin homologue (PTEN) in non-Hodgkin B-cell lymphoma(NHL)of ocular adenexa.

**Methods:** Paraffin sections were collected from patients suffering from B cell NHL in Qingdao Affiliated Hospital from 1995 to 2011 and lymphadenosis (n=10) of ocular adenexa as the control group. The expression of Skp2, p27 and PTEN proteins were detected by Immunohistochemistry and the positive expression rate between lymphomas and lymphadenosis was compared by χ² inspection. Spearman rank correlation was used to estimate the relationships among three proteins in ocular lymphoma. Pathologic grade were evaluated as independent factor of clinical information.

**Results:** The expression of three proteins were related to pathologic type of tumors, but not related to age, gender, pathogenetic locations. In non-Hodgkin B-cell lymphoma of ocular adenexa, the expression of Skp2(76.7%, 23/30) was higher than that in control group(0/10)(χ²=19.79, P=0.001), while the expression of p27(73.3%, 22/30) and PTEN(56.7%, 17/30) was lower than that in the control group(10/10 and 10/10)(χ²=8.37, P=0.039; χ² =13.48,P=0.004). With the increase of pathologic grade, Skp2 labeling frequency increase gradually, while p27 and PTEN labeling frequency decrease gradually; There was a negative correlation between p27 and Skp2 in MALT(r=-0.134, p<0.05) while there was a positive correlation between p27 and PTEN(r=0.828, p<0.05). The relationship between Skp2 and PTEN was also negative (r=-0.883, p<0.05).

**Conclusions:** The high expression of Skp2 and lower expression of p27 and PTEN may play a role in the tumorigenesis and different pathologic type of the B cell NHL of ocular adnexa. The expressions of three proteins correlate with each other in mucosal-associated lymphoid tissue.

**Commercial Relationships:** Guigu Zhao, None; Jing Lin, None; Qian Wang, None; Qiang Xu, None

**116 CNS and Ocular Inflammation and Infection**

Sunday, May 05, 2013 10:30 AM-12:15 PM

606/607 Paper Session

**Program #/Board # Range:** 353-359

**Organizing Section:** Immunology/Microbiology

**Program Number:** 353

**Presentation Time:** 10:30 AM - 10:45 AM

**HLA-B27 affects the gut microbiome of transgenic rats**

Phoebe Lin¹, Mary Bach², Aaron Y. Lee³, Lakshmi Akleswaran¹, Joel D. Taurog⁴, James T. Rosenbaum², ³, Russell N. Van Gelder⁴.

¹Ophthalmology, Oregon Health and Science University, Portland, OR; ²Rheumatology, University of Washington, Seattle, WA; ³Ophthalmology, University of Washington, Seattle, WA; ⁴Ophthalmology, Devers Eye Institute, Portland, OR; ⁴Ophthalmology, Washington University, St. Louis, MO; ⁴Rheumatic Diseases and Internal Medicine, UT Southwestern, Dallas, TX.

**Purpose:** The HLA-B27 gene is a major risk factor for acute anterior uveitis, but its mechanism of risk enhancement is not completely understood. The gut microbiome has been shown to be important in the development of HLA-B27-mediated arthritis in transgenic rats as demonstrated by abrogating clinical disease by rearing animals in a germ-free environment. However, the role of HLA-B27 in shaping the gut microbiome has not been elucidated. In this study, we characterize the differences in the gut microflora mediated by the presence of the HLA-B27 gene.

**Methods:** We identified differences between the cecal microbiome in 45 day-old co-transgenic HLA-B27/beta2-microglobulin Lewis rats (n=7) compared with wild-type Lewis rats (n=6 co-housed, n=3 non-co-housed) using biome representational in situ karyotyping (BRISK). Co-housed rats are housed in the same cages as their transgenic littermates, which is expected to minimize microbiome differences since rats are coprophagic. We confirmed differences by quantitative PCR (qPCR) with normalization based on amplification of 16S ribosomal DNA.

**Results:** Of 909 species of bacteria identified by BRISK, 30 species showed statistically significant differences in HLA-B27/beta2microglobulin transgenic rats compared to wild-type controls as determined by iterative Monte Carlo analysis of the dataset. The three organisms showing the most difference were Faecalibacterium prausnitzii, Bacteroides vulgatus, and Akkermansia muciniphila. A difference in F. prausnitzii could not be confirmed by qPCR. However, there were higher levels of B. vulgatus and lower levels of Akkermansia in transgenic rats compared to non-co-housed control rats (Figure 1). Co-housed control rats did not demonstrate as large a difference in these two organisms compared to their transgenic cage mates. Both B. vulgatus and Akkermansia have been implicated in other immune-mediated diseases.

**Figure 1. Relative abundance of B. vulgatus and Akkermansia}
muciniphila from cecal flora of B27/beta2microglobulin rats compared to control rats. 16s rDNA served as amplification controls. **Conclusions:** The presence of HLA-B27 alters the gut microflora by increasing B. vulgatus and decreasing Akkermansia. While a causal role cannot be proven, a protective effect of Akkermansia and a disease-producing effect of B. vulgatus are plausible.

**Commercial Relationships:** Phoebe Lin, None; Mary Bach, None; Aaron Y. Lee, Congent 14 Productions LLC (threeplus.org) (P); Lakshmi Akileswaran, None; Joel D. Taurog, None; James T. Rosenbaum, Genentech (C), Abbott (F), Xoma (C), Eyeagate (F), Bristol Myers (F), Lux (C), Novartis (C), Regeneron (C), Teva (C), Therakine (F), Mitotech (F), Aquinox (F), Allergan (C), Santen (C); Russell N. Van Gelder, Novartis (F)  
**Support:** NEI grant 1K08EY022948; Burroughs-Wellcome Translational Science Award, Research to Prevent Blindness, and NIH CORE grant EY001730

**Program Number:** 354  
**Presentation Time:** 10:45 AM - 11:00 AM

TL1A is a cytokine that plays a major role in the immune response against helminths and in the pathogenic processes of allergy and inflammation. A subpopulation of T-helper cells, designated "Th9", that selectively produce IL-9, mediates inflammation in mouse eyes expressing the target antigen. TL1A (also known as TNFSF15), a tumor necrosis factor (TNF) family member, co-stimulates T cells and enhances their proliferative responses through its receptor DR3 (TNFRSF25). This study examined the capacity of TL1A to promote the generation of Th9 cells and to enhance their ocular immunopathogenicity.  
**Methods:** Naïve CD4 cells expressing TCR specific against hen egg lysozyme (HEL) were incubated with HEL and Th9-producing cytokines, with or without TL1A and their production of IL-9 and IL-10 was determined by flow cytometry, qPCR and ELISA. The immunopathogenicity of Th9 cells was assessed by their capacity to induce inflammation in recipient eyes expressing HEL. The local effect of TL1A was tested by intraocular injection of the molecule into recipient of Th9 cells, whereas anti-TL1A antibodies were injected into Th9 recipients to determine their inhibitory effect on the disease severity.  
**Results:** TL1A enhanced the generation of Th9 in culture, increasing the proportion of IL-9 producing cells from ~40% to ~90%, while reducing the proportion of IL-10 producing Th9 cells from ~22% to ~2%. Importantly, Th9 cells generated in the presence of TL1A were more efficient than their controls in inducing ocular inflammation. Intracocular administration of TL1A increased moderately the Th9-induced ocular inflammation, whereas systemic treatment with anti-TL1A antibodies reduced significantly the Th9-induced ocular inflammation.  
**Conclusions:** The cytokine TL1A profoundly promotes Th9 differentiation and pathogenicity and might be a therapeutic target in ocular and other immune-mediated diseases.  
**Commercial Relationships:** Cuiyan Tan, None; Francoise Meylan, None; Barbara P. Vistica, None; Richard Siegel, NIH (P); Igal Gery, None

**Program Number:** 355  
**Presentation Time:** 11:00 AM - 11:15 AM  
**Idiopathic Intermediate Uveitis: A 25-year study of visual prognosis**  
William R. Tucker¹, Elizabeth Graham¹, Philip I. Murray², Miles R. Stanford². ¹Ophthalmology Dept, Guys & St Thomas’ NHS Foundation Trust, London, United Kingdom; ²School of Immunity and Infection, University of Birmingham, Birmingham, United Kingdom.  
**Purpose:** Idiopathic Intermediate Uveitis (IIU) is a chronic intraocular inflammatory disorder, which affects over 10% of the uveitis population. This study aims to identify the long-term outcomes and complication rates for patients with IIU.  
**Methods:** A retrospective review of 100 patients from two regional uveitis centres with a follow-up of between 5 and 25 years. The main outcome measure was maintenance of best-corrected visual acuity (BCVA) at 20/40 or better.  
**Results:** The average age at onset of IIU was 38 years (range 4 - 73 years) and 41% were male. Mean follow-up time was 12 years with 26 out of 100 patients having more than 20 years follow-up. Baseline BCVA was recorded after 3 months of treatment with 89% at 20/40 or better, this level of BCVA was maintained over the follow-up period with 82% at 20/40 or better after 5, 10 and 15 years. BCVA was 6/12 or better in 85% at 20 years and 80% after 25 years of follow-up. There was a significant correlation between baseline BCVA and the BCVA at final follow-up with 84% maintaining visual acuity of 20/40 or better (Spearman’s rank correlation coefficient = 0.502 (p=0.0001)).  
**Conclusions:** The visual prognosis for patients with IIU is good with 84% maintaining vision better than or equal to 20/40. Patients who present with visual acuity at this level can be reassured they have a reasonable chance of keeping it long term.  
**Commercial Relationships:** William R. Tucker, None; Elizabeth Graham, Santen (C); Philip I. Murray, None; Miles R. Stanford, None

**Program Number:** 356  
**Presentation Time:** 11:15 AM - 11:30 AM  
**Clinical Profile, Treatment and Visual Outcome of Eales’ Disease, Study of 500 Patients from South India**  
Jyotirmay Biswas¹, Reesha Kr², Bikramjit Pal¹. ¹Uveitis and Ocular pathology, Vision Research Foundation, Sankara Nethralaya, Chennai, India; ²Uveitis and Ocular pathology, Vision Research Foundation, Sankara Nethralaya, Chennai, India.  
**Purpose:** To analyze the clinical profile, treatment and visual outcome of patients with Eales’ disease in a tertiary care centre of South India.  
**Methods:** A retrospective analysis of patients diagnosed clinically and confirmed angiographically as Eales’ disease between 1985 and 1995 with a minimum follow up of 10 years were included in the
study. Eight hundred and ninety eight eyes of 500 patients were analyzed.

**Results:** 500 patients (898 eyes) were included in the study with a mean follow up of 15.8 years. 95.2% were males with male to female ratio of 20:1. Commonest age group affected was 20-40 years (71%), 81% had bilateral involvement. The commonest presenting complaint was defective vision(40%). 53% patients had vision better than 6/12 on presentation. 73% eyes had stable visual outcome at end of the follow up. Causes for poor visual outcomes were end stage disease with optic atrophy (29%) and secondary glaucoma (11%). Factors associated with poor visual outcome were delayed presentation (p=0.038), poor vision on presentation (p=0.000), central variant of Eales’ (0.027), macular involvement (0.000), presence of fibrovascular proliferation, vitreous hemorrhage and retinal detachment (p=0.000).

**Conclusions:** Conclusion: Eales’ disease is common retinal vasculitis which when detected early has a favorable clinical prognosis. Patients require a frequent follow up. Non surgical (systemic steroids and laser) along with surgical management when instituted early in the classical course helps in stabilization of the disease process.

**Commercial Relationships:** Jyotirmay Biswas, None; Reesha Kr, None; Bikramjit Pal, None

**Support:** nil

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**Program Number:** 357
**Presentation Time:** 11:30 AM - 11:45 AM
**Prevalence of Anti-Retinal Autoantibodies in Women with Gynecological Cancers with and without Cancer Associated Retinopathy**

**Grazyna Adamus**, **Dongseok Choi**, **Jade Schiffman**

1. Ophthalmology, Triemli Spital, Zürich, Switzerland.
2. Ophthalmology, Interdisciplinary Uveitis Center, Heidelberg, Germany.
3. Pediatric rheumatology, Interdisciplinary Uveitis Center, Heidelberg, Germany.
4. Ophthalmology, Triemli Spital, Zürich, Switzerland.

**Purpose:** To investigate the prevalence of serum anti-retinal autoantibodies (AAbs) in cancer associated retinopathy (CAR) patients with different types of gynecological cancers in comparison to healthy women and patients with similar cancers but without visual problems.

**Methods:** Sera from repositories at MD Anderson Medical Center and OHSU represented 36 women with an average age of 58 years with CAR and gynecological cancers, including endometrial, cervical, ovarian, and fallopian tubes cancers. Patients mostly presented with progressive loss of vision, with reduced visual acuity, color impairment and photosensitivity and an abnormal ERG. For comparison, sera from 90 patients with endometrial, cervical, ovarian, and fallopian tubes cancers without symptoms of CAR as well as 65 age-matched healthy women were analyzed. Sera were tested for the presence of anti-retinal autoantibodies by western blotting and the P value was calculated from the Fisher's exact test.

**Results:** Anti-retinal AAbs occurred in women with gynecologic malignancies with and without CAR as well as in the age matched controls. Women with gynecologic tumors and CAR had a higher proportion (81%) of seropositivity compared with women with gynecologic malignancies without clinical symptoms of CAR (60%) and healthy normal controls (58%). Four AAbs specific to enolase, carbonic anhydrase II, recoverin and GAPDH were predominant in patients’ sera. Anti-alpha enolase AAbs were detected in 39% CAR patients and 13% control subjects (p=0.006). Anti-CAL antibodies were prevalent in women without CAR (p=0.039). Anti-recoverin AAbs were present only in endometrial CAR (p=0.001). Anti-GAPDH antibodies were significantly higher in patients with CAR compared to patients without CAR (p=0.028). In this cohort, cancer was always diagnosed before diagnosis of retinopathy with latency from 2 months to 30 years with the longest interval in cervical malignancies (on average 21 years). The diagnosis of the ovarian and endometrial cancers and CAR often coincided.

**Conclusions:** CAR associated with different type of gynecological malignancies presented different anti-retinal AAbs and ocular symptoms that manifested months to years after the initial tumor diagnosis. Anti-enolase and anti-recoverin AAbs occurred more often in women with CAR than controls.

**Commercial Relationships:** Grazyna Adamus, None; Dongseok Choi, None; Jade Schiffman, None

**Support:** EY13053

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**Program Number:** 358
**Presentation Time:** 11:45 AM - 12:00 PM
**Use of biologics in pediatric uveitis: changing treatment patterns**

**Friederike Mackensen**, **Matthias D. Becker**, **Juergen Grulich-Henn**, **Thomas Lutz**

1. Ophthalmology, Interdisciplinary Uveitis Center, Heidelberg, Germany.
2. Pediatric rheumatology, Interdisciplinary Uveitis Center, Heidelberg, Germany.
3. Ophthalmology, Triemli Spital, Zürich, Switzerland.

**Purpose:** In 2010 we presented data of 3 European centers with a total of 475 children with uveitis. 182 were included from the uveitis center in Heidelberg. Here we could show that compared to previous studies from the US covering the years 1980-2005, where 6% received treatment with biologics, this increased to 8.8 % in the time period from 1990 to 2007. This was supposed to be due to changing treatment patterns since 2000. The aim of the current study was to analyse further change in treatment patterns and use of newer biologics in pediatric uveitis.

**Methods:** Database search. All children (<18 years) with uveitis seen in the interdisciplinary uveitis center from 08/2007 until 11/2012 were included. Medical treatment was evaluated. This was compared to the previously presented cohort of 457 children and also to a published cohort of 527 children from the US (Smith et al 2009). Parameters analyzed followed published recommendations (MIWGUC and SUN).

**Results:** In the study period 301 children with uveitis were treated at the interdisciplinary uveitis center in Heidelberg. Anatomic localisation was posterior in 21 (7%), intermediate in 65 (21.6%), pan in 10 (3.3%) and anterior in 185 (61.5%).

41(13.6%) children recieved biologies during the studied period: 31 adalimumab (ADA), 8 Etanercept (ETA), 4 Infliximab (INFIL), 3 Abatacept, and one Tocilizumab. Among those were 32 children with juvenile idiopathic arthritis (JIA) (78%).

All 8 patients were put on ETAfor uncontrolled joint disease. Two patients developed uveitis and were switched to ADA or to abatacept (n=1 each). In the remaining 6 patients uveitis remained controlled during ETA treatment.

The other biologics were started for activity of uveitis. Efficacy of treatment was seen in all patients looking at visual acuity, inflammatory activity or prednisone sparing effect at set time points. In the adalimumab group, 3 had secondary loss of efficacy and moved on to infliximab (n=2) or to abatacept (n=1). One patient subsequently also failed infliximab and changed to tocilizumab. Meaning that 5 children received more than 1 biologic during the studied period.

**Conclusions:** Frequency of biologic use further increased to 13.6% in the last 5 years as compared to 8.8% in the previous study. Especially in uveitis assoicated to JIA, which generally is thought to be the most severe form of pediatric uveitis, this has helped to controll inflammation and improve and stabilize visual acuity.

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208 The Untapped Immunomodulating Potential of Neutrophils - Minisymposium

Program Number: 359
Presentation Time: 12:00 PM - 12:15 PM
CAP-Syndrome: Response to Treatment With Canakinumab Manfred Zierhut1, Bianka Sobolewska1, Christoph M. Deuter2, Deshka Doycheva3, Jasmin Kuenmerle-Deschner3. 1Centre for Ophthalmology, University of Tuebingen, Tuebingen, Germany; 2Pediatric Rheumatology, University of Tuebingen, Tuebingen, Germany. Purpose: The cryopyrin-associated periodic syndromes (CAPS) are a group of rare autosomal dominant autoinflammatory disorders associated with mutations in the NLRP3 gene leading to excessive interleukin-1 release. CAPS encompasses three different entities: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological cutaneous and articular syndrome (CINCA/NOMID). Symptoms include rash, arthralgia, arthritis, fever, inflammation of the eyes and hearing loss. We take care for a 5-generation-family with MWS with various symptoms, due to the NLRP3 mutation A439V. Here we report about the response to canakinumab (Ilaris, human monoclonal antibody targeted IL-1-beta).

Methods: A retrospective observational study on 13 family members was performed. NLRP3 gene mutations were determined. All patients had standardized clinical and ophthalmologic assessments. The most common organ manifestations such as rash, arthritis, hypacusis, and uveitis/conjunctivitis were compared before and during therapy with canakinumab (300mg every 8-10 weeks in 10 patients, 150mg every 8 weeks in 2 patients, and 150mg every 2 months in 1 patient).

Results: 13 (7 female and 6 male) symptomatic family members were enrolled in the study. Eleven (85%) of the 13 symptomatic family members carried the A439V mutation. Two (15%) patients and were heterozygous carriers of Alanin 439 (GCG)-Valin (GTG)-p.Ala439Val/A4339V substitution. Before treatment with canakinumab, the most common organ manifestations were arthritis (77%), rash (100%), conjunctivitis (69%), anterior uveitis (69%), and hypacusis (31%). During therapy with canakinumab, neither anterior uveitis nor rash were observed. Moderate conjunctivitis and minimal arthritis were seen in one patient only.

Conclusions: Therapy with canakinumab seems to be effective in mutation-positive family members with MWS.

Commercial Relationships: Manfred Zierhut. None; Bianka Sobolewska. None; Christoph M. Deuter. Novartis (F); Deshka Doycheva. None; Jasmin Kuenmerle-Deschner. Novartis (F), Novartis (R), Novartis (C)

244 Microbial Pathogenesis

Program Number: 1251
Presentation Time: 9:33 AM - 9:54 AM
VIP and SP in Corneal Infection Linda D. Hazlett. Anatomy & Cell Biology, Wayne State Univ Sch of Med, Detroit, MI.

Commercial Relationships: Linda D. Hazlett. None

208 The Untapped Immunomodulating Potential of Neutrophils - Minisymposium

Program Number: 359
Presentation Time: 12:00 PM - 12:15 PM
CAP-Syndrome: Response to Treatment With Canakinumab Manfred Zierhut1, Bianka Sobolewska1, Christoph M. Deuter2, Deshka Doycheva3, Jasmin Kuenmerle-Deschner3. 1Centre for Ophthalmology, University of Tuebingen, Tuebingen, Germany; 2Pediatric Rheumatology, University of Tuebingen, Tuebingen, Germany. Purpose: The cryopyrin-associated periodic syndromes (CAPS) are a group of rare autosomal dominant autoinflammatory disorders associated with mutations in the NLRP3 gene leading to excessive interleukin-1 release. CAPS encompasses three different entities: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological cutaneous and articular syndrome (CINCA/NOMID). Symptoms include rash, arthralgia, arthritis, fever, inflammation of the eyes and hearing loss. We take care for a 5-generation-family with MWS with various symptoms, due to the NLRP3 mutation A439V. Here we report about the response to canakinumab (Ilaris, human monoclonal antibody targeted IL-1-beta).

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Conclusions: Therapy with canakinumab seems to be effective in mutation-positive family members with MWS.

Commercial Relationships: Manfred Zierhut. None; Bianka Sobolewska. None; Christoph M. Deuter. Novartis (F); Deshka Doycheva. None; Jasmin Kuenmerle-Deschner. Novartis (F), Novartis (R), Novartis (C)

244 Microbial Pathogenesis

Program Number: 1251
Presentation Time: 9:33 AM - 9:54 AM
VIP and SP in Corneal Infection Linda D. Hazlett. Anatomy & Cell Biology, Wayne State Univ Sch of Med, Detroit, MI.

Commercial Relationships: Linda D. Hazlett. None
associated risk factors was by medical record review. Keratitis-associated S. aureus strains were assessed for: 1) antibiotic susceptibility by CSLI standards, 2) biofilm robustness by gentian violet staining, 3) genetic lineage by multi-locus sequence typing (MLST), and 4) whole genome sequencing was performed using Illumina sequencing technology.

**Results:** 32 keratitis isolates were identified. Risk factors included trauma, prior surgery, soft contact lens wear, and the presence of a surgical implant or environmental foreign body; 25% had no identifiable risk factor. All isolates were tetracycline- and trimethoprim-sulfamethoxazole-sensitive. Prior antibiotic usage did correlate strongly with methicillin-resistance; all MRSA strains were found to be ciprofloxacin-resistant. More than one-third of all keratitis-associated isolated were in the same lineage, ST-5, with both methicillin-sensitive and -resistant S. aureus strains represented. Using a novel linker and paired-end library construction protocol, whole genome sequencing was performed at a cost of $100 USD per bacterial genome; analysis is currently underway.

**Conclusions:** These results suggest that there may be specific S. aureus lineages that possess genotypic characteristics that enable S. aureus to more effectively cause sight-threatening keratitis and other ocular infections. Additionally, we believe this new genome sequencing technology will give us insight into novel virulence traits that may be uniquely associated with different ocular infections. Further work will explore the feasibility of cost-effective, real-time genomic sequencing technology for clinical application in hospital microbiology laboratories.

**Commercial Relationships:** Irmgard Behlau, None; David Lazinski, None; Jacqueline Martin, None; Susan Heimer, None; Elizabeth M. Leonard, None; Andrew Wright, None; Michael S. Gilmore, Bausch & Lomb (F); Claes H. Dohlman, None; Andrew Camilli, Tufts University (P)

**Support:** Fight-for-Sight, KPro Fund

**Program Number:** 1727
**Presentation Time:** 11:15 AM - 11:30 AM

**Efficacy of an Intravitreal Levofloxacin Implant in an Animal Model of Endophthalmitis**

**Russell Tait**1,4, Richard Prankerd4, Roy Robins-browne4, Penelope J. Allen5,2, Andrew Donohue5,4, Dong Yang6, Louise Adams, Feng Wang4, Asha D’Souza4, Sarah Ng5, PolyActiva Pty Ltd, Melbourne, VIC, Australia; 2Centre for Eye Research Australia, Melbourne, VIC, Australia; 3Microbiology and Immunology, The University of Melbourne, Melbourne, VIC, Australia; 4Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia; 5Royal Victorian Eye and Ear Hospital, Melbourne, VIC, Australia; 6Eye Hospital, Harbin Medical University, Harbin, China.

**Purpose:** Bacterial endophthalmitis is a devastating ocular infection with poor visual outcome. Intravitreal injection of a bolus dose of antibiotic(s) is the preferred method of treatment. Unfortunately, a single intravitreal injection is often insufficient to treat the infection. Use of an intravitreal implant that releases an antibiotic at the appropriate dose for a sustained period may obviate the need for retreatment. We used a rabbit model of endophthalmitis to compare the efficacy of standard treatment (bolus vancomycin/cefazidime) with a levofloxacin(LVX)-releasing implant.

**Methods:** Experiments were performed according to the method described by Ferrer et al. Br J Ophthalmol 2008;92:678-682. The study evaluated treatment with a LVX implant (nominally releases LVX at 100 μg per 24 h) compared with standard treatment of a bolus injection of 1% (w/v) vancomycin + 2.2% (w/v) cefazidime, and an untreated control group. The vitreous of one eye of a New Zealand White rabbit was inoculated with S. aureus ATCC strain 29213. Treatment was administered 1 day after inoculation. On days 1, 3, 7, 10 and 14, samples of vitreous were collected for determination of the number of colony-forming units (CFUs) of S. aureus per ml and the level of LVX. The Peyman classification was used to assess the severity of endophthalmitis.

**Results:** The LVX implant exhibits zero-order release of LVX over at least 30 days, regardless of geometry. LVX concentrations in vitreous matched steady-state levels predicted from pharmacokinetic calculations based on the in vitro release rate, and were well above the MIC for S. aureus for at least a 10-day period. Infection was established in all inoculated eyes. By day 3, CFUs in the vitreous of all treated eyes had fallen to below detection limits. In contrast, CFUs in the vitreous from untreated eyes increased dramatically such that by day 3 symptoms had worsened and rabbit euthanasia was required. For the implant group, mild inflammation was observed on day 3, and by day 7, symptoms of endophthalmitis had subsided. Peyman scores for the implant group were similar to or better than rabbits in the standard treatment group.

**Conclusions:** In a rabbit model of endophthalmitis the LVX implant was at least as effective as the current standard therapy of vancomycin/cefazidime for treating infections of the vitreous cavity caused by S. aureus.

**Commercial Relationships:** Russell Tait, PolyActiva Pty Ltd (I); Richard Prankerd, None; Roy Robins-browne, Polyactiva Pty Ltd. (F), Polyactiva Pty Ltd. (C); Penelope J. Allen, Bionic Vision Australia (P); Andrew Donohue, Polyactiva (E); Dong Yang, None; Louise Adams, None; Feng Wang, Polyactiva (F), Polyactiva (P); Asha D’Souza, Polyactiva Pty Ltd (E); Sarah Ng, Polyactiva Pty Ltd (E)

**Support:** NHMRC Development Grant (APP1000704)

**Program Number:** 1728
**Presentation Time:** 11:30 AM - 11:45 AM

**Matrix Metalloproteiase 13 as a target for suppressing corneal ulceration caused by Pseudomonas aeruginosa infection**

**Nan Gao, Fushin X. Yu. Ophthalmology, Wayne State Univ/Kresge Eye Inst, Detroit, MI.**

**Purpose:** This study investigated whether MMP-13 is involved in P. aeruginosa infection caused corneal tissue destruction and therefore can be targeted for prevention of corneal ulceration associated with microbial keratitis.

**Methods:** Genome-wide cDNA array was performed using epithelial cells isolated from normal, P. aeruginosa infected, and flagellin pretreated/P. aeruginosa infected B6 mouse corneas 6 h post infection. The cDNA array detected MMP expression patterns were verified by realtime PCR and immunohistochemistry. To assess the role of MMP13 B6 mice were subconjunctivally injected with 50 ng MMP-13 inhibitor 16 hours prior to pathogen inoculation and inoculated with 104 ATCC19660 strain. At 1 and 3 dpi, severities of keratitis were monitored. To determine if MMP13 inhibitor can be used for adjuvant therapy, B6 mouse corneas were inoculated with 104 ATCC19660. Topical solution containing gatifloxacin and gatifloxacin/MMP-13 inhibitio were applied 16 hpi and after then every hour in the remaining day, 2 h at day 2 dpi and 4 h 3 days post infection. The corneal opacity was examined and photographed using slit lamp microscopy. At the end of observation, the corneas were processed for H&E stained for morphology examination and immunostaining with collagen IV of and type III.

**Results:** PA01 infection greatly increased MMP-13 expression 6 hpi and this elevated expression was almost totally attenuated by flagellin pretreatment which prevented microbial keratitis in B6 mice. While gatifloxacin applied 24 h or early is sufficient to eradicate pathogens
from the cornea, the inflammation persisted for at least three days. Topical co-application of MMP13 inhibitor with gatifloxacin greatly improved disease outcome including accelerated dissolution of opacity, reduced tissue inflammation, and rapid functional recovery. Fewer disorganization of collagen fibers were seen in antibiotic-MMP-13 co-treated corneas.

**Conclusions:** Downregulation of infection induced MMP13 expression might be an underlying mechanism for flagellin induced protection against microbial infection and MMP-13 inhibitor(s) could potential be used as an adjunctive therapy to treat microbial keratitis and other mucosal infection.

**Commercial Relationships:** Nan Gao, None; Fushin X. Yu, None

**Support:** NIH grants R01 EY017960, EY010869, Midwest Eye Bank

**Program Number:** 1729

**Presentation Time:** 11:45 AM - 12:00 PM

**Loss of HSV-1 induced VEGF-A during acute infection impairs the lymphangiogenic response during later stages of disease**

**Katie M. Hudson**, 1 Min Zheng, 1 Daniel J. Carr, 2-3. 1Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

**Purpose:** To characterize the impact of HSV-1 induced VEGF-A on lymphangiogenesis in the cornea during the development of herpetic stromal keratitis (HSK) and viral latency.

**Methods:** VEGF-A floxed C57BL/6 mice were infected with 200 pfu/cornea of HSV-1, either parental (SC16) or Cre-expressing (SC16 ICPO-Cre). At various time points post-infection (PI) mice were exsanguinated and the corneas were stained for blood (CD31+) and lymphatic (LYVE-1+) vessels. Viral load within the corneas was evaluated by plaque assay. Corneal leukocyte recruitment was determined by flow cytometry. Relative expression of pro-lymphangiogenic growth factors was determined via real-time RT-PCR.

**Results:** VEGF-A floxed mice infected with Cre-expressing HSV-1 had reduced T cell recruitment to the cornea at days 7 and 14 PI compared to floxed mice infected with the parental virus. Lymphatic vessel development into the cornea proper was also reduced at days 14 and 30 PI in the Cre-expressing HSV-1 infected group. Blood vessel development into the central cornea was similar for both groups. VEGF-A and VEGF-C expression was reduced at day 7 PI in ICPO-Cre infected floxed mice compared to the parental strain.

**Conclusions:** Preliminary results indicate loss of HSV-1 induced VEGF-A during acute infection significantly impairs the lymphangiogenic but not hemangiogenic response during the development of HSK and the establishment of viral latency. Reduced lymphatic vessel development may be due to a skewed pattern of leukocyte recruitment and subsequent expression of pro-lymphangiogenic factors.

**Commercial Relationships:** Katie M. Hudson, None; Min Zheng, None; Daniel J. Carr, None

**Support:** NIH/NEI R01 EY021238

**Program Number:** 1730

**Presentation Time:** 12:00 PM - 12:15 PM

**Delivery of Herpes Simplex Virus DNA to Retinal Ganglion Cell Axon is Dependent on Viral Protein Us9**

**Jennifer H. LaVail,** 1 Jolene M. Draper, 1-2 Graham S. Stephenson, 1 Guging Huang, 1 Andrea S. Bertke, 1 Daniel A. Cortez, 1. 1Dept of Anatomy/Ophthalmology, University of California, San Francisco, San Francisco, CA; 2Proctor Foundation, University of California, San Francisco, San Francisco, CA.

**Purpose:** The pathogenic spread of neurotropic virus relies on axonal transport of viral DNA. The role of the Herpes Simplex Virus (HSV) protein Us9 in viral intracellular spread is contentious. We compared the results of Us9 deletions in two HSV strains using a novel quantitative assay to test the hypothesis that Us9 regulates the delivery of viral DNA to the distal axon of retinal ganglion cells in vivo. We also deleted a nine amino acid motif in the Us9 protein of F strain (Us9-30) to define the role of this domain in DNA delivery.

**Methods:** The vitreous chambers of murine eyes were infected with equivalent amounts of F or NS strains of HSV. At three, four or five days post infection (dpi) both optic tracts (OT) were dissected and viral genome was quantified by qPCR.

**Results:** At three dpi the F strain Us9-9 and Us9-30 mutants delivered less than 10% and 1%, respectively, of the viral DNA delivered after infection with the Us9R (control) strain. The effect of the deletion of Us9 was less severe in the NS Us9 deletion experiments. By four and five dpi delivery of viral DNA had partially recovered, although to a greater degree in NS as compared to F experiments.

**Conclusions:** A highly conserved acidic cluster within the Us9 protein plays a critical role for genome transport to the distal axon. The transport is less dependent on Us9 expression in the NS than in F strain virus suggesting compensatory roles for the NS variant. This assay can be used to compare transport efficiency in other neurotropic viral strains.

**Commercial Relationships:** Jennifer H. LaVail, None; Jolene M. Draper, None; Graham S. Stephenson, None; Guging Huang, None; Andrea S. Bertke, None; Daniel A. Cortez, None

**Support:** NIH grant EY019159; Research to Prevent Blindness; That Man May See, Inc.

**Program Number:** 1731

**Presentation Time:** 12:15 PM - 12:30 PM

**Signal peptide peptidase (SPP) is required for HSV-1 infectivity through interaction with glycoprotein K (gK)**

**Sarath J. Allen,** 1 Kevin R. Mott, 1 Yoshilharu Matsuara, 2 Kohji Moriiishi, 1 Konstantin G. Kousoulos, 2 Homayoun Ghiasi, 1. 1Center for Neurobiology & Vaccine Development, Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA; 2Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

**Purpose:** We previously demonstrated that vaccinating mice with expressed glycoprotein K (gK), but not any other of the known HSV 1 glycoproteins results in severely exacerbated corneal scarring and dermatitis following ocular infection with both virulent and avirulent HSV-1 strains. Although gK is not involved in the processes of virus attachment or penetration, it is involved in virus replication and egress. Thus, the goal of this study was to determine if binding of gK to any cellular factor(s) may contribute to HSV-1 infectivity and pathogenesis.

**Methods:** Bacterial two-hybrid assay using gK as bait and a mouse neuronal cDNA library was used to identify binding partners to gK. Binding was confirmed by co-immunoprecipitation and immunohistochemistry (IHC) in HeLa, Vero and RS cell lines. Blocking SPP-gK interaction was assayed using SPP dominant negative mutants, shRNA directed against SPP, and SPP chemical inhibitors (i.e., DAPT, L685,485, (Z-LL)2 ketone, Aspirin or Ibuprofen). The effect of blocking SPP on viral replication was monitored using standard plaque assay, real-time PCR and IHC. The effect of ocular administration of SPP inhibitor (Z-LL)2 ketone on ocular HSV-1 replication was evaluated in BALB/c and C57BL/6 mice by titering virus from tears.

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Results: Using a two-hybrid system we identified signal peptide peptidase (SPP) as a cellular protein which interacts with gK. Binding of gK to SPP was confirmed by pull-down assay and fluorescent colocalization. Blocking SPP binding to gK using SPP dominant negative mutants, shRNA construct against SPP or inclusion of chemical SPP inhibitors significantly reduced HSV-1 replication in vitro. Finally, HSV-1 replication in the eyes of mice that received (Z-LL)2 ketone as an eye drop before and during infection was significantly reduced compared to sham-treated animals.

Conclusions: We have demonstrated for the first time that HSV-1 gK interacts with cellular SPP and this interaction is required for virus infectivity as blocking SPP reduces virus replication in vitro and in vivo. As there is currently no effective treatment for HSV-1 recurrences, blocking SPP-gK interaction may represent a clinically effective and expedient target in developing HSV-1 therapeutics.

Commercial Relationships: None

Support: None; Mitsubishi Tanabe Pharma (F), Celtic Pharmaceuticals Corporation (F), Novartis Pharma Japan (F), Novartis Pharmaceuticals Corporation (C), Xoma (C), Santen Pharmaceutical Corporation (F)

Program Number: 2025 Poster Board Number: D0164
Presentation Time: 11:00 AM - 12:45 PM

T-cell and cytokine investigations in an experimental model of retinal ganglion cell loss
Sandra Kuehn, Rozina Noristani, Mathias Kuehn, Burkhard H. Dick, Stephanie C. Joachim. Experimental Eye Res Inst, Ruhr University Bochum, Bochum, Germany.

Results: Intravenous injection of small RNA molecules helps to alleviate ocular damage induced by cytomegaloviruses and provides a route for alteration of host and/or virus gene expression in the retina.

Commercial Relationships: None

Support: None;

Program Number: 2024 Poster Board Number: D0163
Presentation Time: 11:00 AM - 12:45 PM

IL-2/IL-2 Ab complex plus rapamycin ameliorate experimental autoimmune uveoretinitis associated with expansion of CD4+Foxp3+ regulatory T cells

Purpose: To determine whether injection of IL-2/IL-2Ab together with rapamycin is effective in reducing ocular inflammation in experimental autoimmune uveoretinitis (EAU).

Methods: C57BL/6J mice were immunized with human interphotoreceptor retinoid binding protein peptide (h-IRBP1-20). Mice were injected i.p. with PBS, IL-2/IL-2Ab (JES6-1), rapamycin, or IL-2/IL-2Ab plus rapamycin on days 1, 2, 3, and 4 after immunization. Fundus examination was performed on days 9, 12, 15, 18, and 21 after immunization. The expression of Foxp3 on CD4+ T cells from draining lymph nodes (LNs) was examined by flow cytometry on day 7.

Results: Injection of IL-2/IL-2Ab plus rapamycin significantly reduced the clinical score of EAU on days 12, 15 and 18 compared to PBS, IL-2/IL-2Ab, or rapamycin treated mice. In addition, mice treated with IL-2/IL-2Ab plus rapamycin showed decreased severity of EAU by histopathological analysis. Although the expansion of CD4+Foxp3+ regulatory T cells (Tregs) was observed on day 7 in draining LNs from IL-2/IL-2Ab, rapamycin, and IL-2/IL-2Ab plus rapamycin treated mice, the expansion of Tregs was most prominent in IL-2/IL-2Ab plus rapamycin treated mice.

Conclusions: These results indicate that injection of IL-2/IL-2Ab plus rapamycin is effective in reducing ocular inflammation in EAU and in inducing the expansion of CD4+Foxp3+ Tregs. In vivo expansion of T reggs using IL-2/IL-2Ab plus rapamin may have clinical potential for the treatment of human refractory uveitis.

Commercial Relationships: None

Support: Grants-in-Aid for Young Scientist from the Ministry of Education, Culture, Sports,Science and Technology, Japan

Program Number: 1732
Presentation Time: 12:30 PM - 12:45 PM

Prevention of Cytomegalovirus-Induced Retinitis By Intravenous Administration of Virus-specific siRNA
Brendan Marshall, Jason Covar, Sally S. Atherton, Ming Zhang.
Cellular Biology and Anatomy, Georgia Health Sciences University, Augusta, GA.

Purpose: CMV is a ubiquitous betaherpesvirus which is present in a latent form throughout much of the human population without apparent clinical symptoms. However, during episodes of immune suppression, CMV may escape immune control and induce significant pathology of the eye, including retinal destruction and subsequently, blindness. Retinitis is characterized by both apoptosis and necrosis of the inner and outer retina and a characteristic feature of the apoptotic process is that most of the cells which undergo apoptosis are not infected with virus. We hypothesized that the delivery of virus-inhibiting compounds to the retinal pigment epithelium (RPE) layer of the eye might be beneficial in terms of limiting virus replication and also retinal damage caused by apoptosis of uninfected cells.

Methods: Using a murine cytomegalovirus (MCMV) model of infection we injected immunosuppressed Balb/c mice with MCMV via the supraciliary route. Two days following infection, we intravenously injected 1 nanomole of either control siRNA or siRNA specific for the MCMV immediate early protein-3 (IE-3). We then harvested eyes at several subsequent days for a period of two weeks in order to measure virus titters and levels of the IE-3 protein as well as the number of apoptotic cells in the retina, using both TUNEL and immunoblot assays for the cleaved caspase 3 protein.

Results: IE-3 specific siRNA produced a substantial decrease in levels of the IE-3 protein as well as MCMV titters over the 14 day time course of virus infection, compared to control siRNA. The amount of apoptosis in anti IE-3 siRNA treated mice, as measured by TUNEL assay, was also greatly reduced compared to control siRNA treated mice. Furthermore, consistent with the results of TUNEL assays, levels of cleaved caspase 3 were also greatly reduced in IE-3 siRNA treated mice compared to those treated with control siRNA. Overall, retinitis was substantially decreased as a result of treatment with IE-3 specific siRNA. Our data indicate that an siRNA, which targets the MCMV IE-3 protein, is effective in substantially reducing the levels of both virus and apoptosis in MCMV infected eyes of immunosuppressed mice.

Conclusions: Intravenous injection of small RNA molecules helps to alleviate ocular damage induced by cytomegaloviruses and provides a route for alteration of host and/or virus gene expression in the retina.

Commercial Relationships: Brendan Marshall, None; Jason Covar, None; Sally S. Atherton, None; Ming Zhang, None

Support: This work was supported by Public Health Service Grant EY009169 from the National Eye Institute to SSA.
**Purpose:** In the last years immune activities of destructive nature were detected in retina of glaucoma patients. Evidence of T-cell activities was noted in blood of patients. These immune cells can act via pro-apoptotic cytokines, which are great contributors in the mechanism of cell death. In order to investigate the role of T-cells in a glaucomatous model we detected cells and we looked at the cytokines to define the T-cell status.

**Methods:** Rats were immunized with optic nerve homogenate antigen (ONA, n=6) or NaCl (Co, n=6). Over a 4 week period the intraocular pressure (IOP) was measured in ONA and Co group. 14 days post immunization (p.i.) the CD3+ T-cell number was measured in the retina with a Cy flow FACS and the groups were compared to a sham immunized group (Naïve: n=6). At 28 days cryo-cross sections of the eyes were stained with Brn-3a in combination with CD3, TNF-α or Fasl. The serum TNF-α concentration was measured with an ELISA Kit. Groups were compared with student t-test.

**Results:** IOP remained constant in Co and ONA (p=0.4) group throughout the project. 28 days p.i. the retinal ganglion cell (RGC) density of the immunized group decreased compared with Co (Co: 5±3.3/section; ONA: 3.6±2.5/section, p=0.001). In comparison with the Naïve group the retinal CD3+ T-cell number increased significantly in the ONA group (p=0.04) after 14 days. 28 days p.i. no T-cells and hardly any TNF-α signal were detected in the retinae of Co and ONA. In the serum of both groups the TNF-α concentration is extremely low, with no differences between the groups. For this reason the concentration wasn’t detectable with the ELISA at the point of time 4 weeks p.i.. The FasL signal was quantifiable in all retina layers, but no significant differences between the groups were noted (Co: 15.6±9.2; ONA 9.7±4.6, p=0.4) at 4 weeks.

**Conclusions:** In the ONA group the RGC loss increases significantly without an elevation of the IOP after 28 days. While the ONA animals displayed a slight T-cell infiltration in the retina at 14 days, no T-cell activities were detectable at 28 days. The T-cell effect seems to be an early event, because the 28 days hardly any T-cell dependent immune activities were noted: no CD3+ signal in the retina, no sign for retinal and systemic TNF-α, and no changes in retinal FasL formation. The antigen ONA seems to have a time dependent influence to the immune mechanism of T-cells.

**Commercial Relationships:** Sandra Kuehn, None; Rozina Noristani, None; Mathias Kuehn, None; Burkhard H. Dick, None; Stephanie C. Joachim, None

**Support:** German research foundation, DFG JO-886/1-1, FoRUM Program (Ruhr University Bochum)

**Program Number:** 2026 Poster Board Number: D0165
**Presentation Time:** 11:00 AM - 12:45 PM

**Altered CD8+ T cell function in human non-infectious uveitis**


**NEI, NIH, Bethesda, MD; School of clinical sciences, University of Bristol, Bristol, United Kingdom.**

**Purpose:** Althought CD8+ T cells are primarily implicated in the clearance of viral infections, they have also recently been shown to be a key determinant of clinical prognosis in patients with non-ocular autoimmune diseases. This challenges the conventional paradigm of CD4+ T cell driven autoimmune, and raises new questions about the role of CD8+ cells in non-infectious inflammation. We therefore interrogated the phenotype and function of CD8+ cells in patients with sight threatening autoimmune uveitis.

**Methods:** Fresh whole blood from uveitis patients (n=25) and healthy controls (HCs, n=25) was analyzed using flow cytometry. Three subsets of CD8+ cells were distinguished based on surface expression of CD45RA, CD28 and CCR7, representing naïve (CD28+CCR7+CD45RA+), effector memory (CD28+CCR7-CD45RA-), and late differentiated (CD28-CCR7+CD45RA+) cells. The cytotoxic potential of these subsets was then assessed by CD107a expression, and functional killing was quantified by determining the percentage of CFSE+Propidium Iodide+ cells after 6 hours of CD8+ cell co-culture with a CFSE labeled target K562 cell line. Intracellular expression of the transcription factor T-bet , which is a master regulator of CD8+ effector function, was also assessed by flow cytometry.

**Results:** The proportions of all three CD8+ cell subsets were similar in both uveitis patients and HCs (CD28+CCR7+ ~25%, CD28+CCR7- ~33%, and CD28+CCR7+ ~25%). However, there was a significantly higher percentage of CD107a+ CD8+ cells in uveitis patients (p=0.03). CD8+ cells from uveitis patients also demonstrated a greater capacity to kill target cells (10% vs 3% in HCs) and, in addition, the percentage of cells expressing T-bet was significantly increased in effector memory CD8+ cells from uveitis patients (p<0.0001). A higher percentage of CD8+ cells from uveitis patients were TNF-α+ and IFN-γ+ (62% vs 43% in HCs) but a lower percentage of cells expressed IL-2 (13% vs 25% in HCs).

**Conclusions:** The increased expression of CD107a and T-bet in effector memory (CD28+CCR7-CD45RA+) CD8+ T cells from uveitis patients indicates a greater capacity for cytotoxicity and this is reflected in enhanced CD8+ T cell killing. This is the first report of altered CD8+ cell function in patients with sight-threatening non-infectious uveitis, and further investigations are now needed to determine whether this contributes to the pathogenesis of intraocular inflammation.

**Commercial Relationships:** Simia Hirani, None; Ping Chen, None; Shayma Jawad, None; Ian A. Thompson, None; Baoying Liu, None; Lai Wei, None; H Nida Sen, None; Richard W. Lee, Genentech (C); Robert B. Nussenblatt, None

**Support:** NEI Intramural Research Program

**Program Number:** 2027 Poster Board Number: D0166
**Presentation Time:** 11:00 AM - 12:45 PM

**The contribution of T cells to retinopathy of prematurity in mice**

**Dean Talia, Tong Zhu, Alex Agrotis, Robyn M. Slattery, Melanie Le Page, Fabienne Mackay-Fisson, Jennifer L. Wilkinson-Berka.**

**Monash University, Melbourne, VIC, Australia.**

**Purpose:** Inflammation is known to contribute to the vascular pathology which develops in retinopathy of prematurity (ROP). Previous studies have indicated a causative role for the adaptive immune system in ROP; however the mechanisms by which this occurs are unknown. We evaluated the contribution of T and B cells to experimental ROP using mice deficient in these cell populations.

**Methods:** RAG-1-/- mice (T and B cell deficient) and MuMT-/- mice (T cell sufficient, B cell deficient) were studied and comparisons made to C57BL/6 mice. ROP was induced by exposure to 75% oxygen from postnatal day (P) 7 to P12 and room air until P13 (acute ROP) or P18 (established ROP). Control mice were in room air from birth until P13 or P18. Neovascularization and the area of central avascular retina were quantitated in retinal wholemounts using ImageJ (NIH, USA). Flow cytometry was used to determine the number and percentage of T cells, B cells and dendritic cells in the spleen and pooled lymph nodes. The distribution and number of CD3+ T cells were evaluated in retinal wholemounts using immunofluorescence.

**Results:** Retinal neovascularization was reduced in RAG-1-/- ROP mice, but not in MuMT-/- ROP mice compared to C57BL/6 ROP controls, suggesting a predominant role for T cells in ROP. FACS analysis revealed an approximately 3.6-fold increase in
CD4+/CD25+ Foxp3+ Tregs in the spleens of C57BL/6 ROP mice at P13 compared to room air controls (p<0.0001). By P18 the number of CD4+/CD25+ Foxp3+ Tregs in spleen and lymph nodes had declined, whereas the number of CD8+ T cells increased approximately 6.5-fold compared to room air controls (P<0.0001). Immunolabelling for CD3+ T cells was increased in C57BL/6 ROP retina at P18 compared to room air controls and was located adjacent to the vasculature.

**Conclusions:** Particular populations of T cells may contribute to the development of ROP, suggesting the potential role of immune-based therapies for treatment of the disorder.

**Commercial Relationships:** Dean Talia, None; Tong Zhu, None; Alex Agrotis, None; Robyn M. Slattery, None; Melanie Le Page, None; Fabienne Mackay-Fisson, None; Jennifer L. Wilkinson-Berka, National Health and Medical Research Council of Australia (F), JDRF (F)

**Program Number:** 2028 Poster Board Number: D0167
**Presentation Time:** 11:00 AM - 12:45 PM
**Fas-dependent release of high-mobility group protein B1 (HMGB1) in the eye is critical for the development of experimental autoimmune uveitis (EAU) initiated by uveitogenic T cells**

**Guomin Jiang, Amir R. Hajrasouliha, Henry J. Kaplan, Hui Shao.**
Department of Ophthalmology, University of Louisville, Louisville, KY.

**Purpose:** We have previously reported that HMGB1, an important member of damage-associated molecular patterns (DAMPs), is an early and critical mediator in the eye in response to transferred uveitogenic T cells and that transfer of uveitogenic T cells into Fas-deficient (lpr) mice did not induce HMGB1 release by retinal tissue cells nor result in intraocular inflammation. In this study, we wanted to test if HMGB1 release in the eye is Fas-dependent.

**Methods:** Retinal explants from Fas-deficient (Faslpr) and wild-type (wt) C57BL/6 (B6) mice were cultured with a Fas receptor agonist (Jo2 Ab) or interphotoreceptor retinoid-binding protein (IRBP) 1-20 peptide-specific T cells, and then the level of HMGB1 in culture supernatants were detected by ELISA. In addition, uveitis was evaluated after IRBP-specific T cell transfer into both Faslpr mice intra-vitreous injected with recombinant HMGB1, and in wt B6 mice intra-vitreous injected with a Fas signaling antagonist (Met12).

**Results:** Compared to Faslpr retinal explants, high level of HMGB1 was detected in the culture supernatants of wt retinal explants cocultured with either IRBP-specific T cells or Jo2 Ab for 2 and 4 hours. The increased HMGB1 release was suppressed by Fas signaling pathway inhibitor Met12. Moreover, IRBP-specific T cells induced uveitis in Faslpr mice intravitreally injected with HMGB1, whereas, uveitis induced by IRBP-specific T cells in wt B6 mice was attenuated by Met12 treatment.

**Conclusions:** Except the fact that Fas could mediate apoptotic cell death, we have found that Fas signaling is also involved in HMGB1 secretion from intact retinal cells in response to uveitogenic T cells. Blockade of Fas signaling pathway reduced HMGB1 release, thus, suppressing the development of uveitis. Met12, a Fas antagonist, might be a potential alternative for the treatment of autoimmune uveitis in man.

**Commercial Relationships:** Guomin Jiang, None; Amir R. Hajrasouliha, None; Henry J. Kaplan, None; Hui Shao, None
**Support:** NEI EY12974 (HS), Research to Prevent Blindness Lew R Wasserman Merit Award (HS), the Commonwealth of Kentucky Research Challenge Trust Fund (HK), Grant from University of Louisville School of Medicine (GJ), Research Initiation Grant from University of Louisville (GJ).

**Program Number:** 2029 Poster Board Number: D0168
**Presentation Time:** 11:00 AM - 12:45 PM
**Regulatory T cell levels and cytokine production in active non-infectious uveitis: in vitro effect of anti-TNF-α, dexamethasone, and cyclosporine**

**Blanca Molins1, Víctor Llorens1, Marina Mesquida1, Laura Pellegrin1, Alfredo Adan Civera1.**
Ophthalmology, Hospital Clinic Barcelona, Barcelona, Spain; 2Ophthalmology, Fundació Clinic Recerca Biomedica, IDIBAPS, Barcelona, Spain.

**Purpose:** To evaluate circulating regulatory T-cell (Treg) levels and cytokine production by peripheral blood mononuclear cells (PBMC) in patients with active non-infectious uveitis and to evaluate the effect of in vitro treatment with infliximab, dexamethasone, and cyclosporine on Treg levels and cytokine production in PBMC from uveitis and healthy subjects.

**Methods:** We included 12 patients with active non-infectious uveitis from Hospital Clinic of Barcelona (Spain) and 11 age-matched healthy subjects. PBMC were obtained by Ficoll gradient and cultured for 24 h in the presence or absence of dexamethasone (0.1-1x10-6M), cyclosporine (100-200 ng/ml), and infliximab (5-20 μg/ml). Treg (CD3+CD4+Foxp3+CD25highCD127low) levels in PBMC were determined by flow cytometry using the following fluorochrome-labeled monoclonal antibodies: AF700-CD3, Fitc-CD4, APC-CD25, PeCy7-CD127, and PE-Foxp3 (eBiosciences). Supernatants of cultured PBMC were collected and IL-10 and TNF-α levels were measured by ELISA (R&D Duoset).

**Results:** No significant differences were observed in Treg levels between uveitis patients and control subjects. However, PBMC from uveitis patients produced significantly higher amounts of TNF-α (20.3 ±8.9 pg/mL vs. 6.9 ± 3.4 pg/ml, P<0.05) and lower amounts of IL-10 (12.7± 3.9 pg/mL vs. 20.0±6.0 pg/mL, P<0.05). In vitro treatment with infliximab and cyclosporine did not modulate the levels of CD3+CD4+Foxp3+, CD3+CD4+CD25high, CD3+CD4+Foxp3+CD25high, and CD3+CD4+Foxp3CD25highCD127low in both groups. However, dexamethasone treatment significantly reduced CD25high in CD3+CD4+ cells from uveitis patients, decreasing Treg levels. Furthermore, dexamethasone also increased in a dose-dependent manner CD127 expression in CD3+CD4+ cells in both uveitis and control subjects, further reducing Treg levels. On the other hand, dexamethasone and infliximab treatment significantly reduced TNF-α production in PBMC from both groups without affecting IL-10 production.

**Conclusions:** Our results suggest that TNF-α and IL-10 production by PBMC rather than altered Treg levels are associated with active uveitis and that in vitro treatment with infliximab and dexamethasone lower the inflammatory response without increasing Treg levels.

**Commercial Relationships:** Blanca Molins, None; Víctor Llorens, None; Marina Mesquida, None; Laura Pellegrin, None; Alfredo Adan Civera, None

**Program Number:** 2030 Poster Board Number: D0169
**Presentation Time:** 11:00 AM - 12:45 PM
**Local Regulatory T Cells of the Retina Protect Against Spontaneous and Induced Autoimmune Disease**

**Scott W. McPherson, Neal D. Heuss, Mark J. Pierson, Dale S. Gregerson.**
Department of Ophthalmology and Visual Neurosciences, University of Minnesota, Minneapolis, MN.

**Purpose:** Previously we showed that local depletion of regulatory T cell (Tregs) from the retina enhanced autoimmune disease against beta-galactosidase (bgal) expressing photoreceptor cells induced by activated, bgal-specific T cells (McPherson, et. al., ARVO 2012 and manuscript submitted). Here we examine whether retinal Tregs 1)

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limit interphotoreceptor retinoid-binding protein (IRBP) induced autoimmunity and 2) protect against spontaneous retinal autoimmunity.

**Methods:** FDG mice, which express green fluorescent protein (GFP) and diphtheria toxin receptor (DTR) under control of the FoxP3 promoter, were used to track and/or deplete Tregs. These mice were also crossed with T cell receptor transgenic mice (BG2) specific for bgal and/or mice expressing bgal in photoreceptor cells (bgal mice). IRBP-mediated autoimmune disease was induced in FDG mice by immunization with IRBP peptides. Local (retinal) depletion of Tregs was done with anterior chamber (AC) injections of diphtheria toxin (DTx). Systemic depletion of Treg was done with i.p. injections of DTx. Tregs were analyzed by FACs on blood or perfused retinas. Retinal autoimmune disease was analyzed by histology.

**Results:** IRBP immunized FDG mice were given regular AC injections of DTx in the right eye, (3x per week) and occasional injections in the left eye (day 5, day 8, or day 5 and 8). Examination of eyes 21 days post immunization showed elevated incidence and severity of autoimmune disease in the right eyes compared to either the left eyes and eyes from immunized only mice, which were similar. Spontaneous retinal autoimmune disease developed more often in otherwise naive and healthy FDG-BG2-bgal mice given regular AC DTx injections compared to similarly treated naive FDG, FDG-BG2, or FDG-bgal mice. Conversely, when given systemic DTx, none of the mice develop retinal autoimmune despite having systemic autoimmune symptoms.

**Conclusions:** Tregs are an important component of retinal immune privilege. We found that only that sustained local depletion of Tregs enhanced retinal autoimmunity whether induced or spontaneous. This suggests that Tregs within the retina act locally to maintain retinal immune homeostasis.

**Commercial Relationships:** Scott W. McPherson, None; Neal D. Heuss, None; Mark J. Pierson, None; Dale S. Gregerson, None

**Support:** NIH Grants RO1-EY021996, RO1-EY016376, P30-EY011374; Research to Prevent Blindness; Minnesota Lions Club

**Program Number:** 2031 Poster Board Number: D0170

**Presentation Time:** 11:00 AM - 12:45 PM

**Investigating the response of naive T-cells to the three isoforms of TGFβ**

Robert J. Barry1,2, David Withers3, Philip I. Murray1,2, Peter J. Lane1, John Curnow1,2, 1Academic Unit of Ophthalmology, University of Birmingham, Birmingham, United Kingdom; 2Centre for Translational Inflammation Research, University of Birmingham, Birmingham, United Kingdom; 3Institute for Biomedical Research, University of Birmingham, Birmingham, United Kingdom.

**Purpose:** In both human and experimental animal models of disease, uveitis is thought to be mediated by CD4+ T-cells that on entering the eye fall under the control of TGFβ. Of the three TGFβ isoforms, TGFβ2 is found at high levels in the aqueous humour of uninfamed eyes, whereas TGFβ1 predominates in episodes of inflammation. The presence of TGFβ3 has not been demonstrated in uveitic eyes. Given the apparent importance of differential TGFβ signalling in the ocular microenvironment we set out to investigate the response of purified naive CD4+ T-cells to the three mammalian isoforms of TGFβ at a range of physiological dilutions.

**Methods:** Cells were extracted from the lymph nodes of C57Bl/6 mice and purified to naive CD4+ T-cells (CD4+CD25-CD62L-CD44hi) using flow cytometry. CD25+ cells were removed to exclude all regulatory T-cells (<1% Fox-P3+) and the CD62L-CD44hi population selected to exclude central memory cells. Cells were labelled with a proliferation dye (eFluor450) and cultured in 96-well plates (200,000 cells in 200ul) in serum-free media with IL-2, stimulated with plate-bound anti-CD3 and soluble anti-CD28. TGFβ1, 2 or 3 was added at 0.05 - 5.0 ng/ml. Cells were harvested following 4 days of culture with proliferating and apoptotic cells identified from forward/side-scatter profile on flow cytometric analysis, and cell count and cycles of proliferations calculated.

**Results:** Increasing concentrations of all three TGFβ isoforms resulted in increased cell proliferation and decreased apoptosis at the dilutions tested. At the highest concentrations of TGFβ (5.0ng/ml) cells underwent >4 cycles of proliferation. No statistically significant difference between TGFβ isoforms was observed (average % cells undergoing proliferation 2.52 at 0.05ng/ml, 14.22 at 0.5ng/ml, 45.38 at 5.0ng/ml and apoptosis 55.45, 49.95, 20.11 respectively).

**Conclusions:** Our data illustrates that all isoforms of TGFβ are equal in their ability to induce proliferation and inhibit apoptosis of a highly purified population of naïve T-cells, possibly through inhibition of activation-induced cell death. As TGFβ also determines immune activation through its ability to generate both immunosuppressive Treg and pathogenic Th17 cells further work is underway to investigate the effect of different isoforms of TGFβ on Treg / Th17 differentiation.

**Commercial Relationships:** Robert J. Barry, None; David Withers, None; Philip I. Murray, None; Peter J. Lane, None; John Curnow, None

**Support:** Fight for Sight Clinical Fellowship

**Program Number:** 2032 Poster Board Number: D0171

**Presentation Time:** 11:00 AM - 12:45 PM

**V-domain Ig Suppressor of T Cell Activation (VISTA) is Necessary for Corneal Allograft Survival**

Tomoyuki Kunishige1, Hiroko Taniguchi1, Tatsukuni Ohno2, Miyuki Azuma1, Junko Hori1. 1Nippon Medical School, Bunkyo, Japan; 2Tokyo Medical and Dental University, Bunkyo, Japan.

**Purpose:** A novel and structurally distinct Ig superfamily inhibitory ligand, whose extracellular domain bears homology to the B7 family ligand PD-L1, was identified. This molecule is designated V-domain Ig suppressor of T cell activation (VISTA). VISTA is primarily expressed on hematopoietic cells, and VISTA expression is highly regulated on myeloid antigen-presenting cells (APCs) and T cells. The expression and function of VISTA in the eye remain largely unknown. The purpose of the present study was to determine the role of VISTA in immune privilege of corneal allografts.

**Methods:** Normal corneas of C57BL/6 were transplanted orthotopically into normal eyes of BALB/c mice. Recipients were administrated intraperitoneally with 0.2 mg of anti-VISTA monoclonal antibodies (mAb) or control rat IgG, three times a week for 8 weeks after grafting. Graft survival was assessed clinically and was compared. Expression of VISTA in allografts was assessed immunohistochemically by confocal microscopy.

**Results:** Survival of allografts treated with anti-VISTA mAb was less than that of the control. Control allografts were infiltrated by small number of CD4+cells which strongly expressed VISTA. On the other hand, blockade of VISTA by mAb led to infiltration of a lot of CD4+ T cells which did not express VISTA, and resulted in allograft rejection.

**Conclusions:** VISTA plays important role in acceptance of corneal allografts. It is suggested that VISTA contribute to Immune privilege of the corneal allografts.

**Commercial Relationships:** Tomoyuki Kunishige, None; Hiroko Taniguchi, None; Tatsukuni Ohno, None; Miyuki Azuma, None; Junko Hori, None

**Support:** Grants-in-Aid for Scientific Research(c) from Japan Society for the Promotion of Science

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Leukocyte Infiltrate in Rabbit Tears Post Intravenous Muramyl Dipeptide

Marilyn P. Langford, Tyler W. Plauche, Claude Y. Bundrick, Thomas B. Redens. Ophthalmology, Louisiana State Univ Hlth Sci Ctr, Shreveport, LA.

Purpose: To characterize the ocular immune response to intravenous muramyl dipeptide (MDP) adjuvant.

Methods: Adult rabbits were injected intravenously with MDP (1 mg/kg). Ocular exams, tear fluid collection and Schirmer’s tear test were performed through 48 h. Tear production, total tear protein, western blots for IgA and IgG, immunofluorescence antibody (IFA) detection of gamma-glutamyl transpeptidase (GGT; cellular marker for PMN), calreticulin (CRT, MDP binding protein) and nod2 (intracellular ligand for MDP), GGT activity and tear cytology (H&E staining) were determined.

Results: Clinical signs of uveitis and mild conjunctivitis were detected, but tear production, total tear protein and IgA/G levels were only slightly increased. The number of leukocytes in tear fluid and levels of GGT activity on Schirmer’s tear test strips were significantly higher (Ps<0.01) than pre-treatment control levels at 12-48h post intravenous MDP. Serum GGT levels peaked at 24 h. Immunofluorescent staining of cellular infiltrate in tears revealed numerous leukocytes, predominantly heterophils (PMN) positive for GGT, calreticulin (CRT) and nod2.

Conclusions: The results suggest intravenous MDP induces inflammatory changes in the conjunctiva tissues and that the increase in GGT activity in the tear fluid at 16-48 h post intravenous MDP is likely due to nod2, CRT, and GGT-positive heterophils.

Commercial Relationships: Marilyn P. Langford. None; Tyler W. Plauche. None; Claude Y. Bundrick. None; Thomas B. Redens. None

Program Number: 2033 Poster Board Number: D0172
Presentation Time: 11:00 AM - 12:45 PM

Dendritic cells from uveitis patients have a mature phenotype and a reduced capacity to take up antigen

Ping Chen1, Baoying Liu1, Richard W. Lee2, H Nida Sen1, Zhiyu Li1, Shayma Jawad1, Sima Hirani1, Lai Wei1, Robert B. Nussenblatt1

1Lab of Immunology, National Institute of Health, National Eye Institute, Rockville, MD; 2Department of Clinical Sciences, University of Bristol, Bristol, United Kingdom.

Purpose: In experimental animal models of uveitis, specialized subsets of dendritic cells (DCs) have been shown to either induce or suppress inflammation in the eye. However, little is known about the phenotype and function of these DC subsets in the context of clinical uveitis in man. The purpose of this study was to characterize DCs in the peripheral blood of patients with uveitis, and also to assess their functional capacity to present antigen, secrete cytokines and induce T helper cell activation and proliferation.

Methods: Fresh peripheral blood from uveitis patients (n=92) and healthy controls (HCs, n=112) was analysed by flow cytometry. HLA-DR+ myeloid DC1 (mDC1), mDC2 and plasmacytoid DCs (pDC) were then identified by their BDCA-1, BDCA-3 and BDCA-2 expression. Monocyte-derived DCs (MoDCs) were also generated from uveitis patients and HCs by culturing isolated CD14+ cells for 6 days with GM-CSF and interleukin (IL)-4. The capacity of DCs to process antigen and respond to lipopolysaccharide (LPS) was then quantified by measuring FITC-labeled albumin uptake, intracellular cytokine expression and co-cultured CD4+ T cell activation.

Results: The mDC1 pool is expanded in the peripheral blood of uveitis patients both as a percentage of all DCs (p=0.007) and in terms of absolute numbers of cells (p=0.012), whereas the absolute number of mDC2 and pDCs was unchanged compared with HCs. This expansion was independent of the diagnostic category of uveitis (either by disease description or anatomical classification) and treatment. However, the intensity of HLA-DR expression was increased in mDC1 cells from patients with uveitis (p=0.02), and this increase was most pronounced in patients in disease remission (p=0.01). Myeloid DC1 cells from uveitis patients also had a greater capacity to upregulate HLA-DR in response to LPS (p=0.005), but their upregulation of IL-6, IL-10 and IL-12p40/70 expression was reduced compared with HCs. Antigen uptake was also decreased in mDC1 cells from uveitis patients (p=0.01). Albumin/low) MoDCs from HCs induced greater CD4+ cell activation in T-cell co-cultures.

Conclusions: DCs in the peripheral blood of uveitis patients are skewed to a mature, HLA-DR(high) mDC1 phenotype, with a reduced capacity for antigen uptake, and exhibit a blunted upregulation of IL-6, IL-10 and IL-12p40/70 following LPS stimulation.

Commercial Relationships: Ping Chen. None; Baoying Liu. None; Richard W. Lee. None; H Nida Sen. None; Zhiyu Li. None; Shayma Jawad. None; Sima Hirani. None; Lai Wei. None; Robert B. Nussenblatt. None
Support: intramural research program of NEI

Program Number: 2035 Poster Board Number: D0174
Presentation Time: 11:00 AM - 12:45 PM

Identifying and classifying nonspecific orbital inflammation (NSOI) by gene expression array

James T. Rosenbaum1,2, Dongseok Choi1, Christina A. Harrington, Gerald J. Harris2, Craig N. Czyz2, Valerie A. White3, Eric A. Steele3, Bobby S. Korn3, David J. Wilson3, Stephen R. Planck1. 1Casey Eye Institute, Oregon Health & Science University, Portland, OR; 2Devers Eye Institute, Legacy Research Institute, Portland, OR; 3Public Health and Preventive Medicine, Oregon Health & Science University, Portland, OR; 4Integrated Genomics, Oregon Health & Science University, Portland, OR; 5Department of Ophthalmology, Medical College of Wisconsin, Milwaukee, WI; 6Department of Ophthalmology, Ohio University, Columbus, OH; 7Department of Pathology, University of British Columbia, Vancouver, BC, Canada; 8Department of Ophthalmology, University of California, San Diego, CA.

Purpose: NSOI is presumed to be a heterogeneous collection of diseases that are difficult to classify by conventional histology and sometimes difficult to distinguish from other causes of orbital inflammation.

Methods: We analyzed mRNA from 38 formalin-fixed anterior orbital biopsies taken from 14 patients with NSOI, 12 patients with thyroid eye disease (TED), 2 patients with granulomatosis with polyangiitis, and 4 patients with sarcoidosis as well as 6 biopsies from healthy controls. Gene expression was analyzed with Affymetrix Gene Chip U133 plus 2.0 arrays that detect approximately 47,400 transcripts.

Results: Principal coordinate analysis indicated that NSOI demonstrated disease heterogeneity which could be readily distinguished from controls or tissues from patients with other orbital diseases. 230 genes were expressed at least two-fold greater than in control tissue using a false discovery rate (FDR)<0.05 and 1930 genes were reduced by at least two fold relative to controls with an FDR <0.05. All of the 25 probe sets with signals most enhanced in tissue from patients with NSOI were immunoglobulin related. Other prominently upregulated genes included CXCR4, YKL-40, SLAM Family 7, CXCL9, and IL-7 receptor. Prominently down regulated genes included alcohol dehydrogenase 1B, perlipin 1, adiponectin,
leptin receptor, and C1Q. Many transcripts were up or down regulated by ten-fold or more compared to normal tissue. Transcripts detected by 6839 probe sets were differentially regulated in NSOI (FDR <0.05; >1.5-fold change) compared to either TED or sarcoid tissues.

Conclusions: We believe that this is the first study to analyze gene expression from patients with NSOI and the first study to compare gene expression among orbital diseases. Transcripts related to inflammation often distinguished NSOI from normal tissue. Our results support the hypothesis that gene expression array will become an essential tool in the classification and understanding of NSOI.

Commercial Relationships: James T. Rosenbaum, Genentech (C), Abbott (F), Xoma (C), Eyegate (F), Bristol Myers (F), Lux (C), Novartis (C), Regeneron (C), Teva (C), Therakine (F), Mitotech (F), Aquinox (F), Allergan (C), Santen (C); Dongseok Choi, None; Christina A. Harrington, None; Gerald J. Harris, None; Craig N. Czyz, None; Valerie A. White, None; Eric A. Steele, None; Bobby S. Korn, None; David J. Wilson, None; Stephen R. Planck, None

Support: NIH Grant EY020249

Program Number: 2036 Poster Board Number: D0175
Presentation Time: 11:00 AM - 12:45 PM

Association of Thrombospondin-1 Polymorphism with Predisposition to Chronic Dry Eye
Laura Contreras-Ruiz1, Bruce Turpie1, Denise S. Ryan2, Rose K. Sia2, Kraig S. Bower1, Darlene A. Darr1, Sharmila Masli1, 1Department of Ophthalmology, Boston University School of Medicine, Boston, MA; 2U.S. Army Warfighter Refractive Surgery Research Center, Fort Belvoir, VA; 3The Wilmer Eye Institute, The Johns Hopkins School of Medicine, Baltimore, MD; 4Scheppens Eye Research Inst and Massachusetts Eye and Ear, Department of Ophthalmology Harvard Medical School, Boston, MA.

Purpose: Thrombospondin-1 (TSP-1) is a matricellular protein with immunomodulatory properties. Our previous work showed that TSP-1 deficient mice develop ocular surface inflammation. The purpose was to determine if polymorphism in TSP-1 gene (THBS-1) correlates with the development of the chronic ocular surface inflammatory conditions of dry eye that develops after refractive surgery.

Methods: Genomic DNA and RNA were obtained from conjunctival impression cytology samples of 75 patients before and 3 months after refractive surgery. The samples were assigned to 2 groups: patients with post surgery dry eye (n=59) or healthy controls (n=16). Five TSP-1 single nucleotide polymorphisms (SNPs) were assessed using Sequenom iPLEX Gold platform: SNP1 (rs2228262) A>G, SNP2 (rs2228261) C>T, SNP3 (rs229305) A>G, SNP4 (rs1478604) T>C and SNP5 (rs3743125) G>A. Their association with the development of dry eye was analyzed with gPLINK software and Fisher’s exact test. In addition, expression of TSP-1, and IL-1β (inflammatory marker associated with dry eye) were quantified by RT-PCR on both dry eye and control samples.

Results: Frequencies of TT or CT genotypes (T allele carriers) of SNP2, as well as the GG or GA genotypes (G allele carriers) of SNP3, were 39% in dry eye patients compared to 25% in controls (p = 0.048, odds ratio [OR] 1.9, 95% confidence interval (95% CI) 1.1 - 3.5). The G allele of SNP1 was detected in 19% of subjects in both groups (p = 1.0, OR 1.0, 95% CI 0.5 - 2.0). The frequency of SNP4 C allele carriers in dry eye patients was 63% as against 44% in control group (p = 0.003, OR 2.5, 95% CI 1.4 - 4.5). The SNP5 was excluded from analysis due to departure from Hardy-Weinberg equilibrium. Allelic association analysis indicated significant association between SNP4 and development of dry eye (p=0.03) while remaining SNPs did not show any significant allelic associations. Expression of TSP-1 in SNP4 C allele carriers (CC+CT) compared to normal allele (TT) carriers was significantly reduced, while expression of inflammatory cytokine IL-1β was significantly increased.

Conclusions: Our results indicate a significant association between polymorphism in THBS-1 gene and ocular surface inflammation as seen in dry eye. This study provides the very first evidence of a genetic association in dry eye disease and suggests THBS1 as a susceptibility factor of this ocular condition.

Commercial Relationships: Laura Contreras-Ruiz, None; Bruce Turpie, None; Denise S. Ryan, None; Rose K. Sia, None; Kraig S. Bower, None; Darlene A. Darrt, None; Sharmila Masli, None

Support: DoD grant W81XWH-10-1-0392

Program Number: 2037 Poster Board Number: D0176
Presentation Time: 11:00 AM - 12:45 PM

Expression and Function of the NLRC4/NAIP5 Inflammasome in the Ocular Surface: Ramifications of Eicosanoid Storms
Karsten Gronert, Kyle M. Hu, David W. Lin, Yuning Wang, Samantha B. Wang, Vision Science, School of Optometry, University of California, Berkeley, Berkeley, CA.

Purpose: Inflammasomes have reemerged as key players in the innate immune system. The NLRC4/NAIP5 inflammasome complex acts as an intracellular sensor that detects flagellin and mounts an inflammatory response that includes pyroptosis, activation of caspase 1, cleavage of IL-1 β and IL-18. We recently discovered a novel early and critical effector function of inflammasome activation, namely the rapid formation of an eicosanoid storm by resident macrophages. In the eye, inflammasomes have been implicated in the progression of age-related macular degeneration; however its role in the ocular surface still remains to be explored. Hence, we investigated the effects of NLRC4/NAIP5 activation in the ocular surface during an acute inflammatory/reparative response.

Methods: MyD88/Trif KO, NLRC4/NAIP5 KO and C57BJ/6 wild type mice were used to directly assess the role the inflammasome. A fusion protein (FlaTox) consisting of flagellin attached to the pore forming, amino-terminal domain of the B. anthracis lethal factor was topically applied to the eye following full corneal epithelial abrasion. The control group received an inactive mutant construct (AAA). Wound healing was measured with ImagePro Express and inflammation assessed by leukocyte infiltration by enzyme assays, FACS and immunohistochemistry. Eicosanoid formation was quantified using LC/MS/MS-based lipidomics and gene expression by QPCR.

Results: NLRC4 and NAIP5 are expressed and functional in the ocular surface. 4 days after injury, MyD88/Trif KO corneas that received FlaTox exhibited markedly decreased wound healing (1.8-fold greater epithelial defect) when directly compared to the control AAA group, consistent with pronounced corneal opacity, inflammatory neovascularization, and greater neutrophil infiltration. Targeted inflammasome activation triggered an early eicosanoid storm in the conjunctiva, which was abrogated in NLRC4/NAIP5 KO mice.

Conclusions: These findings provide the first evidence for a role of the NLRC4/NAIP5 inflammasome in ocular surface innate immune responses. NLRC4/NAIP5 activation lead to the rapid formation of an eicosanoid storm and triggered a far reaching and perilous immune response, which can have severe ramifications to ocular health. The molecular mechanisms and regulation of inflammasomes are of great interest to the health of the visual axis and remain to be elucidated.

Commercial Relationships: Karsten Gronert, None; Kyle M. Hu, None; David W. Lin, None; Yuning Wang, None; Samantha B. Wang, None

Support: NIH Grant EY022208

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Purpose: To evaluate the molecular and cellular mechanisms involved in the disruptive immune effect of benzalkonium chloride (BAK) on the ocular surface.

Methods: A previously validated in vivo murine model was used (Mucosal Immunol. 2012 Jun 13. doi: 10.1038/mi.2012.44). In brief, Balb/c mice were given ovalbumin (OVA) alone or in combination with 0.01% BAK (B+O) daily for 5 days in both eyes, with and without nuclear factor xB (NFkB) pathway inhibitors: pyrrolidine dithiocarbamate (PDTC) and sulfasalazine (SSZ). On day 7, mice were immunized intraperitoneally with OVA in alum to evaluate the systemic response on day 14 as OVA-induced splenocyte proliferation by thymidine incorporation. To model in vitro the ocular surface epithelium, Pam212 cells were exposed for 15 min to different BAK concentrations (0.00001%-0.01%), then cultured for 96 h with syngeneic splenocytes and finally cell viability and expression of major histocompatibility complex class II (MHC-II) as activation marker were assayed.

Results: In the in vivo model, OVA instillation induced conjunctival tolerance (43±9% of control systemic response, p<0.05), in contrast to B+O (90±8%), which did not affect the subsequent cellular response. Addition of NFkB inhibitors restored tolerance in B+O mice (PDTC 39±3%, SSZ 54±8%, p<0.05) but did not significantly affect it in OVA mice (PDTC 31±5%, SSZ 69±5%, p<0.05). In the epithelial cultures, exposure to BAK decreased cell viability after 96 h but did not modify the MHC-II+ fraction (5% control, <8% BAK). Both γ interferon and splenocyte coculture increased MHC-II expression (15% and 19%, respectively, p<0.05). In BAK-exposed Pam212 cells, coculture with splenocytes increased even further the MHC-II+ fraction (24%-46% with increasing BAK concentrations, p<0.05). Epithelial pretreatment with PDTC or SSZ did not affect MHC-II expression in any case.

Conclusions: In the in vivo model, BAK-induced conjunctival tolerance breakdown could be reverted by the instillation of two known inhibitors of NFkB activation. Regarding the increased MHC II expression observed in conjunctival biopsies from preservative-exposed patients, BAK indirectly induced a similar change in cultured epithelial cells that involved their interaction with lymphocytes. These results suggest that NFkB modulation could have a therapeutic role in ocular surface immunity.
Commercial Relationships: Stephen R. Planck, None; Dongseok Choi, None; Christina A. Harrington, None; Craig N. Czyz, None; Roger A. Dailey, None; Peter J. Dolman, None; Gerald J. Harris, None; Patrick Stauffer, None; David J. Wilson, None; James T. Rosenbaum, Genentech (C), Abbott (F), Xoma (C), Eyegate (F), Bristol Myers (F), Lux (C), Novartis (C), Regeneron (C), Teva (C), TheraVance (C), Mitotech (F), Aquinox (F), Allergan (C), Santen (C)

Support: NIH Grant EY020249

Program Number: 2041 Poster Board Number: D0180
Presentation Time: 11:00 AM - 12:45 PM

An optical coherence tomography based *in vivo* scoring system for experimental autoimmune uveoretinitis


1. Department of Genetics, UCL Institute of Ophthalmology, London, United Kingdom; 2. NIHR Biomedical Research Center for Ophthalmology, Moorfields Eye Hospital, London, United Kingdom; 3. Unit of Ophthalmology, School of Clinical Sciences, University of Bristol, Bristol, United Kingdom.

**Purpose:** Despite advances in assessing immune responses affecting the retina, post-mortem histology remains the standard for quantifying disease severity in murine experimental autoimmune uveoretinitis (EAU). Therefore, progression or the effect of therapeutic intervention cannot be observed in real time. We wished to ascertain whether optical coherence tomography (OCT) could detect intraretinal changes during inflammation and determine its utility as a tool for accurate *in vivo* scoring of EAU.

**Methods:** Three independent cohorts of *C57BL/6j* mice were immunised with IRBP 1-20, CFA and pertussis toxin. Contemporaneous Spectralis-OCT scanning, fluorescein fundus angiography (FFA), topical endoscopic fundal imaging (TEFI) and CD45-immunolabelled histology were performed at 15, 26, 36 and 60 days post-induction. OCT features were characterised on corresponding retinal flat-mounts using immunohistochemistry for T-cells (CD4 & CD8), activated myeloid cells (lectin B4), microglia (Iba1) and vascular changes (collagen IV). Imaris software was used for 3D reconstructions. All eyes were scored by three masked assessors, using our newly developed OCT-based EAU scoring system as well as established TEFI and histology-based analyses.

**Results:** OCT identified optic disc swelling and vitreous opacities, which corresponded to CD45+ cell infiltration on histology. Vasculitis detected by FFA and OCT matched inflamed vessels surrounded by myeloid and T-cell infiltrates in retinal flat-mounts and could be differentiated from unaffected vessels. Evolution of these changes could also be followed over time in the same eye. Structural changes such as retinal folds were visible and encapsulated mixed populations of activated myeloid cells, T-cells and microglia as shown by 3D reconstruction. Using these features, an OCT-based EAU scoring system was developed, with significant correlation to histological (Pearson r=0.6392, P<0.0001, n=31 eyes) and TEFI-based scoring systems (r=0.6784, P<0.0001).

**Conclusions:** OCT distinguishes key features of murine EAU *in vivo*, permits dynamic assessment of intraretinal changes, accurate disease stage synchronisation prior to histological or cellular assessment and more efficient animal usage. By correlating OCT signals with other orbital diseases, Many of the up regulated transcripts detected by us have also been implicated by other investigators, thus supporting the validity of the approach. Our results indicate that TED is a distinct form of orbital inflammation. Transcripts related to inflammation are relatively under-represented in TED compared to other forms of orbital inflammation.

Commercial Relationships: Stephen R. Planck, None; Dongseok Choi, None; Christina A. Harrington, None; Craig N. Czyz, None; Roger A. Dailey, None; Peter J. Dolman, None; Gerald J. Harris, None; Patrick Stauffer, None; David J. Wilson, None; James T. Rosenbaum, Genentech (C), Abbott (F), Xoma (C), Eyegate (F), Bristol Myers (F), Lux (C), Novartis (C), Regeneron (C), Teva (C), TheraVance (C), Mitotech (F), Aquinox (F), Allergan (C), Santen (C)

Support: NIH Grant EY020249
immunohistochemistry in EAU, our findings may also inform the interpretation of OCT changes in human uveitis.

**Commercial Relationships:** Colin J. Chu, None; Philipp Herrmann, None; Livia S. Carvalho, None; Sidath E. Liyanage, None; James W. Bainbridge, Novartis (F), Alimera (C), Genesignal (C), Advanced Cell Technology (F), Targeted Genomics (P), Oxford Biomedica (C), GSK (F); Robin R. Ali, None; Andrew D. Dick, Novartis (C), Novartis (F), GSK (F), Abbott (F); Ulrich F. Luhmann, None

**Support:** Medical Research Council (MRC), UK. Fight for Sight, UK

**Program Number:** 2042 Poster Board Number: D0181
**Presentation Time:** 11:00 AM - 12:45 PM

**Age related aqueous cytokine and growth factor changes in cataract patients**

Jing Li, Yan Zheng, Yu Xu, Shin-Jye Lee, Yuqing Rao, Qi Zhang, Peiquan Zhuo. Ophthalmology, Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China.

**Purpose:** To examine changes of aqueous cytokines and growth factors in human subjects of different ages.

**Methods:** Aqueous humor was collected from cataract patients with no other ocular abnormalities, no systemic inflammatory diseases at the start of cataract surgery. Levels of 48 different proinflammatory mediators, including cytokines, chemokines and growth factors were measured using Bio-Plex Pro Human Cytokine panels and analyzed using Predictive Analytics SoftWare (PASW) Statistics 18. This study was performed in accordance to the tenets of the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Xinhua Hospital.

**Results:** We collected 157 aqueous samples, 34 from patients with congenital cataract patients, 123 from adult cataract patients. The median age was 0.67 years (range 0.2-6.5 yrs) for the former group and 67.5 years (range 22-108 yrs) for the latter group. Initial analysis of 34 congenital and 53 adult cataract patient aqueous samples revealed 29 factors which existed in over 90% of all samples tested. Mann-Whitney U test revealed significantly lower concentration of IL-7, IL-12p70, GROα, IFN-α2 and MCP-3 in the infantile group than in the adult cataract group. FGF-basic, IL-1Ra, TNF-α, IL-9 and SCGF-β was significantly higher in the infantile group than the adult cataract group. In adult cataract group, a positive correlation was found between age and axial length. Regression analysis showed significant positive correlation between age and aqueous concentration of IL-7 (p=0.00, beta=0.69), VEGF (p=0.023, beta=-0.392), IL-12 (p=0.022, beta=-0.393), IL-10 (p=0.052, beta=0.34), IFN-γ (p=0.013, beta=0.434), IFN-α2 (p=0.022, beta=0.485), MIP-1α (p=0.007, beta=0.45), SCF (p=0.012, beta=0.496), CXCL9 (p=0.006, beta=0.629).

**Conclusions:** This study revealed a basic profile of aqueous cytokines, chemokines and growth factors in cataract aqueous, providing a reference for further study using cataract aqueous as control. So far our results showed an increasing Th1 dominant proinflammatory activity and increasing proangiogenic activity with aging in the aqueous of adult cataract patients. The cytokine profile also suggested age-related activation of resident macrophages and structural cells of the intraocular tissue.

**Commercial Relationships:** Jing Li, None; Yan Zheng, None; Yu Xu, None; Shin-Jye Lee, None; Yuqing Rao, None; Qi Zhang, None; Peiquan Zhao, None

**Support:** Ministry of Science and Technology of China (the National Key Scientific Program 2012CB966901), National Natural Science Foundation of China (No.81070760)

**Program Number:** 2043 Poster Board Number: D0182
**Presentation Time:** 11:00 AM - 12:45 PM

**The Herpes Simplex Virus-1 Latency-Associated Transcript Promotes Functional Exhaustion of Virus-Specific CD8+ T Cells in Trigeminal Ganglia of Latently Infected “humanized” HLA-transgenic rabbits**


**Purpose:** HSV-1-specific CD8+ T cells that reside in latently infected trigeminal ganglia (TG), appear to control recurrent herpetic disease by aborting or reducing spontaneous viral reactivations. The HSV-1 Latency-Associated Transcript (LAT), the only viral gene that is abundantly transcribed during latency, increases reactivation. In latently infected C57BL/6 mice: 1) HSV-specific (HSV-gB498-505) CD8+ T cells are induced and selectively retained in TG; and 2)more of these CD8+ T-cells expressed markers of exhaustion with LAT(+) compared to LAT(-) virus. However, mice do not mimic spontaneous viral shedding or recurrent disease as occurs in human. Thus, here we examined CD8+ T-cell exhaustion in latently infected “humanized” rabbits.

**Methods:** We used Human Leukocyte Antigen- (HLA-) transgenic rabbits, which: (1) have spontaneous reactivation with clinical features relevant to human disease; and (2) mount “humanized” CD8+ T cell responses specific to HLA-restricted epitopes. HLA-transgenic rabbit were infected with LAT(+) or LAT(-) HSV-1 and the number and function of CD8+ T cells, specific to human gB epitopes was determined in TG at different times.

**Results:** HLA-transgenic rabbits latently infected with LAT(+) had more HLA-restricted CD8+ T cells than from HLA-transgenic rabbits latently infected with LAT(-) viruses. Thus, the previous findings in mice were not an artifact of species. Importantly, to our knowledge we show here for the first time that during LAT(+) virus latency most of the HSV-1 specific TG-resident CD8+ T-cells specific to human epitopes were phenotypically and functionally exhausted, as judged by high expression of PD-1, low proliferation and decreased IFN-γ production. This resulted in LAT(-) TG having more functional HLA-restricted, HSV-gB441-449- and gB561-569-specific CD8+ T cells, compared to LAT(+) TG.

**Conclusions:** These findings in “humanized” HLA-transgenic rabbits (1) confirm that the HSV-1 LAT promotes functional exhaustion (i.e., dysfunction) of HSV-specific CD8+ T cells in latently infected TG. This appears to be a LAT dependent immune evasion mechanism that results in increased reactivation; and (2) Suggest that TG are an immunological battleground where herpes-specific CD8+ T cells are continuously stimulated.

**Commercial Relationships:** Lbachir BenMohamed, None; Xavier Dervillez, None; Huma Qureshi, None; Aziz A. Chentoufi, None; Chelsea Nguyen, None; Oscar R. Diaz, None; Anthony B. Nesburn, None; Steven L. Wechsler, None

**Support:** This work is Supported by Public Health Service research grants EY019896, EY14900, EY14017 from the NIH, by the Discovery Eye Foundation and an unrestricted grant from Research to Prevent Blindness. L.B.M. is an RPB Special Award Investigator

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**Purpose:** The importance of the toll-like receptor 4 (TLR4) in endotoxin-induced uveitis (EIU) from administration of lipopolysaccharide (LPS) is established, but the role of endogenous TLR4 ligands in the activation of this system is not well-characterized. We focus on one endogenous TLR4 ligand, S100A8/A9, and study its potential contribution to the pathogenesis of the EIU mouse model.

**Methods:** We injected S100A8/A9 (7.8 μg) or saline intravitreally in BALB/c mice (n=6 mice/treatment/timepoint) and monitored ocular inflammation with intravitreal microscopy at 3 and 6 hours post-injection. Iris tissue explants were stimulated with LPS or increasing concentrations of S100A8/A9, and the IL-6 response was measured by ELISA at 24 hours post-stimulation. We administered intravitreal LPS (250 ng) to BALB/c mice or to S100A9 knockout mice and corresponding C57BL/6 controls (n = 4 mice/genotype/timepoint) and measured inflammation by histology at 6 and 24 hours post-injection. S100A8/A9 levels in whole eye explants from BALB/c mice injected intravitreally with LPS or saline (n = 8 mice/treatment/timepoint) were also measured by ELISA.

**Results:** Intravitreal microscopy showed that intravitreal injection of S100A8/A9 resulted in an increase of rolling and adhering cells in the iris vasculature at both 3 and 6 hours post-injection as well as an increase in cells infiltrating surrounding iris tissue at 6 hours compared to saline-injected controls. ELISA of whole eye explants at 2, 6, and 24 hours after intravitreal LPS showed an increase in S100A8/A9 levels in LPS-injected eyes over time. Stimulation of iris tissue explants with S100A8/A9 resulted in an increase in IL-6 production in a dose-dependent manner, although the highest concentration of S100A8/A9 induced less IL-6 production than a relatively low dose of LPS. Unexpectedly, S100A9 knockout mice showed increased levels of infiltrating cells in both posterior and anterior segments after LPS stimulation compared to wild type C57BL/6 mice.

**Conclusions:** Our data show that S100A8/A9 can induce or enhance ocular inflammation in mice, but paradoxically suggest that its absence, analogously to the absence of TLR2 or NOD2 in some models, can also lead to increased inflammation. The ability of endogenous TLR ligands to induce eye inflammation broadens the importance of the innate immune system in the eye.

**Commercial Relationships:** Christina Metea, None; Joe E. Ensign-Lewis, None; Alec Amram, None; Holly L. Rosenzweig, None; Thomas Vogl, None; Johannes Roth, None; Stephen R. Planck, None; James T. Rosenbaum, Genentech (C), Abbott (F), Xoma (C), Eyeprint (F), Bristol Myers (F), Lux (C), Novartis (C), Regeneron (C), Teva (C), Therakine (F), Mitotech (F), Aquinox (F), Allergan (C), Santen (C)

**Support:** NIH Grant EY010572

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**Program Number:** 2046 Poster Board Number: D0185
**Presentation Time:** 11:00 AM - 12:45 PM

**Chemokine C-C motif ligand 7 as a Costimulatory Signal on Mast Cell Activation and Motility**

**Molecules that are necessary for ocular hypersensitivity reactions include the chemokine receptors CCR1 and CCR3; CC chemokine ligand (CCL) 7 is a genetic target and ligand for these receptors. The goal of this study is to explore the role of CCL7 in mast cell activity and motility response to costimulation via FceRI and CCR1 engagements.**
Purpose: To assess the efficacy of immunomodulatory therapy in to reproduce any abstract, contact the ARVO Office at arvo@arvo.org.

Results: CCL7 was upregulated in conjunctiva tissue in OVA-induced anaphylaxis compared to controls. The presence of recombinant human CCL7, combined with IgE and antigen treatment, synergistically enhanced degranulation when compared to cells treated with only IgE and its respective antigen in RBL-CCR1 and BMMC. RBL-CCR1 cells stimulated with CCL7 showed chemotactic increase in a dose dependent manner and dramatic membrane ruffling. In contrast, RBL-CCR1 cells costimulated with antigen and CCL7 exhibited less membrane ruffling and inhibited chemotaxis when compared with CCL7-stimulated cells.

Conclusions: Our results demonstrate that the chemokine CCL7 is upregulated in OVA-induced ocular anaphylaxis in vivo and can enhance mast cell chemotaxis in vitro. The cross-talk between FceRI-mediated and CCR-mediated signaling pathway induces activation and arrested chemotaxis of mast cells, thus contributing to allergic inflammation. Combined treatment with antigen and CCL7 decreases membrane ruffle formation in RBL-CCR1 cells, suggesting that the decreased ruffling response involves the inhibition of chemotaxis due to the costimulation. A better understanding of the roles played by CCL7 will provide insights into mast cell function and ideas for novel treatments for allergic ocular diseases.

Commercial Relationships: Chuan-Hui Kuo, None; Andrea Collins, None; Masaharu Ohbayashi, None; Santa Ono, None

Support: R01 EY019630-01

Program Number: 2047 Poster Board Number: D0186

Presentation Time: 11:00 AM - 12:45 PM

Immunomodulatory therapy and multiple sclerosis-associated uveitis

Gueorgui T. Markov1,2, Kittikamon Vongpaisarnsin3, C. Stephen Foster1,3, Ophthalmology, University Eye Hospital "Professor Pashev", Sofia, Bulgaria; 1Massachusetts Eye Research and Surgery Institution, Cambridge, MA; 2Harvard Medical School, Boston, MA.

Purpose: To assess the efficacy of immunomodulatory therapy in achieving remission and long-term control of inflammation in patients with multiple sclerosis-associated uveitis.

Methods: A retrospective case series study on the clinical records of 10 consecutive patients with uveitis and multiple sclerosis, treated at the Massachusetts Eye Research and Surgery Institution. Period of study was from July 2005 to November 2012. Evaluation of effectiveness was based on findings from the clinical exam and specialized imaging tests.

Results: All 10 patients (100%) were female, white. Mean age of presentation at our institution was 49.3 years. Mean follow-up was 70.4 months. Intermediate uveitis was diagnosed in 6 cases (60%), panuveitis - in 3 (30%), posterior - in 1 (10%). Bilateral involvement was present in all (100%) patients. Active uveitis was observed in 6 (60%) and quiescent in 4 (40%), initially. Immunomodulatory medications as monotherapy included azathioprine in 1 patient (10%), methotrexate - in 5 (50%), mycophenolate mofetil - in 4 (40%), cyclosporin - in 1 (10%), Daclizumab - in 2 (20%), and cyclophosphamide - in 2 (20%). Combined therapy was used with mycophenolate mofetil and cyclosporin in 4 cases (40%), cyclosporin and azathioprine - in 2 (20%), and methotrexate and cyclosporin - in 1 (10%). Corticosteroids, by various routes, were utilized in all patients (100%). 6 patients (60%) were corticosteroid-dependent. 7 patients (70%) had systemic therapy for multiple sclerosis with Glatiramer acetate in 3 (30%) of them, interferon beta-1a - in 3 (30%), and interferon beta-1b - in 1 (10%). At the end of follow-up, 1 patient (10%) was in remission for 19 months following azathioprine therapy, 2 (20%) - quiescent with no immunomodulatory therapy or corticosteroids for 6 and 12 months, with no previous stable remission, 1 (10%) - stable on mycophenolate mofetil and cyclosporin for 21 months, 2 (20%) - maintained on immunomodulatory therapy and corticosteroids for 8 and 37 months, 5 eyes of 3 patients - quiescent after fluocinolone acetone intravitreal implant for as long as 60 months, 3 eyes of 2 patients had signs of active disease.

Conclusions: Uveitis, associated with multiple sclerosis, is characterized by a protracted and complicated course. Remission is possible with the use of immunomodulatory agents. In addition, the intravitreal fluocinolone implant could present a reasonable alternative in recalcitrant cases.

Commercial Relationships: Gueorgui T. Markov, None; Kittikamon Vongpaisarnsin, None; C. Stephen Foster, Abbott Medical Optics (C), Abbott Medical Optics (F), Alcon Laboratories, Inc. (C), Alcon Laboratories, Inc. (F), Allergan, Inc. (C), Allergan, Inc. (F), Eyegate Pharmaceuticals, Inc. (I), Eyegate Pharmaceuticals, Inc. (F), IOP Opthalmics (C), Ista Pharmaceuticals (C), Lux Biosciences, Inc. (C), Lux Biosciences, Inc. (F), Novartis Pharmaceuticals Corporation (C), Novartis Pharmaceuticals Corporation (F), XOMA Ltd (C)

Program Number: 2048 Poster Board Number: D0187

Presentation Time: 11:00 AM - 12:45 PM

CD163+ and CD68+ cells in the adult human eye

SVETLANA CHEREPANOFF1,2, Enisa Hasic3, Paul G. McMenamin1, Mark C. Gillies1, 1Anatomical Pathology, Prince of Wales Hospital, Randwick, NSW, Australia; 2School of Medical Sciences, University of NSW, Randwick, NSW, Australia; 3Anatomy and Developmental Biology, Monash University, Melbourne, VIC, Australia; 4Save Sight Institute, University of Sydney, Sydney, NSW, Australia.

Purpose: To describe the distribution of CD163+ and CD68+ cells, markers of M1 and M2 macrophages and microglia, in the normal adult human eye in order to test the hypothesis that intraocular immune competent cells are TH2/M2 polarised.

Methods: Six eyes (4 donors, aged 52-59 years) from the NSW Lions Eye Bank, with corneas removed for transplantation, were processed for routine H&E and histological examination. Eyes had no history of ocular disease and were normal on macroscopic examination. Sections were stained with anti-human CD163 (clone 10D6) and anti-human CD68 (clone 514H12) using the proprietary Bond Polymer Red Detection kit from Leica (Catalogue No DS 9390). Nuclei were counterstained with haematoxylin. CD163 and CD68 staining was assessed in all compartments of the globe (except cornea).

Results: CD163+ macrophages were abundantly distributed throughout the choroid while CD68+ macrophages were clustered in the outer choroid. In one eye, CD163+ choroidal macrophages were seen beneath small hard drusen and beneath an anteriorly migrated hypertrophied retinal pigment epithelial cell. Within the retina, CD163+ microglia were infrequently seen in the inner retina around
retinal vessels, within the ganglion cell layer, abutting the internal limiting membrane, within Henle's fibre layer and very occasionally in the inner nuclear layer. CD163+ cells with plump epitheloid morphology were seen in the subretinal space of the peripheral retina near the ora serrata. CD68+ cells were entirely absent in the neural retina. CD163+ macrophages were also frequently seen within ciliary body stroma and anterior iris stroma, between fibres of the optic nerve, and within conjunctival epithelium and stroma. CD68+ cells were absent from these sites.

**Conclusions:** Excepting the outer choroid, intraocular microglia and macrophages in this series of normal human eyes expressed CD163 and not CD68, supporting the idea that the normal intraocular immune environment is TH2/M2 polarised.

**Commercial Relationships:** SVETLANA CHEREPANOFF
None; Enisa Hasic, None; Paul G. McMenamin, None; Mark C. Gillies, Novartis (R), Pfizer (R), Allergan (F), Bayer (F)

**Support:** Ophthalmic Research Institute of Australia

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**Program Number:** 2049 **Poster Board Number:** D0188
**Presentation Time:** 11:00 AM - 12:45 PM

**Cytokine Production in the Endotoxin-Induced Uveitis Model**

Abrar A. Rageh1, Michael Jordan1, Neal D. Heuss1, Dale S. Gregerson2, Deborah A. Ferrington1, Sandra R. Montezuma1.

1Ophthalmology and Visual Neuroscience, University of Minnesota, Minneapolis, MN; 2Medical School, North Dakota University, Grand Forks, ND.

**Purpose:** The purpose of this study is to determine the tissue-specific response and temporal kinetics of the inflammatory response in the mouse eye, using the endotoxin-induced uveitis (EIU) murine model.

**Methods:** Two groups of age-matched C57BL/6J mice (N=12) received ocular injections (10 µL) of either lipopolysaccharide (LPS, 1µg/µL) or phosphate buffered saline (PBS) into the anterior chamber. Control (untreated) eyes received no injection. Mice were sacrificed at 0 (untreated controls), 3, 6, or 16 hours after injection. The mice were perfused with PBS and the eyes were enucleated. Ocular tissue was dissected, separating the iris, retina and retinal pigment epithelial (RPE). Tissue homogenates underwent analysis by Cytometric Bead Array (CBA, BD Biosciences) to determine the inflammatory response at each interval. The markers used to determine the extent of inflammation were Tissue Necrosis Factor-alpha (TNF-α) and Interleukin 6 (IL-6).

**Results:** Injection with LPS induced expression of IL-6 in all ocular tissues. In the iris, peak IL-6 response occurred at 6 hours, and was ~400 fold higher than PBS injected controls. In the retina, peak IL-6 response occurred at 16 hours, and was ~120 fold higher than PBS injected controls. In the RPE, peak IL-6 response occurred at 16 hours, and was ~140 fold higher than the PBS injected controls. For TNF-α, peak production occurred at 3 hours in the iris and retina, and 6 hours in the RPE. Values were 10 to 80% higher than untreated tissue; however, these values were unchanged when comparing PBS to LPS. Injection with either PBS or LPS increased cytokine production over untreated controls, suggesting the trauma induced by the injection was sufficient to elicit a cytokine response.

**Conclusions:** Our results indicate significant tissue-specific responses in the EIU animal model. The inflammatory response, as determined by IL-6 production, was the greatest in the iris, and slightly less in RPE and the retina. Peak response of IL-6 occurred at 6 hours in the iris and RPE, and 16 hours in retina.

**Commercial Relationships:** Abrar A. Rageh, None; Michael Jordan, None; Neal D. Heuss, None; Dale S. Gregerson, None; Deborah A. Ferrington, None; Sandra R. Montezuma, None

**Support:** Minnesota Lions Club. Unrestricted grant to the Department of OVNS from Research to Prevent Blindness

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**Program Number:** 2050 **Poster Board Number:** D0189
**Presentation Time:** 11:00 AM - 12:45 PM

**Osteopontin is Expressed by Microglia and T Cells and Regulated by STAT3**

Chengrong Yu, Waynekid Kam, Ivy M. Dambuzza, Bernadette Marrero, Rashid M. Mahdi, Charles E. Egwuagu. Laboratory of Immunology, NEI, Bethesda, MD.

**Purpose:** Osteopontin (OPN) is a SIBLING family protein that binds to integrin receptors including αβ1, α9β1, and α9β4 and it promotes chemotactic properties of leukocytes. In previous studies, we showed that mice with STAT3 deletion in T cells do not develop EAU or EAE because their uveotigogenic or encephalotigogenic T cells are defective in the expression of OPN and αβ1 and could not traffic into the retina or brain during CNS inflammation (J Immunol. 180:6070-6; J Immunol. 187:3338-46). Furthermore, OPN and αβ1, along with αβ crystalline, have been implicated in mechanism that mediate relapsing/remitting CNS autoimmune diseases. In this study, we have investigated whether OPN is expressed in the retina and whether its expression is directly regulated by STAT3.

**Methods:** Expression of OPN in T cells, microglia, and retinal tissues was determined by FACS, confocal microscopy (using anti-IBa 1 and anti-OPN abs), and RT-PCR. Naïve CD4 T cells were polarized under Th0, Th1 and Th17 conditions and their lineage makers and intracellular cytokine expression were analyzed by FACS. Experimental autoimmune uveitis (EAU) was induced in C57BL/6 mice by immunization with IRBP/CFA. Bioinformatic tools were used to analyze whether there are potential STAT binding sites within distal and proximal core OPN promoter regions of the OPN gene. Chromatin immunoprecipitation (CHIP) analysis was performed using STAT3 and STAT1 Abs to determine potential STAT binding to OPN promoter.

**Results:** We detected constitutive OPN expression by the microglia cell line. BV-2 and analysis of the various T-helper subsets revealed that OPN is preferentially expressed in Th17 and Th1 but barely detectable in Th0 cells. By CHIP assays, we identified 2 potential STAT binding sites and have demonstrated here that STAT3 preferentially binds the TTTcagGAA (-1162). By immunohistochemistry we detected increased OPN expression in perivascular cells of retinal blood vessels and retinal microglial cells of mice with EAU.

**Conclusions:** We provide for the first time direct evidence that STAT3 binds to the OPN promoter and is a positive regulator of OPN gene expression. Increased OPN expression by microglia and perivascular cells during uveitis, taken together with our previous finding that defective OPN expression correlates with resistance to EAU, suggest that OPN is a potential therapeutic target in uveitis.

**Commercial Relationships:** Chengrong Yu, None; Waynekid Kam, None; Ivy M. Dambuzza, None; Bernadette Marrero, None; Rashid M. Mahdi, None; Charles E. Egwuagu, None

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**Program Number:** 2051 **Poster Board Number:** D0190
**Presentation Time:** 11:00 AM - 12:45 PM

**Cataract and uveitis: Comparison of two different anti inflammatory regimens for the prevention of post-operative complications**

Nathalie Butel1,2, Emmanuelle Champion1, Valerie Touitou1, Christine Fardreau1, Bahram Bodaghi2, Phuc Le Hoang2.

1Osteopontin is Expressed by Microglia and T Cells and Regulated by STAT3

**Purpose:** To compare the efficacy of the classical anti inflammatory regimen and a simplified protocol in patients with uveitis, undergoing cataract surgery.

**Methods:** Retrospective monocentric, nonrandomized, open study, unrestricted grant to the

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Conducted in 2012 and including patients followed for uveitis, requiring cataract surgery. Patients received either a complete regimen (pre and post operative oral corticosteroids, intraoperative iv methylprednisolone and subconjunctival dexamethasone) (group A), or a simplified regimen including iv and subconjunctival corticosteroids (group B). Each regimen was attributed based on the severity of posterior synchiae and the risk of macular edema.

Anterior chamber inflammation was analyzed based on SUN criteria and laser flare photometry. Central macular thickness was evaluated by OCT preoperatively and postoperatively (45 days +/- 10).

**Results:** The study included 34 eyes of 29 patients with a mean age of 53.9 years. After a median follow up of 6 months (3-9 months), VA improved significantly in both groups: 0.7 to 0.26 logmar in group A, and 0.44 to 0.15 logmar in group B. Flare values went from 56.8 ph/ms to 50.36 ph/ms (group A) and from 21.4 ph/ms to 12.38 ph/ms in group B. Central macular thickness increased from 220.9 microns to 242.2 microns (group A) and from 224 microns to 242.4 microns (group B). Postoperative complications were predominantly observed in group A: 3 early inflammatory relapses, 2 macular edema, 1 epipapillary membrane and a retinal detachment with vitreoretinal traction in a case of toxocariasis. In group B, we only deploire 1 early anterior uveitis. Inflammatory complications were related to the history of uveitis, its etiology, the preoperative inflammatory state and a past medical history of macular edema. They were not related to the protocol.

**Conclusions:** Even though uveitis was less severe in group A, the results are encouraging. Therefore, a simplified prophylactic anti-inflammatory regimen may be considered in patients with prolonged quiescence and without macular edema.

**Commercial Relationships:** Nathalie Butel, None; Emmanuelle Champion, None; Valerie Toutou, None; Christine Fardeau, None; Bahram Bodaghi, None; Phuc Le Hoang, allergan (C), bausch Lomb (R), santen (C)

**Program Number:** 2052 Poster Board Number: D0191
**Presentation Time:** 11:00 AM - 12:45 PM
**Response of uveitis-related retinal vasculitis to therapy with systemic infliximab**

Pramod K. Sharma1,2, Gueorgui T. Markov1,4, C. Stephen Foster3,4

1Uveitis, Massachusetts Eye Research and Surgery Institution, Cambridge, MA; 2Ophthalmology, VSS Medical College, Burla, Sambalpur, India; 3Ophthalmology, University Eye Hospital "Professor Pashev", Sofia, Bulgaria; 4Harvard Medical School, Boston, MA.

**Purpose:** To assess the effect of infliximab on inducing remission in patients with uveitis associated with retinal vasculitis.

**Methods:** Retrospective case series study of the clinical records of 48 consecutive patients, who received infliximab for uveitis with associated vasculitis at MERSI, between 7/2005 and 7/2012, for at least 12 months. Effect was assessed on the basis of findings from fluorescein angiography and optical coherence tomography.

**Results:** From all 48 patients (100%) with uveitis and retinal vasculitis, 12 (25%) were male, and 36 (75%) - female. Age varied from 10 to 70 years. A total of 91 eyes were affected. Follow-up was from 12 to 78 months. Anterior uveitis was diagnosed in 3 (6.25%), intermediate - in 7 (14.58%), posterior - in 1 (22.91%), panuveitis - in 12 (25%), scleritis in 1 (2.08%). In addition we had birdshot retinochoroidopathy in 7 cases (14.58%), Behcet’s disease in 6 (12.5%), and sympathetic ophthalmia in 1 (2.08%). Average dose of Infliximab was 5mg/kg. Sustained control of inflammation was achieved in 40 (83.3%) out of 48 patients. Average time for control of vasculitis was from 3 to 6 months. In 2 cases infliximab was discontinued due to rise of liver enzymes. One (2.08%) case improved after rescue therapy with cyclophosphamide. One (2.08%) developed optic nerve demyelination. 2 (4.1%) cases developed lupus-like rash. One patient (2.08%) improved after pars plana vitrectomy and/or pericentral or intravitreal corticosteroid injections. Relapses occurred due to temporary discontinuation of infliximab secondary to flu or pneumonia in one patient (2.08%). 1 (2.08%) had a relapse after stretching of infusion interval but subsequently improved on decreasing the interval. One patient (2.08%) showed good response but had to stop due to insurance issues. 1 (2.08) achieved remission without needing further therapy.

**Conclusions:** Uveitis associated with retinal vasculitis is a sight-threatening condition necessitating immunomodulatory therapy in a large percentage of patients. Infliximab appears to be a useful therapeutic agent which can lead to a sustained control of inflammation over a long period of follow-up.

**Commercial Relationships:** Pramod K. Sharma, None; Gueorgui T. Markov, None; C. Stephen Foster, Abbott Medical Optics (C), Abbott Medical Optics (F), Alcon Laboratories, Inc. (C), Alcon Laboratories, Inc. (F), Allergan, Inc. (F), Eyegate Pharmaceuticals, Inc. (I), Eyegate Pharmaceuticals, Inc. (F), IOP Ophthalmics (C), Ista Pharmaceuticals (C), Lux Biosciences, Inc. (C), Lux Biosciences, Inc. (F), Novartis Pharmaceuticals Corporation (F), Novartis Pharmaceuticals Corporation (F), XOMA Ltd (C)

**277 Corneal Infection and Inflammation**

Monday, May 06, 2013 2:45 PM-4:30 PM
606/607 Paper Session
**Program #/Board # Range:** 2156-2162
**Organizing Section:** Immunology/Microbiology

**Program Number:** 2156
**Presentation Time:** 2:45 PM - 3:00 PM
**Adenoviral Pathogenesis in Epidemic Keratoconjunctivitis: the Failure of Hexon Gene Sequence to Predict Corneal Tropism**

James Chodosh1, Gurdeep Singh2, Xiaohong Zhou1, Jaya Rajaiya1, Mohammad A. Yousuf1, Jeong Yoon Lee1, Christopher M. Robinson1, Don Seto2, David Dyerv, Morris S. Jones2, Ophthalmology, Mass Eye & Ear - Harvard Medical School, Boston, MA; 1School of Systems Biology, George Mason University, Manassas, VA; 3Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

**Purpose:** In the last century, human adenoviruses (HAdVs) were differentiated from one another by serum neutralization. This methodology depends on a stereotypical humoral immune response by the host to two adjacent hypervariable loops on the hexon protein, the most abundant adenovirus capsid component. Epidemic keratoconjunctivitis (EKC) is caused by HAdVs within species D, including HAdV-D56. Recent advances in genomic sequencing and bioinformatics led us to discover three other HAdVs with identical hexon genes to HAdV-D56.

**Methods:** Purified DNA from virus stocks were sequenced on a Roche 454 DNA sequencer by Operon to at least 17-fold depth, with an accuracy of greater than 99% (Q20 or better). The sequencing reads were assembled using CLC Genomics Workbench, with an N50 average of 5,260. Annotation was performed using the GenScan web server at MIT. Phylogenetic analysis was performed using MEGA. mVISTA LAGAN was used for global pair-wise sequence alignment, and SimPlot and Bootscan were used to identify possible recombination events. Confocal microscopy with Cy3-labeled virus was used to

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identify differential entry into corneal epithelial and stromal cells in vitro.

**Results:** Four HAdV-Ds with a highly (>99%) identical hexon gene were identified, HAdV-D15, HAdV-D29, HAdV-D56, and a fourth previously untyped virus, which was identified from a second lot of HAdV-D15 (ATCC). All four viruses differed from one another across the remainder of their genomes. Homologous recombination was shown for the penton base RGD loop between the novel HAdV-D and HAdV-D53, another known EKC pathogen. By confocal microscopy, of the four viruses with the same hexon only HAdV-D56 readily entered both human corneal epithelial cells and fibroblasts.

**Conclusions:** Our findings show that the hexon gene hypervariable regions can be shared between otherwise disparate viruses, leading to mistyping if only partial genome sequencing is employed. These data further reinforce the importance of homologous recombination to the evolution of HAdV-Ds, including those viruses associated with EKC, and confirm an absence of contribution of the hexon to adenoviral tropism in the cornea.

**Commercial Relationships:** James Chodosh, Alcon (C), Allergan (C), 3-V Biosciences (C), Novabay (C); Gurdeep Singh, None; Xiaohong Zhou, None; Jaya Rajaiya, None; Mohammad A. Yousef, None; Jeong Yoon Lee, None; Christopher M. Robinson, None; Don Seto, None; David Dyer, None; Morris S. Jones, None

**Support:** R01 EY013124, R01 EY021558, and P30 EY014104 from NEI, and Research to Prevent Blindness

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**Program Number: 2157**

**Presentation Time:** 3:00 PM - 3:15 PM

**Establishing a New Role for the Enzyme Heparanase in Ocular Herpes Infection**

**Deepak Shukla.** Ophthalmal/Visual Sciences, University of Illinois at Chicago, Chicago, IL.

**Purpose:** Heparanase is a metalloproteinase that is released from infected cells as a result of viral infection. It mediates the release of the virions from the infected cells. Our hypothesis is that heparanase, an enzyme, is a novel mediator of HSV-1-induced cell death and virus release. We demonstrate that HSV-1 infection results in enhanced expression of heparanase, an enzyme that hydrolyzes heparan sulfate. We found a direct role for enhanced heparanase expression and exogenous treatments with the enzyme in HSV-1 release from infected cells. Enhanced expression of heparanase did not affect HSV-1 entry into host cell, or HSV-1 induced cell-to-cell fusion, suggesting that heparanase activation is tightly regulated, and possibly more sensitive to HS modification utilized for virus egress compared to those that are utilized for virus entry. Furthermore, HSV-1 resulted in an increase in active heparanase expression, which was accompanied by a decrease in the inactive heparanase expression. Active heparanase has also been shown to mediate heparan sulfate proteoglycans (HSPGs) shedding including syndecans; where syndecan ectodomain is released in a soluble form to the extracellular space. Enhancing syndecan shedding by the shedding agonist PMA resulted in increased HSV-1 release from infected cells to the culture supernatant. This suggests that not only heparanase induces virus release from infected cells through HS cleavage, but also by mediating the shedding of HSPGs including syndecans.

**Conclusions:** Our study defines a novel mechanism for viral release from the cells of the corneal epithelium and implicates heparanase, an enzyme, in control of the release. Thus, heparanase may be used as a new target to control corneal complications originating from HSV-1 infection.

**Commercial Relationships:** Deepak Shukla, None

**Support:** NIH Grant RO1 AI057860

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**Program Number: 2158**

**Presentation Time:** 3:15 PM - 3:30 PM

**The role of corneal Plasmacytoid Dendritic Cells in acute herpes simplex virus infection**

**Kai Hu,1,2, Desheea L. Harris,2, Takefumi Yamaguchi,1,2, Homayon Ghiasi,3, Ulrich von Andrian,1, Pedram Hamrah,1,2,3**

**Ophthalmology, Massachusetts Eye & Ear Infirmary, Boston, MA; 3Schepens Eye Research Institute, Boston, MA; 1Immune Disease Institute, Program in Cellular and Molecular Medicine at Children’s Hospital, Boston, MA; 3Ophthalmology Research, Cedar Sinai Medical Center, Los Angeles, CA.

**Purpose:** We have recently demonstrated the presence of resident plasmacytoid dendritic cells (pDCs) in the murine cornea. In order to determine the function of corneal pDC, our purpose was to investigate the role of pDCs in acute herpes simplex virus (HSV-1) keratitis.

**Methods:** Murine corneas were inoculated with HSV-1 after scarification. Corneal pDCs were depleted with subconjunctival (s.c.) injection of diphertheria toxin (DT) into BDCA-2-DTR or wild type (WT, C57BL/6) controls (sham-depleted). Clinical opacity scores were graded, and corneas were collected for immunofluorescence staining for pDC and analysis for HSV-1 virus titers. Corneas were analyzed for IFN-a mRNA and protein levels. CPG-ODN-1862, a Toll Like Receptor 9 (TLR9) agonist, was applied to the cornea after mechanical debridement in the presence and absence of pDC to test if increased IFN-a by pDC is TLR9-mediated.

**Results:** As early as day 1 post inoculation (p.i.) with HSV-1, pDC density of central and peripheral corneas were significantly increased as compared to sham infection (p<0.05), and continuously increased on days 2, 4, 6 p.i. pDC were successfully depleted (>90%) by s.c. DT injections every 3 days. The corneal opacity scores and corneal virus titers in pDC-depleted mice [pDC(-)] were significantly increased compared to sham-depleted corneas (p<0.05) on days 1, 3, 5, 7 p.i. Corneal IFN-a mRNA and protein levels significantly increased on day 1, 3, 5, p.i. (p<0.05), with a peak 18-fold (mRNA) and 12-fold (protein) expression on day 3 p.i. compared with sham infection (p<0.01). However, pDC-depletion resulted in significant decrease (p<0.05) in both mRNA and protein levels of IFN-a. CPG-ODN-1862 application resulted in 5-fold (mRNA) (p<0.01) and 4-fold (protein) (p<0.01) increase in corneal IFN-a levels vs. CPG-ODN control, which was nearly abolished with depletion of pDC.

**Conclusions:** Corneal pDCs are the main producers of corneal IFN-a in HSV keratitis in a TLR9-mediated fashion and thus participate in the first-line defense against viral pathogens.

**Commercial Relationships:** Kai Hu, None; Desheea L. Harris, None; Takefumi Yamaguchi, None; Homayon Ghiasi, None; Ulrich von Andrian, None; Pedram Hamrah, None

**Support:** NIH K08-EY020575 (PH), Research to Prevent Blindness Career Development Award (PH), Falk Medical Research Trust (PH), MEEI Foundation (PH), Japanese Eye Bank (TY).

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**Program Number: 2159**
In Vivo Confocal Microscopy Demonstrates Bilateral Increase in Epithelial Corneal Dendritic Immune Cells in Unilateral Herpes Zoster Ophthalmicus

Bernardo M. Cavalcanti1, 2, Andrea Cruzat1, 2, Deborah Pavan-Langston1, Erik A. Samaya1, 2, Pedram Hamrah1, 2. 1Department of Ophthalmology - Cornea & Refractive Surgery, Massachusetts Eye & Ear Infirmary, Harvard Medical School Department of Ophthalmology, Boston, MA; 2Department of Ophthalmology - Ocular Surface Imaging Center, Massachusetts Eye & Ear Infirmary, Harvard Medical School Department of Ophthalmology, Boston, MA.

Purpose: Herpes zoster ophthalmicus (HZO), a unilateral disease, results in recurrent immune-mediated keratitis and neurotrophic keratopathy. This study aimed to analyze by laser in vivo confocal microscopy (IVC M) bilateral corneal immune cell as correlated to subbasal nerve changes in patients with HZO.

Methods: A prospective, cross-sectional study was conducted in 24 patients with clinical diagnosis of HZO, their contralaterally clinically unaffected eyes, and 24 normal age matched controls. IVC M (HRT3/RCM) and corneal sensation (Cochet-Bonnet) of the central cornea were performed bilaterally in all patients and controls. Confocal images were evaluated by 2 masked observers for the presence of dendritiform immune cells (DC), subbasal nerve density and numbers. For each variable, results for 3 frames were averaged, and analyzed utilizing ANOVA and Pearson’s correlation coefficient.

Results: The mean DC density was significantly higher in patients with HZO (147.4±33.9 cells/mm2; p<0.001) as compared to controls (23.0±3.6). Surprisingly, in the contralaterally clinically unaffected eyes, DC density was significantly increased (120.1±21.2; p<0.001). Patients with immune-mediated keratitis at the time of the visit, had a 60% higher DC density than patients with no clinical keratitis. DC in HZO eyes showed a significant increase in number of dendrites (4.1±0.8 dendrites/cell) and were larger in size (232.4±47.4µm2) as compared to controls (2.3±0.5, 57.2±11.7; p<0.001). DC increase was correlated (R=0.43; p<0.001) with diminishment in total nerve density (9052.6±1151.8, 14959.8±903.2, and 22851.4±1117.8µm/mm2 respectively; p< 0.001), total number of nerves (5.8±0.9, 11.9±1.2, and 26.6±1.2/mm2; p<0.001), main trunks (2.4±0.3, 3.8±0.3, and 4.4±0.2; p<0.001), and branches (3.4±0.7, 8.2±1.1, and 22.2±1.2; p<0.001) as compared to controls. Further, reduction in nerves was strongly correlated with corneal sensation (p<0.05).

Conclusions: Patients with unilateral HZO demonstrate a profound and significant bilateral increase in corneal DC as compared to controls, which is correlated to decrease in corneal subbasal nerve. The results may explain bilateral ocular surface disease observed in patients with HZO and suggest a direct interaction between the immune and nervous system in the cornea and a bilateral nerve and immune alteration in an apparently unilateral disease.

Commercial Relationships: Bernardo M. Cavalcanti, None; Andrea Cruzat, None; Deborah Pavan-Langston, SAGE Consulting Group (C); Erik A. Samaya, None; Pedram Hamrah, None

Support: NIH K08-EY020575, New England Corneal Transplant Research Fund, Falk Medical Research Trust, Research to Prevent Blindness Career Development Award Stevens Fund, Johnstone Fund

Program Number: 2160

Presentation Time: 3:45 PM - 4:00 PM

Penetrating keratoplasty to one eye abolishes immune privilege and promotes corneal allograft rejection in the opposite eye, even to grafts from unrelated donors

Jerry Y. Niederkorn, Kathryn Paunicka, Jessamine Mellon, Ophthalmology, Univ Texas Southwestern Med Ctr, Dallas, TX.

Purpose: To determine the effect of penetrating keratoplasty (PK) on immune privilege of subsequent corneal allografts in both eyes.

Methods: Corneas from BALB/c, C57BL/6, C3H, or A/J donors were grafted orthotopically to BALB/c mice. A 2 mm trephine was used to make shallow circumferential incisions in left eyes prior to applying corneal allografts to right eyes. Ocular neuropsychology levels were evaluated by enzyme immunosassays. Substance P (SP) was blocked by daily administration of Spantide II (72 micrograms/day).

Results: PK to one eye abolished immune privilege and exacerbated graft rejection in the same eye or the unmanipulated eye. C57BL/6 corneal allografts normally undergo rejection in 50% of BALB/c hosts. However, BALB/c hosts that previously rejected C3H or A/J allografts in the right eye had 100% rejection of genetically unrelated C57BL/6 corneal allografts placed into the left eye (P <0.001).

Moreover, syngeneic BALB/c corneal grafts placed in the right eye induced 88% rejection of C57BL/6 allografts placed in the left eye (P = 0.032). Even a 360 degree corneal surface incision in the right eye induced 100% rejection of corneal allografts in the left eye (P < 0.001), but did not affect survival of BALB/c syngeneic grafts (100% survival; P> 0.05). Surgery-induced graft rejection was associated with severing corneal nerves and not due to trauma, as insertion of sutures in the right eye did not affect graft survival in the left eye. Likewise, "X" shaped corneal incisions did not affect graft survival in the other eye (P > 0.05). A trephine incision in one eye induced upregulation of SP in both eyes. Trehpine

Conclusions: Circular corneal surface incisions, and not simple ocular trauma (e.g., sutures or "X" shaped incisions), abolish immune privilege in both eyes. Sympathetic abolition of immune privilege is associated with upregulation of SP in the contralateral eye. Blocking SP with Spantide II restores immune privilege and suggests that SP is a key mediator that abolishes immune privilege in response to severing corneal nerves during PK.

Commercial Relationships: Jerry Y. Niederkorn, Allergan (C); Kathryn Paunicka, None; Jessamine Mellon, None

Support: NIH Grant EY007641 and Research to Prevent Blindness Program Number: 2161

Presentation Time: 4:00 PM - 4:15 PM

IL-23 and CCL20 in the corneal response to epithelial abrasion

Yuan Gao1, 2, Alan R. Burns3, Clifton W. Smith3, 4. 1Pediatrics, Baylor College of Medicine, Houston, TX; 2College of Optometry, University of Houston, Houston, TX; 3Clinical College of Ophthalmology, Tianjin Medical University, Tianjin, China.

Purpose: γδ T cells responding to corneal abrasion in mice express receptors for Interleukin 23 (IL-23) and chemokine ligand 20 (CCL20). The current work assesses the influence of IL-23 and CCL20 on epithelial and sensory nerve regeneration in a murine model of corneal injury.

Methods: Central corneal epithelial abrasion (2 mm) was performed in female C57BL/6 mice and γδ T cell-deficient (TCRδ-/-) mice. Wildtype mice received anti-IL-23 and anti-CCL20, and TCRδ-/- mice received recombinant IL-23 (rIL-23) and recombinant CCL20 (rCCL20) dissolved in PBS topically on the wounded corneas every 4 hours for 24 hours. The control animals received an equal concentration of IgG in PBS or PBS alone. Corneas were analyzed for epithelial cell division by immunofluorescence at 24 hrs post wounding. Nerve regeneration was evaluated using fluorescence deconvolution microscope to assess each field of view (150x150µm) across the cornea from limbus to limbus with a deconvolved z-stack (0.2-µm steps) encompassing the corneal epithelium, the subbasal nerve plexus, and an adjacent portion of the corneal stroma.

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Results: Dividing epithelial cells in wild-type mice had a 15.2% and 39.2% decrease after topical treatment of anti-IL-23 and anti-CCL20 (p<0.01, n=6). TCRδ-/- mice exhibited marked depression of epithelial division at 24 hours after injury, and treatment of TCRδ-/- mice with cCL20 elevated epithelial division 28.4% (p<0.01, n=6) while RIL-23 had no effect. Early nerve regeneration is evident at 24 hours after injury, and wounded wildtype corneas after topical treatment with anti-IL-23 and anti-CCL20 had 51.0% and 31.4% reductions in nerve regeneration at 24 hours after injury, respectively (p<0.01, n=6). Nerve regeneration is significantly depressed in TCRδ-/- mice (Zhijie Li et al. Am J Path, 2011), but increased 27.2% (p<0.01, n=6) in TCRδ-/- mice after topical treatment with cCL20. In contrast, RIL-23 treatment did not significantly increase nerve regeneration of TCRδ-/- mice.

Conclusions: IL-23 appears to act in corneal wound healing through the γδ T cells, while CCL20 appears to have a broader range of activity.

Commercial Relationships: Yuan Gao, None; Alan R. Burns, None; Clifton W. Smith, None
Support: EY018239, EY007551 and EY017120; U.S. Department of Agriculture Grant 6250-51000-046

Program Number: 2162
Presentation Time: 4:15 PM - 4:30 PM

Molecular Epidemiology of Methicillin Resistant Staphylococcus aureus (MRSA) Causing Ocular Infections in South India
Nithya Velusamy*, Rathinam Sivakumar*, Lalitha Prajna*
1Microbiology, Aravind Medical Research Foundation, Madurai, India; 2Department of Ovea, Aravind Eye Hospital and Post Graduate Institute of Ophthalmology, Madurai, India.

Purpose: The purpose of this study is to perform molecular characterization and epidemiological analysis of MRSA causing ocular infections in South India.

Methods: Between Jan 2012 and Oct 2012, 33 MRSA isolates were collected at Aravind eye Hospital, Madurai, India, from the patients who presented with various ocular infections like orbital infections, infective keratitis and lacrimal sac abscess. Preliminary screening was performed by antibiotic susceptibility method using oxacillin and cefoxitin discs and further confirmed by mecA screening. Staphylococcal chromosome mec typing, Staphylococcal protein A typing and detection of Panton-Valentine leukocidin toxin were done for 17 isolates by PCR.

Results: A total of 127 Staphylococcus aureus were isolated, among that 33 were confirmed as MRSA. 17 of 33 were screened, in which 10 were SCCmec type V, 5 were SCCmec type IV and 2 were not typeable. Most of the SCCmec type V isolates belonged to spa type t657 (7/10) and 3 isolates had spa type t037, t3387, t836. SCCmec type IV had the spa type t852 (2/5), t363, t7181 and t1598. 82% (14/17) isolates were found to be positive for the toxin Panton-Valentine leukocidin.

Conclusions: Community acquired MRSA strains (SCCmec type IV and V) are more prevalent than hospital acquired MRSA in causing ocular infections. Severe and non severe opthalmic manifestations are characterized with the emergence of an epidemic spa type t657. Most of the community acquired MRSA isolates are harboring the PVL gene which may be responsible for the pathogenicity and ocular morbidity.

Commercial Relationships: Nithya Velusamy, None; Rathinam Sivakumar, None; Lalitha Prajna, None
Support: Aravind Medical Research Foundation

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Landon Grange, Monica D. Dalal, Yujuan F. Wang, Chi-Chao Chan, Robert B. Nussenblatt, H Nida Sen. National Eye Institute, National Institutes of Health, Bethesda, MD. 

**Purpose:** Non-paraneoplastic autoimmune retinopathy (AIR) is a rare immune-mediated disease characterized by the presence of serum antiretinal autoantibodies. The present study seeks to quantify HLA allelic frequencies and autoimmunity markers in the peripheral blood for a cohort of 24 patients with antiretinal antibody-confirmed non-paraneoplastic AIR.

**Methods:** A retrospective review was performed on 24 patients with non-paraneoplastic AIR who were seen and evaluated at the NIH. HLA markers of 18 Caucasian patients were assessed and subtyped by molecular biology, and were compared to the levels in the Caucasian population at large using the Allele Frequency Net Database. All patients underwent systemic work-up to rule out malignancy and infectious etiology. Additionally, patients were screened for the following autoimmune markers: anti-nuclear antibodies (ANA), anti-extractable nuclear antigen (Anti-ENA), anti-double stranded DNA (Anti-ds DNA), Rheumatoid Factor (RF), C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), Anti-cyclic citrullinated peptide (Anti-CCP). These results were analyzed in an effort to detect peripheral markers that could aid in the diagnosis of AIR.

**Results:** All 24 confirmed cases of AIR had positive serum antiretinal antibody testing by either Western blot or immunohistochemistry. Of these, 20 (83%) were Caucasian, 19 (79.2%) were female, with an average age at presentation of 54.9 years (range 37-88, median 51.5). The majority (96%) had bilateral disease. Fourteen (58%) had a personal or family history of autoimmune disease.

Analysis of blood samples from 18 Caucasian patients with confirmed AIR disclosed that HLA DRB1-03, and HLA DRB1-15 (found in 56% and 44% of subjects respectively) were more prevalent than the general population (18.6% and 17.2%) (p=0.0025 and 0.0209, respectively), and that haplotype HLA A2 (found in 17%) was significantly less prevalent than the general population (50%) (p=0.0183). Two of the 18 patients tested for ANA and Anti-ENA(11%) were strongly positive, and both of these patients had personal as well as family histories of autoimmune diseases.

**Conclusions:** Our results reveal significant association of AIR with HLA DRB1-03, and HLADRB1-15 alleles. None of the peripheral antibodies were significantly elevated in AIR. Larger cohorts are needed to confirm the significance of these findings.

**Commercial Relationships:** Landon Grange, None; Monica D. Dalal, None; Yujuan F. Wang, None; Chi-Chao Chan, None; Robert B. Nussenblatt, None; H Nida Sen, None

Support: NEI Intramural Research Program

Clinical Trial: NCT01086631

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**Program Number:** 2516 Poster Board Number: D0121
**Presentation Time:** 2:45 PM - 4:30 PM
**Optical Coherence Tomography, Fundus Autofluorescence, and Fluorescein Angiography in Non-Paraneoplastic Autoimmune Retinopathy**

Monica D. Dalal, Landon Grange, Yujuan Wang, Chi-Chao Chan, Robert B. Nussenblatt, H Nida Sen. Laboratory of Immunology, NEI, Bethesda, MD.

**Purpose:** Non-paraneoplastic autoimmune retinopathy (AIR) is a rare immune-mediated disease characterized by serum antiretinal autoantibodies with often normal or subtle fundus findings that can be difficult to diagnose. Our aim is to evaluate the imaging techniques of optical coherence tomography (OCT), fundus autofluorescence, and fluorescein angiography (FA) in patients with AIR to better characterize the disease.

**Methods:** A retrospective review of patients with a diagnosis of AIR evaluated at the NEI who underwent optical coherence tomography (OCT), fundus autofluorescence (FAF), and fluorescein angiography (FA).

**Results:** Twenty-four patients were identified with a clinical diagnosis of AIR. All patients had positive serum antiretinal antibody testing by either Western blot or immunohistochemistry. The group was predominantly female (19, 79.2%) and Caucasian (20, 83.3%). The mean age at presentation was 54.9 years old (range 37–88, median 51.5). All but 1 patient had bilateral disease (95.8%) and the most common symptom was decreased vision and photopsias. On clinical exam and review of fundus photography the most common interphotoreceptor retinoid-binding protein peptide (IRBP) in male C57Bl/6 mice. Ocular Coherence Tomography (OCT) was performed using the Heidelberg Spectralis HRA+OCT system before induction of EAU, and days 7, 14, and 20 post-EAU induction. Retinas were collected 21 days following immunization, sections stained with H&E and the extent of inflammation and associated pathology graded using a standard histopathological scale (Chan, 1990). An OCT scoring system was devised based on the above histopathological scoring method, and OCT images were scored by a masked observer. Inflammatory cell clusters and retinal thickness were also quantified separately by OCT. Three independent experiments were conducted, and correlations between OCT and histological scores were determined.

**Results:** By Day 20, signs of inflammation and retinal damage were evident in the OCT images taken from IRBP immunized mice, including retinal edema, inflammatory cell clusters, sub-retinal lesions, retinal folds, vascular dilation, vasculitis, retinal layer disruptions and retinal detachment. In Study1 (n=17): the average OCT score on Day 20 (2.5 ± 0.34) was similar to that obtained by the standard histological scoring system (3.0 ± 0.30), with a correlation coefficient (r) of 0.88 between methods. In Study2 (n=15): the average OCT score on Day 20 (2.3 ± 0.34) was identical to that of the histological score (2.3 ± 0.34), with an r of 0.91. In Study3 (n=36): average OCT score (1.4 ± 0.14) was again very similar to that of the histological score (1.6 ± 0.16), having an r of 0.88.

**Conclusions:** Results showed a strong correlation between OCT scores assessed in vivo and the standard histological method of scoring inflammation and retinal damage in mice with EAU. Thus, OCT imaging can serve as a rapid and accurate means of assessing inflammation and associated retinal damage in mice.

**Commercial Relationships:** Thomas C. MacPherson, Regeneron Pharmaceuticals (E); Jingtai Cao, Regeneron Pharmaceuticals, Inc. (E); George D. Yancopoulos, Regeneron Pharmaceuticals (E), Regeneron Pharmaceuticals (I), Regeneron Pharmaceuticals (P); Stanley J. Wiegand, Regeneron Pharmaceuticals, Inc (E)

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**Program Number:** 2515 Poster Board Number: D0120
**Presentation Time:** 2:45 PM - 4:30 PM
**Novel Non-invasive in vivo Quantification of Ocular Inflammation using Optical Coherence Tomography in Mice**


**Purpose:** Ocular Coherence Tomography (OCT) is a non-invasive method of imaging the retina in vivo, most commonly used in clinical practice to assess anatomical changes associated with vascular eye diseases. We evaluated the potential utility of OCT as a means for in-life quantitation of inflammatory and associated pathological changes in retinas of mice with experimental autoimmune uveitis (EAU).

**Methods:** EAU was induced by immunization with human interphotoreceptor retinoid-binding protein peptide (IRBP) in male C57Bl/6 mice. Ocular Coherence Tomography (OCT) was performed using the Heidelberg Spectralis HRA+OCT system before induction of EAU, and days 7, 14, and 20 post-EAU induction. Retinas were collected 21 days following immunization, sections stained with H&E and the extent of inflammation and associated pathology graded using a standard histopathological scale (Chan, 1990). An OCT scoring system was devised based on the above histopathological scoring method, and OCT images were scored by a masked observer. Inflammatory cell clusters and retinal thickness were also quantified separately by OCT. Three independent experiments were conducted, and correlations between OCT and histological scores were determined.

**Results:** By Day 20, signs of inflammation and retinal damage were evident in the OCT images taken from IRBP immunized mice, including retinal edema, inflammatory cell clusters, sub-retinal lesions, retinal folds, vascular dilation, vasculitis, retinal layer disruptions and retinal detachment. In Study1 (n=17): the average OCT score on Day 20 (2.5 ± 0.34) was similar to that obtained by the standard histological scoring system (3.0 ± 0.30), with a correlation coefficient (r) of 0.88 between methods. In Study2 (n=15): the average OCT score on Day 20 (2.3 ± 0.34) was identical to that of the histological score (2.3 ± 0.34), with an r of 0.91. In Study3 (n=36): average OCT score (1.4 ± 0.14) was again very similar to that of the histological score (1.6 ± 0.16), having an r of 0.88.

**Conclusions:** Results showed a strong correlation between OCT scores assessed in vivo and the standard histological method of scoring inflammation and retinal damage in mice with EAU. Thus, OCT imaging can serve as a rapid and accurate means of assessing inflammation and associated retinal damage in mice.

**Commercial Relationships:** Thomas C. MacPherson, Regeneron Pharmaceuticals (E); Jingtai Cao, Regeneron Pharmaceuticals, Inc. (E); George D. Yancopoulos, Regeneron Pharmaceuticals (E), Regeneron Pharmaceuticals (I), Regeneron Pharmaceuticals (P); Stanley J. Wiegand, Regeneron Pharmaceuticals, Inc (E)
findings were RPE mottling in 14 (58.3%), attenuated vessels in 10 (41.7%), bone spicules in 3 (12.5%), and a normal fundus in 4 (16.7%). OCT revealed 3 (12.5%) patients with cystoid macular edema. Drusen were present in 2 (8.3%) patients. Disturbance of the inner-segment/outer-segment junction on OCT was noted in 14 (58.3%) patients and an epiretinal membrane was present in 6 (25%) patients. FAF demonstrated speckling of the RPE limited to the macula in 13 (54.2%) patients and more extensive changes in 8 (33.3%) patients. One patient (4.2%) demonstrated evidence of retinal vasculitis and 2 (8.3%) patients revealed staining of the vessels on FA. All 3 patients with macular edema demonstrated mild perifoveal leakage.

Conclusions: A number of changes can be seen on imaging in patients with AIR, however there are no definite findings on OCT, FAF, or FA specific to the diagnosis. Imaging can help to rule out other diagnoses and understand causes of visual acuity loss, however psychophysical testing such as electroretinogram and visual field remain important for aiding in diagnosis and monitoring.

OCT demonstrating loss of the inner-segment/outer-segment junction.

FAF demonstrating abnormal autofluorescence, "speckling", in the macula.

Conclusions: This study is the first to investigate the use of flow cytometry as a method for confirming a clinical diagnosis of AIR. Our findings suggest that, similar to other inflammatory conditions, monocytes may be involved in the pathogenesis of AIR. With an unknown pathophysiology, and a positive response to immunosuppression, it logically follows that an improved diagnostic system will directly target the immune system. This provides an important avenue for investigation, as PBMC and flow cytometry can be used to look at extracellular cytokines along with additional cell types, such as B-cells or T-cells. Further investigation, with additional sampling, is required to achieve statistical significance. Longitudinal studies that track patient disease progression may also shed valuable insight on individuals' response to immunosuppression.

Commercial Relationships: Ryan E. Tsuchida, None; Jillian Huang, None; John R. Heckenlively, None; Kanishka T. Jayasundera, None

Program Number: 2518 Poster Board Number: D0123
Presentation Time: 2:45 PM - 4:30 PM
Investigation of murine experimental autoimmune uveoretinitis by Optical Coherence Tomography
Kouzo Harimoto1, Masataka Ito2, Masaru Takeuchi2.
1Ophthalmology, National Defense Medical College, Tokorozawa, Japan; 2Developmental Anatomy and Regenerative Biology, National Defense Medical College, Tokorozawa, Japan.
Purpose: Experimental autoimmune uveoretinitis (EAU) is an animal model of human endogenous uveitis, and the onset and severity are evaluated clinically and histologically. Morphological findings reflect more inflammatory processes, however can not be observed consecutively in the same individuals or tissues. Spectral domain-Optical coherence tomography (SD-OCT) is non-invasive and useful for evaluating fundus findings presented in human uveitis. In this study, we examined whether SD-OCT can be utilized for in vivo monitoring of EAU in mice.
Methods: Six- to eight-week old female C57BL/6 mice were immunized subcutaneously with 0.2 ml of emulsion containing 200 µg of hIRBP-p in CFA containing 5 mg/ml Mycobacterium tuberculosis H37Ra. Concurrent with immunization, 1 µg of PTX was injected intraperitoneally. Examinations by funduscopy and SD-OCT (Heidelberg®) were performed on days 7, 14, 21, and 28 after immunization, and the EAU clinical scores were graded on a scale of 0 to 4 according to the previous report. Eyes were then collected and ocular inflammation was assessed histologically.
Results: Retinal lamella structure and inner segment/outer segment (IS/OS) line was observed in the normal mouse retina. After the established role of macrophages in autoimmune-related disorders, coupled with the fact that many AIR patients respond positively to immunosuppression.
Methods: Participants were immunosuppression naïve AIR (n=4) or age-matched controls (n=1). Inclusion criteria included age over 40 years, onset of visual symptoms less than six months, no personal or family history of retinal dystrophy, and no illness or surgery in the past two months. Serological samples were prepared for peripheral blood mononuclear cells (PBMC) isolation. Cells were stained for surface CD14 and CD16 antibodies that characterized unique subsets of macrophage populations and were processed through flow cytometry. Finally, these cells were analyzed and compared against controls for relative expression of their respective surface receptors.

Results: Our findings suggest that AIR patients may contain a subset of PBMC that increasingly express CD14+/CD16+ surface receptors compared to healthy-controls.

Conclusions: This study is the first to investigate the use of flow cytometry as a method for confirming a clinical diagnosis of AIR. Our findings suggest that, similar to other inflammatory conditions, monocytes may be involved in the pathogenesis of AIR. With an unknown pathophysiology, and a positive response to immunosuppression, it logically follows that an improved diagnostic system will directly target the immune system. This provides an important avenue for investigation, as PBMC and flow cytometry can be used to look at extracellular cytokines along with additional cell types, such as B-cells or T-cells. Further investigation, with additional sampling, is required to achieve statistical significance. Longitudinal studies that track patient disease progression may also shed valuable insight on individuals’ response to immunosuppression.

Commercial Relationships: Ryan E. Tsuchida, None; Jillian Huang, None; John R. Heckenlively, None; Kanishka T. Jayasundera, None

Program Number: 2517 Poster Board Number: D0122
Presentation Time: 2:45 PM - 4:30 PM
Characterizing Peripheral Biomarkers in Patients with Autoimmune Retinopathy by Flow Cytometry
Purpose: Autoimmune retinopathy (AIR), a rare disease, lacks an understood pathophysiology and the standard serological anti-retinal antibody test lacks both specificity and sensitivity. To date, there is no published research on the ability of flow cytometry, a reliably fast and robust technique, to define or diagnose AIR. We characterized the profile of circulating monocytes through flow cytometry due to the established role of macrophages in autoimmune-related disorders, coupled with the fact that many AIR patients respond positively to immunosuppression.
development of EAU, segmental dormancy of the internal retinal layers was observed in mice developing EAU at grade 2 level, and destruction of total retinal layers, disappearance of IS/OS line, and partial retinal detachment were presented in EAU mice of grade 3.

**Conclusions:** We conclude that OCT is available for evaluating the development of murine EAU and reflects the histopathological changes.

**Purpose:** Experimental autoimmune uveoretinitis (EAU) is the animal model of human uveoretinitis, and the onset and severity are evaluated clinically and histopathologically. Histological finding reflects more inflammatory processes, however cannot be observed consecutively in the same animals or tissues. Spectral domain-OCT (SD-OCT) is non-invasive and useful for evaluating fundus findings presented in humans. In this study, we examined whether SD-OCT can be utilized in vivo to monitor changes in EAU mice.

**Methods:** Six to eight-week-old female C57BL/6 mice were vaccinated subcutaneously with 50 μl of emulsion consisting 200 μg of MBP or CFA containing 5 mg pertussis toxin intranasally (challenged). Experimental and control groups were performed as days 1, 3, 5, 7, 10, and 14 after immunization, and the eyes challenged were graded on a scale of 0 to 5 according to the previous report. Eyes were then enucleated and corneal inflammatory was measured histologically.

**Results:** Retinal lamellar structure and inner segment-retinal segment (IS/OS) line was observed in the normal control eyes. After development of EAU, segmentation of the inner retinal layers was observed in mice developing EAU at grade 3 level, and destruction of total retinal layers, disappearance of IS/OS line, and partial retinal detachment were presented in EAU mice of grade 3. These SD-OCT findings correlated with the histological observations.

**Conclusions:** We conclude that OCT is suitable for evaluating the development of murine EAU and reflects the histopathological changes.

**Commercial Relationships:** Kouzo Harimoto, None; Masataka Itô, None; Masaru Takeuchi, None

**Program Number:** 2519 **Poster Board Number:** D0124

**Presentation Time:** 2:45 PM - 4:30 PM

**Intraocular leukocyte response after IL-23 intravitreal injection as measured by intravitreal microscopy**

**Hyun Woong Kim**, 1, 2, **Christina Matea**, 3, 2, **Stephen R. Planck**, 2, **James T. Rosenbaum**, 1, 2

1. Icahn School of Medicine at Mount Sinai, New York, NY, USA; 2. Bascom Palmer Eye Institute, University of Miami, Miami, FL, USA; 3. Department of Ophthalmology, Busan Paik Hospital, Inje University, Busan, Republic of Korea; 4. Casey Eye Institute, Oregon Health & Science University, Portland, OR; 5. Devers Eye Institute, portland, OR.

**Purpose:** Uveitis is frequently associated with spondyloarthritides. Innate lymphoid cells that express the receptor for interleukin-23 (IL-23) have been recently implicated in the pathogenesis of ankylosing spondylitis (Sherlock et al., Nature Medicine, 2012). However, it is unclear whether IL-23 plays a major role in the intraocular inflammation characteristic of spondyloarthropathies. We examined the leukocyte response within the eyes of mice after intraocular injections of IL-23.

**Methods:** We injected IL-23 (100 ng/2 μl) into the vitreous of the right eye of BALB/c mice. Using intravitreal microscopy, we observed the leukocyte responses in the iris vessels at 6 hours (n=10) and at 24 hours (n=7) after IL-23 injection. We also injected different concentration of IL-23, 1 μg/2 μl (n=5) and monitored the same response at 3 hours and 6 hours after the injection.

**Results:** Intravitreal microscopy revealed slow blood flow of iris vessels in 4 out of 10 mice at 6 hours and in 3 out of 7 mice at 24 hours in the IL-23 100 ng group. The average number of rolling leukocytes was 1803/mm² at 6 hours and 1310/mm² at 24 hours in the 100 ng group, compared with 1310/mm² at 6 hours and 750/mm² at 24 hours in the contralateral, saline-injected control eye. Leukocyte rolling was significantly increased at 24 hours after 100 ng of intravitreal IL-23 (p<0.05), but rolling was not significantly increased at other time points or after other concentrations of IL-23. The average number of sticking leukocytes was 91.7/mm² at 6 hours and 108/mm² at 24 hours in the 100 ng group, compared with 80.5/mm² at 6 hours and 75.2/mm² at 24 hours in the contralateral control eye. The average number of extravasated infiltrating leukocytes was 199/mm² at 6 hours and 133/mm² at 24 hours in the 100 ng group, compared with 141/mm² at 6 hours and 129/mm² at 24 hours in the contralateral control eye. Leukocyte sticking and infiltration were not significantly increased at 6 hours and 24 hours after 100 ng of intravitreal IL-23 (p>0.05) and at other concentrations of IL-23.

**Conclusions:** Our data show blood flow changes and a transient increase in rolling leukocytes in iris vessels after IL-23 intravitreal injection. Further studies for the presence of resident CD3+ IL23R+ cells within eye will be informative with regard to the role of IL-23 in the uveitis.

**Commercial Relationships:** Hyun Woong Kim, None; Christina Matea, None; Stephen R. Planck, None; James T. Rosenbaum, Genentech (C), Abbott (F), Xoma (C), Eyegete (F), Bristol Myers (F), Lux (C), Novartis (C), Regeneron (C), Teva (C), Therakine (F), Mitotech (F), Aquinox (F), Allergan (C), Santen (C)

**Program Number:** 2520 **Poster Board Number:** D0125

**Presentation Time:** 2:45 PM - 4:30 PM

**Steroid refractory Th17 cells have unperturbed glucocorticoid receptor expression and trafficking**

**Philippa J. Laiti**, 1, 2, Lauren P. Schewitz-Bowers, 1, 2, Ashwin Dhandia, 1, 2, Becky L. Conway-Campbell, 1, 2, Andrew D. Dick, 1, 2, Richard W. Lee, 1, 2

1. Inflammation and Immunotherapy Theme, National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, University Hospitals Bristol NHS Foundation Trust, London, United Kingdom; 2. Academic Unit of Ophthalmal, University of Bristol, Bristol, United Kingdom.

**Purpose:** We have previously reported that both human and murine Th17 cells maintain phenotype and proliferation in the face of steroid treatment corroborated by the observations that CD4+ T cells from patients with steroid refractory (SR) uveitis are biased to express IL-17 following TCR engagement. Such IL-17 bias in the presence of steroids may be due to failure of glucocorticoid receptor (GR) nuclear translocation and over-expression of the receptor isoform GRβ. The purpose of this study was to extend our previous observations to determine whether these mechanisms confer the steroid refractory Th17 phenotype we observe in uveitis.

**Methods:** We first optimised the quantification of GR trafficking in T cells by testing a panel of GR antibodies and image analysis techniques in freshly isolated CD4+ cells from normal volunteers. Subsequently, CD4+ CCR6+ (Th17) and CD4+ CCR6- (Th0) cells were sorted (BD Influx) from the PBMCs of normal volunteers (n=4) and cultured for 2 weeks in Th17 and Th0 (unpolarised) conditions respectively, with or without exposure to the synthetic glucocorticoid dexamethasone (Dex) or RU486 (which induces GR translocation). Intracellular IL-17 and IFN-γ expression was determined by flow cytometry (BD LSR II). Nuclear translocation of GR was quantified using laser scanning confocal microscopy of paraformaldehyde fixed cells after 30 minutes exposure to Dex (anti-GR mAb, Santa Cruz) and 4:30 PM. Nuclear translocation of GR was quantified using laser scanning confocal microscopy of paraformaldehyde fixed cells after 30 minutes exposure to Dex (anti-GR mAb, Santa Cruz 3D5). Full depth z-stack images were acquired and fluorescence analyses using Velocity 6.2 (Perkin Elmer) within delineation via nuclear staining (DAPI) to facilitate quantification of red (GR) staining and expressed as total red divided by nucleus volume (nuclear density). Matched cell samples from the same volunteers were collected into RNA later after Dex treatment and the relative expression of GR isoforms was quantified when normalised to GAPDH (Applied Biosystems).

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**Results:** Fig 1 Nuclear density of GR increased significantly in both Th0 and Th17 cells after treatment with dex or positive control RU486 (multivariate ANOVA). Fig 2 Expression of total GR and the GRβ isoform is equal in human Th0 and Th17 cells, and this is not affected by exposure to Dex.

**Conclusions:** There was no significant difference in GR translocation or the expression of GR isoforms in human Th17 and Th0 cells and neither mechanism explains the glucocorticoid refractory Th17 phenotype observed clinically.

![Figure 1](image1.png)

**Figure 1**

**Figure 2**

**Figure 2**

**Commercial Relationships:** Philippa J. Lait, None; Lauren P. Schewitz-Bowers, None; Ashwin Dhand, None; Becky L. Conway-Campbell, None; Andrew D. Dick, Novartis (F), Novartis (C), Abbott (F); Richard W. Lee, Genentech (C)

**Support:** This work was funded by the NIHR Moorfields Biomedical Research Centre

**Program Number:** 2521 **Poster Board Number:** D0126 **Presentation Time:** 2:45 PM - 4:30 PM

Human monocyte subsets differentially drive T helper cell polarization: implications for the pathogenesis and treatment of autoimmune uveitis

Baoying Liu1, Ashwin Dhand2, Zhiyu Li2, Rafael Villasmil1, Richard W. Lee2, Robert B. Nussenblatt3, 1Lab Immunology, National Eye Institute/NIH, Bethesda, MD; 2Inflammation and Immunotherapy Theme, National Institute for Health Research Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, University Hospitals Bristol NHS Foundation Trust and University of Bristol, Bristol, United Kingdom.

**Purpose:** T helper 1 (Th1) and Th17 cells are thought to be the main pathogenic effectors in autoimmune uveitis. However, how human innate immunity interacts with adaptive immunity and whether this interaction contributes to uveitis pathogenesis are less well understood. Peripheral monocytes can be categorized into 3 groups: CD14dimCD16+; CD14highCD16-; and CD14highCD16+. We have previously reported that circulating monocytes from patients with autoimmune uveitis exhibit a skewed phenotype, and in this study we sought to investigate the effect this could have on T helper cell polarization.

**Methods:** Human peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy donors using a Ficoll gradient centrifugation protocol. Untouched naïve and memory CD4+ T cells were isolated based on magnetic depletion protocols (Mitenyi Biotec). The subsets of monocyte described above were purified from the same donors by flow cytometry (BDFACSaria II) based on CD14 and CD16 staining. Each monocyte subset was then co-cultured in the presence of anti-CD3 with naïve or memory T cells for 5 days, after which T cell polarization was assessed by intracellular staining with anti-interferon γ (IFN γ, Th1) and anti-IL-17 (Th17). T cell activation was also monitored by CD25 surface staining.

**Results:** CD14dimCD16+ monocytes were the only subset of monocytes to significantly increase CD25 expression on naïve CD4+ T cells. CD14dimCD16+ monocytes also polarized both naïve and memory CD4+ T cells in Th1 direction. The principal effect of CD14highCD16- monocytes was to polarize memory Th cells towards the Th17 phenotype.

**Conclusions:** In this study, which is the first to examine the effect of CD14dim cells on human CD4+ T cell polarization, we have demonstrated that human monocyte subsets differentially define both naïve and memory CD4+ T cell polarization. This highlights the critical role monocytes play in driving Th cell differentiation, and underlines their potential value, both for the stratification of disease, and as targets for immunomodulation in the treatment of human autoimmune uveitis.

**Commercial Relationships:** Baoying Liu, None; Ashwin Dhand, None; Zhiyu Li, None; Rafael Villasmil, None; Richard W. Lee, Genentech (C); Robert B. Nussenblatt, None

**Support:** National Eye Institute intramural research program

**Program Number:** 2522 **Poster Board Number:** D0127 **Presentation Time:** 2:45 PM - 4:30 PM

The spectrum of inflammatory ocular involvement in systemic lupus erythematosus in a multidisciplinary uveitis unit

Laura Pelegrin1, Alfredo Montehernoso1, Marc Figueras2, Maite Sainz de la Mata1, Bernardo Sanchez-Dalmau3, Victor Llorens1, Blanca Molins4, Marina Mesquida1, Gerard Espinosa2, Alfredo Adam Civera2, 1Ophthalmology, Hospital Clinic de Barcelona, Barcelona, Spain; 2Autoimmune Disease, Hospital Clinic de Barcelona, Barcelona, Spain; 3Autoimmune Disease, Hospital Clinic de Barcelona, Barcelona, Spain.

**Purpose:** To describe the inflammatory ocular manifestations of patients with systemic lupus erythematosus (SLE) at a multidisciplinary uveitis unit

**Methods:** Retrospective chart review of patients with SLE in a tertiary referral center between 2007 and 2012 was performed. All patients have undergone complete rheumatologic and ophthalmic examination including visual acuity, slit-lamp examination of the anterior segment and fundus examination. Fluorescein angiography and optical coherence tomography were performed if they were required.

**Results:** Twenty-three patients presented inflammatory ocular manifestations related to SLE. Patients complained of ophthalmologic disturbances with blurry vision and ocular redness as the most common symptoms. A decrease in the visual acuity was detected in 16 patients (69.6%) mostly due to retinal involvement, optic neuritis and anterior uveitis. Anterior uveitis was found in 8 patients (34.8%), peripheral ulcerative keratitis in 1 patient and diffuse scleritis in 4 patients (17.4%). Changes in retina were found in 7 patients (30.4%); the most frequent was retinal vein occlusion (central retinal vein occlusion in 2 patients and branch retinal vein occlusion in 2 patients) followed by hypertensive retinopathy with serous retinal detachment in 1 patient, occlusive vasculopathy in 1 patient and central serous choroidopathy due to corticosteroids in 1 patient. Three patients (13%) showed neuro-ophthalmological symptoms. 1 patient showed rotatory nistagmus related to central nervous system involvement, 1 patient showed optic neuritis and the remaining presented bitemporal hemianopsia.

**Conclusions:** Ocular manifestations in SLE can affect any structure in the eye. The most visually devastating damage occurs secondary to...
optic nerve involvement and retinal vaso-occlusion. Anterior uveitis is not an uncommon manifestation of SLE; physicians must be aware of this involvement since it can be treated without serious visual loss.

**Commercial Relationships:** Laura Pelegrin, None; Alfredo Montethermoso, None; Marc Figueras, None; Maite Sainz de la Maza, Allergan (C), Alcon, Labs (R), Merck Sharp and Dohme (R); Bernardo Sanchez-Dalmau, None; Victor Llorens, None; Blanca Molins, None; Mara Mesquida, None; Gerard Espinosa, None; Alfredo Adan Civera, None

**Purpose:** A recently identified gene NOD2 is genetically associated with Blau syndrome, a granulomatous uveitis that is accompanied by arthritis and dermatitis. NOD2 belongs to a family of innate immune receptors characterized by a high affinity for peptidoglycan from bacterial cell walls. NOD2 is upregulated in many different uveitides, and a recent study in the mouse model of experimental autoimmune uveitis (EAU) demonstrated that cardiac-derived NOD2 rich macrophages can promote a Th17 cytokine profile and inflammation.

**Methods:** The contribution of NOD2 was investigated using Nod2 knockout (KO) mice and C57BL/6J congenic wild type (WT) controls, immunized for EAU with interphotoreceptor retinoid-binding protein (IRBP). Disease was followed clinically by topical endoscopic fundus imaging (TEFI) on d14 and d21 post-immunization and confirmed by histopathology (d21). IRBP-specific cytokine production by splenocytes was measured by ELISA. To determine the cellular compartment associated with uveitis susceptibility in the Nod2 KO animals, bone marrow (BM) chimeras of WT and Nod2 KO mice were subjected to EAU induction.

**Results:** IRBP-immunized Nod2 KO mice developed significantly more severe EAU than their WT controls (p<0.01, n=13-14 mice/group). TEFI revealed NOD2-associated retinal damage in IRBP-immunized mice that was manifest by severe papillary and peripapillary inflammation, extensive retinal vascular cuffing, and florid retinitis. Eyes of mice injected with adjuvant alone (Nod2 KO and WT) mice did not exhibit any signs of inflammation. Histology in Nod2 KO mice revealed marked increase in severity of uveitis (p<0.01 vs. WT, n=13-14 mice/group) characterized by increased retinal hemorrhages, folding, detachment, and structural damage that were accompanied by perivascular, optic disc, and vitreous infiltration. Notably, granulomatous inflammation was a predominant immunopathological feature of Nod2 KO mice. Interestingly, although IL-17A rather than IFN-γ was shown to be critical for IRBP-EAU development, the deleterious effects of NOD2 deficiency were associated with augmented IRBP-specific T cell production of IFN-γ and diminished IL-17A production. BM chimera studies suggested that NOD2 expression in the hematopoietic compartment predominately contributed to inflammation protection.

**Conclusions:** These data reveal an unexpected and critical role for NOD2 in the hematopoietic cell compartment to dampen uveitogenic T cell responses.

**Commercial Relationships:** Ellen J. Lee, None; Joao M. Furtado, None; Brieneann Brown, None; Emily E. Vance, None; John Paul Sacdal, None; Varunika Bhargava, None; Justine R. Smith, None; Phyllis Silver, None; Rachel R. Caspi, None; Holly L. Rosenzweig, None

**Support:** NEI/NIH Grant EY019020, Research to Prevent Blindness Foundation, NEI Intramural support (project # EY000184-30 LI)

**Program Number:** 2524 Poster Board Number: D0129

**Presentation Time:** 2:45 PM - 3:40 PM

**Exhausted effector memory CD8 T cells expand in chronic EAU Joanne Boldison1, David A. Copland2, Philippa J. Lait3, Tarnjit K. Khera2, Andrew D. Dick1,2, Lindsay B. Nicholson1,2.

1Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom; 2Academic Unit of Ophthalmology, University of Bristol, Bristol, United Kingdom.

**Purpose:** Using CD4 T cell mediated Experimental Autoimmune Uveitis (EAU) as a model for human non-infectious intraocular inflammation (uveitis) we have demonstrated short and long scale immunopathological feature of temporal variation in leukocyte populations that infiltrate the retina during RBP-3 (IRBP) peptide induced disease. CD8 T cells are particularly prominent in the late phase of disease, where their role in retinal inflammation is unclear. As CD8 T cells may be both pathogenic and regulatory in autoimmunity our purpose was to interrogate the function and phenotype of our recently identified CD8 T cell population during secondary progressive EAU.

**Methods:** C57BL/6J mice were immunised with RBP-3 1-20 peptide. Disease progression was assessed by Topical Endoscopic Fundal Imaging (TEFI) and retinal infiltrate quantified by flow cytometry. Cytokine production was measured by intracellular cytokine staining. Cytotoxic potential and function was assessed by flow cytometric CD107a and granzyme B expression. Monoclonal antibodies YTS 169.4 and 156.7 directed against CD8 were used for in vivo depletion of CD8 T cells.

**Results:** In the secondary progressive phase of EAU the infiltrating effector memory CD8 T cells expressed cell surface markers associated with recent antigen stimulation. From day 35 post immunisation there was a significant expansion of CD8 T cells that lacked cytokine production and evidence of recent degranulation, as determined by CD107a expression. Surface expression of PD-1 increased and was associated with a lack of effector function, with approximately 90% of PD-1- CD8 T cells phenotypically CD69lowLy6c+.

**Conclusions:** In contrast to peak disease, the retinal infiltrate during persistent disease was characterised by CD8 T cells that lacked effector function and showed some signature changes of exhaustion (increased PD-1) associated with chronic antigen stimulation.

**Commercial Relationships:** Joanne Boldison, None; David A. Copland, None; Philippa J. Lait, None; Tarnjit K. Khera, None; Andrew D. Dick, Novartis (C), Novartis (F), GSK (F), Abbott (F); Lindsay B. Nicholson, None

**Support:** National Eye Research Centre, Bristol, UK

**Program Number:** 2525 Poster Board Number: D0130

**Presentation Time:** 2:45 PM - 4:30 PM

**Intratracheal Administration of Interphotoreceptor Retinoid-Binding Protein Peptide Suppress Murine Experimental Autoimmune Uveitis Toshikatsu Kaburaki1, Xiangyuan Jin2, Masateru Uchiyama3, Mitsuko Takamoto4, Hisae Nakahara5, Hidetoshi Kawashima5, Shiho Amano3, Masanori Nishi3.

1Ophthalmology, Univ of Tokyo School of Medicine, Bunkyo-Ku, Japan; 2Surgery, Teikyo University School of Medicine, Itabashi-ku, Japan; 3Ophthalmology, Jichi Medical University, Shimotsuke-shi, Japan.

**Purpose:** To reproduce any abstract, contact the ARVO Office at arvo@arvo.org.
Purpose: Mucosal administration of autoantigens results in the development of a state of peripheral immunological tolerance. Previous study demonstrated that intranasal administration of retinal antigens induced transient T cell activation and apoptosis within drainage lymph nodes but not spleen in murine experimental autoimmune uveitis (EAU) model. In this study, we examined the effects of intratracheal administration of retinal antigens in murine EAU and clarified the mechanisms of the suppression of uveitis.

Methods: Murine EAU (C57BL/6) was induced with subcutaneous injection of human interphotoreceptor retinoid-binding protein (IRBP) peptide mixed with complete Freund’s adjuvant. In IRBP-treated group (I-Group), 100µg of IRBP peptide was administrated intratracheally 7 days before immunization, whereas in control group (C-group), 100µg of saline was administrated. Clinical and histopathological scoring of EAU, cytokine production, T cell proliferation assay and numbers of CD4+CD25+Fox-p+ regulatory T (Treg) cells in regional lymph nodes and spleen were examined.

Results: The average histopathological EAU score was 2.5±1.5 in C-group and 1.1±1.2 in I-group at 21 days after immunization (p<0.01, Student's unpaired t-test), respectively. Interferon-γ and IL-12 production in regional lymph nodes cells and splenocytes were significantly suppressed in I-group compared to those in C-group (p<0.05). Antigen-specific T cell proliferation of regional lymph nodes cells and splenocytes were also significantly suppressed in I-group compared to those in C-group (p<0.05).

The percentages of Treg cells in the whole regional lymph nodes cells and splenocytes were significantly increased in I-group (3.87±0.20% and 3.90±0.76%) compared to those in C-group (2.93±0.14% and 2.67±0.67%, p<0.01).

Conclusions: Intratracheal administration of IRBP peptide has an inhibitory effect on murine EAU, probably through the increase of Treg cells in regional lymph nodes and spleen and the decrease of antigen-specific T cells.

Commercial Relationships: Toshikatsu Kaburaki, None; Xiangyuan Jin, None; Masateru Uchiyama, None; Mitsuco Takamoto, None; Hisae Nakahara, None; Hidetoshi Kawashima, None; Shiro Amano, Topcon (P); Masanori Niimi, None

Support: This study was supported in part by Grants-in-Aid for Japanese Foundation for Applied Enzymology in 2010.

Program Number: 2526 Poster Board Number: D0131
Presentation Time: 2:45 PM - 4:30 PM

Incidence of Endogenous Intraocular Inflammation in the Central Tokyo of Japan for 8 Years from 2004 to 2012

Purpose: To report the clinical statistical analysis of patients with endogenous intraocular inflammation in the central Tokyo for 8 years from 2004 to 2012.

Methods: This retrospective study involved 789 new patients with endogenous intraocular inflammation who visited Nippon Medical School Hospital for 8 years from April 2004 to October 2012.

Results: The subjects comprised 374 men and 415 women. The ratio of men to women was 1.1:1. The age averaged 50.7±16.7 years. Definitive diagnosis was made in 503 cases (63.8%). The most frequent clinical entity was sarcoidosis (17.5%), followed by scleritis (12.9%), Vogt-Koyanagi-Harada disease (4.6%), herpetic iridocyclitis without acute retinal necrosis (4.6%), human leukocyte antigen (HLA)-27-associated uveitis (3.0%), Behçet’s disease (2.5%). The subjects were classified into four groups; adolescent (0–19 years), young (20–39), middle-aged (40–59), and old (60 years and older). The most frequent clinical entity of adolescent group was juvenile rheumatoid arthritis -associated iridocyclitis. In other age group, sarcoidosis was the most frequent. This study was also classified into 4 parts of intraocular inflammation. Anterior uveitis was the most frequent, compared with intermediate, posterior, and pan-uveitis. The incidence of secondary glaucoma was 26.5%, and steroid responder was about 30% among of them.

Conclusions: Generally, sarcoidosis, Vogt-Koyanagi-Harada disease, and Behçet’s disease are the most frequent intraocular inflammations in Japan. The characteristic of this study was that scleritis and herpetic iridocyclitis were also frequent intraocular inflammation at Nippon Medical School Hospital.

Commercial Relationships: Motoko Serizawa, None; Yukiko Ito, None; Reiko Tsukada, None; Hiroshi Takahashi, None; Hiroko Taniguchi, None; Junko Hori, None

Program Number: 2527 Poster Board Number: D0132
Presentation Time: 2:45 PM - 4:30 PM

Non-infectious Uveitis: Emotional and personality findings

Purpose: 1)To determine if the presence of emotional factors related to the personality predispose to the occurrence of non-infectious uveitis. 2)To assess if previous stressing situations in personal life are associated with the onset of non-infectious uveitis.

Methods: This is a prospective study performed in the Uveitis Section and the Department of Mental Health, at the University of Buenos Aires, Argentina. Thirty-six patients divided into two groups (18 with non-infectious uveitis and 18 without uveitis) were asked to fill out the Type D Scale-14 (DS14) in order to assess Type D personality, consisting in negative affectivity (NA) plus social inhibition (SI) and the Social Readjustment Rating Questionnaire(SRRQ) so as to determine whether stressful life events contribute to the onset of uveitis. NA is related to dysphoria worry and irritability, SI covers discomfort in social interactions, reticence and lack of social poise. Data concerning socio-demographics as gender, age, best-corrected visual acuity at time of questionnaire, anxiety and depressiveness were collected. Disease following the SUN-criteria, age at onset, time since diagnosis, uni- or bilateral disease (in the group with uveitis) were collected. Data were analyzed using Mann-Whitney test. The significance level was 0.05.

Results: The frequency of Type D personality was 44.4% in the uveitis group and 27.8% in the group of patients without uveitis being this difference non statistically significative (P=0.298). Beside, although NA subscale showed similar medium values in the two groups(P=1.00), the SI subscale yielded greater values in the uveitis group. In this case, the difference was statistically significative (P= 0.024).The risk of illness characterized by the presence of stressful events in the person’s life measured by the SRRQ was comparable in both groups (P= 0.235).

Conclusions: The presence of stressful events in personal life has not shown signs of being a determinant factor in triggering non-infectious uveitis. On the other hand, patients with non-infectious uveitis show an inhibition in the expression of emotions/behaviors in social interactions and a tendency towards negative affectivity. Although Type D personality has not reached a significative difference in this sample, the results suggest the fact that this personality type should be taken into account as another issue to be approached in the therapeutic management of these patients.

Commercial Relationships: Matilde Lopez, None; Cristobal A. Couto, None; Maria de las Mercedes Frick, None; Erika Miolet Hurtado Jalala, None; Bernardo A. Schlaen, None; Nora Taubenslag, None

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Conjunctival biopsy analyzed by: A) direct immunofluorescence with positive BMZ; B) avidin-biotin complex immunoperoxidase with positive BMZ.

Program Number: 2529 Poster Board Number: D0134
Presentation Time: 2:45 PM - 4:30 PM
Incidence and Prevalence of Episcleritis and Scleritis in Northern California
Grace Honik1, Ira G. Wong2,3, David C. Gritz4, 1Montefiore Medical Center, Bronx, NY; 2Proctor Foundation UCSF, San Francisco, CA; 3Stanford University, Stanford, CA.

Purpose: Evaluate the incidence and prevalence of episcleritis and scleritis in a large, well-defined population in Northern California.

Methods: Secondary data analysis was performed on data from the Northern California Epidemiology of Uveitis Study (NCEU). The patient database of a large, regional health maintenance organization was searched for all patients who potentially experienced ocular inflammatory disease during the 12-month study period. Medical records were reviewed for all potential patients to confirm ocular inflammatory disease and the specific diagnosis, establish time of onset, and collect additional data. Age- and gender-stratified quarterly study population data were used to calculate incidence rates and prevalence ratios.

Results: The midperiod population was 731,895 for the study population. After reviewing 2011 possible cases, 297 new onset cases of episcleritis, 39 prior onset cases of episcleritis, 25 new onset cases of scleritis, and 8 prior onset cases of scleritis were confirmed. For patients with episcleritis, overall the incidence was 40.7 per 100,000 person-years. Females between the ages of 45-64 had the highest incidence rate (74.0 per 100,000 person years) and the highest prevalence ratio (91.5 per 100,000 persons). Men from the ages of 25-44 had the highest incidence rate (41.1 per 100,000 person years) and prevalence ratio (43.6 per 100,000 persons).

The overall incidence of scleritis was 3.4 per 100,000 person-years. Females aged 65 years or older had the highest incidence of scleritis (8.1 per 100,000 person-years) and the highest prevalence ratio (12.1 per 100,000 persons). For men, those from 45-64 years of age had the highest incidence rate (5.5 per 100,000 person-years) and prevalence ratio (8.7 per 100,000 persons) for scleritis.

Scleritis patients were older than episcleritis patients, with a mean age of 45.6 and 52.6, respectively (p=0.017).

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**Conclusions:** This report found that scleritis patients were older than episcleritis patients, and that women had higher rates of both episcleritis and scleritis compared to men.

**Commercial Relationships:** Grace Honik, None; Ira G. Wong, None; David C. Gritz, None

**Support:** Research to Prevent Blindness Foundation unrestricted institutional grant award

**Program Number:** 2530 Poster Board Number: D0135

**Presentation Time:** 2:45 PM - 4:30 PM

**Application of the diagnostic criteria of the International Workshop on Ocular Sarcoidosis in patients with sarcoid uveitis in a tertiary center**

Eva Jakob\(^1\), Regina Max\(^1\), Matthias D. Becker\(^2\), Friederike Mackensen\(^1\). \(^1\)Interdisciplinary Uveitis Center, University of Heidelberg, Heidelberg, Germany; \(^2\)Eye Hospital, Triemli Hospital, Zürich, Switzerland.

**Purpose:** Presenting in various forms and leaking of pathognomonic signs, the diagnosis of sarcoid uveitis is challenging. The recently published results of the First International Workshop on Ocular Sarcoidosis (IWOS)* suggest 4 diagnostic categories to standardize the criteria and nomenclature of ocular sarcoidosis. Goal of this study is to assess the clinical application of these criteria in a tertiary center and to analyse the distribution of the different diagnostic subgroups. 

* International Criteria for the Diagnosis of Ocular Sarcoidosis: Results of the First International Workshop on Ocular Sarcoidosis, C. Herbort et al., Ocul Immunol Inflamm 2009

**Methods:** After identifying all patients in our electronic database who presented 2005 and later to our center with sarcoid uveitis we retrospectively applied IWOS criteria. In our records, we counted bilateral smoldering chronic inflammation, good response to steroids and presence of scd25 also to the diagnostic criteria. So far, we defined patients with biopsy proven or radiologic diagnosis of sarcoidosis as systemic sarcoidosis, patients with a typical uveitis and elevated ACE or scd25 in absence of systemic findings as ocular sarcoidosis. As suggested by IWOS we then classified the detected patients (with and without systemic disease) in the following levels of certainty: Definite, presumed, probable and possible ocular sarcoidosis.

**Results:** So far, we analyzed the data of 179 patients presenting the first time between 2005 and 2011. They were classified as following: 26% definite, 35% presumed: 8% probable; and 1% possible ocular sarcoidosis. Not fulfilling all criteria for one of the groups 25% of the patients who in our records were clinically diagnosed as sarcoidosis were not suitable for one of the groups. For the conference, we will be able to present the complete data of all patients who presented from 2005 until end of 2012.

**Conclusions:** Taking into account the difficulties of a retrospective analysis, the criteria of IWOS were applicable in most of our patients. However, we had several patients diagnosed with sarcoid uveitis by us, who could not be classified in one of the suggested subgroups. Reason for this might be that we take into consideration more symptoms (smoldering chronic inflammation, good response to steroids, scD25 elevation, skin symptoms) for the diagnosis.

**Commercial Relationships:** Eva Jakob, None; Regina Max, Roche (R), Abbott (R); Matthias D. Becker, Novartis (F), Bayer (F), Allergan (F); Friederike Mackensen, Abbott (F), Heidelberg Engineering (F), Heidelberg Engineering (R)

**Program Number:** 2531 Poster Board Number: D0136

**Presentation Time:** 2:45 PM - 4:30 PM

**Telomere Length of peripheral leukocytes is shortened in Ocular Sarcoidosis patients**

Ian A. Thompson\(^1\), Baoying Liu\(^1\), H Nida Sen\(^1\), Bogdan Dumitriu\(^2\), Rodrigo Calado\(^2\), Sima Hirani\(^1\), Mary Morgan\(^3\), Shayma Jawad\(^2\), Neal Young\(^2\), Robert B. Nussenblatt\(^1\). \(^1\)Immunology, NEI, Bethesda, MD; \(^2\)Hematology Branch, NHLBI, Bethesda, MD.

**Purpose:** Chronic inflammatory activity has been associated with leukocyte telomere shortening, and short telomere length may occur with higher frequency in autoimmune diseases including sarcoidosis. Tumor necrosis factor - alpha (TNF-alpha), a marker of systemic inflammation, has been reported to promote telomere attrition in lymphocytes through the inhibition of telomerase (hTERT) activity. The aim of this study was to determine if telomere length is shortened in peripheral blood leukocytes of ocular sarcoidosis patients as compared to healthy controls, and to investigate the relationship between lymphocyte telomere length and TNF-a serum level.

**Methods:** Telomere length of leukocytes was measured by quantitative polymerase chain reaction in 44 ocular sarcoidosis patients, and compared to 300 age-matched healthy control samples. 35 of the 44 ocular sarcoidosis patients and 40 healthy controls had their serum levels of TNF-alpha measured by an enzyme-linked immunosorbent assay. TNF-alpha levels were then correlated with leukocyte telomere lengths.

**Results:** Ocular sarcoidosis patients demonstrated significantly shorter leukocyte telomere lengths when compared with age-matched controls. There was weak negative correlation between leukocyte telomere length and age (r = - .24, p = 0.11) in ocular sarcoidosis patients. Very short peripheral blood leukocyte telomere lengths (below the first percentile as compared to age-matched controls) were identified in six out of 44 patients. Ocular sarcoidosis patients did not differ from healthy controls in TNF-alpha serum levels.

**Conclusions:** Telomere attrition in leukocytes is a plausible cause for abnormal leukocyte cell function in ocular sarcoidosis patients. Functional studies are underway to address this.

**Commercial Relationships:** Ian A. Thompson, None; Baoying Liu, None; H Nida Sen, None; Bogdan Dumitriu, None; Rodrigo Calado, None; Sima Hirani, None; Mary Morgan, None; Shayma Jawad, None; Neal Young, None; Robert B. Nussenblatt, None

**Support:** NIH Intramural Research Program
CD11c+ cells were present in Tenon's layer; and C3 and immunoglobulin (Ig)G and IgM were seen in the anterior sclera in contact with the ciliary body. In the cornea, CD31 and LYVE-1 expression was increased compared to untreated mice; and infiltration of CD4+, CD11b+, and CD11c+ cells was present.

**Conclusions:** With enhanced immunization in a collagen-induced anterior scleritis model, infiltration of T cells, macrophages, and dendritic cells was seen not only in the sclera, but also in the cornea; and blood and lymphatic vessels were increased.

**Commercial Relationships:** Hiroko Taniguchi, None; Yuki Kitahara, None; Junko Hori, None

**Support:** Grants-in-Aid for Scientific Research (C) from Japan Society for the Promotion of Science

**Program Number:** 2533 **Poster Board Number:** D0138

**Presentation Time:** 2:45 PM - 4:30 PM

**Factors in the Initial Presentation to Predict Subsequent Ocular Complications of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis**

**Shin-yi Chen**, Wen-Hung Chung, David H. Ma. 1 Ophthalmology, Chang Gung Memorial Hospital, Taoyuan, Taiwan; 2 Dermatology, Chang Gung Memorial Hospital, Taoyuan, Taiwan.

**Purpose:** To evaluate the severity of ocular complications of patients with Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and SJS/TEN overlap; and to investigate the correlation between it and clinical characteristics, hemogram, and serum chemistry data of the patients at initial presentation.

**Methods:** Retrospective observational case series. We review the charts of all patients admitted to Chang Gung Memorial Hospital, Taiwan, with the diagnosis of SJS, TEN and SJS/TEN overlap between 1998 and 2011. Patients who met the criteria from clinical presentation, positive skin biopsy, and received ocular exams from ophthalmologic consultations were included. Acute and chronic ocular complications were graded as mild, moderate, or severe. Chronic complication was defined as ocular manifestation after 6 months. The hemogram and serum chemistry data at admission was used.

**Results:** A total 334 patients were included. Age and gender were not significantly correlated with the severity of ocular complications. The severity of acute ocular involvement was correlated with oral mucosal involvement (P=0.006), genital mucosa involvement (P=0.003), fever in the first three days (P=0.051) and the total body surface area (TBSA) of epidermal detachment (P=0.017). There was a high association between the severity of acute ocular involvement and the severity of chronic ocular complications (P<0.001). Multivariate analyses of the initial hemogram and serum chemistry data, platelet count and C-reactive protein (CRP) levels are strongly associated with chronic ocular complications (platelet count: odds ratio=0.41, P=0.001; CRP: odds ratio=1.01, P=0.052). These two factors combined with genital mucosal involvement yield 76% predictive rate for chronic ocular complications.

**Conclusions:** The severity of acute ocular involvement is associated with oral and genital mucosal involvement, fever, and TBSA of epidermal detachment at the initial presentation. Lower platelet count and higher CRP level at admission increase the risk of subsequent chronic ocular complication.

**Commercial Relationships:** Shin-yi Chen, None; Wen-Hung Chung, None; David H. Ma, None

**Program Number:** 2534 **Poster Board Number:** D0139

**Presentation Time:** 2:45 PM - 4:30 PM

**Conceal Pathology Profile in the Absence of a Functional Type I Interferon Pathway Following HSV-1 Infection**

Ana J. Chucair-Elliott, Christopher D. Conrady, Min Zheng, Daniel J. Carr. 1 Ophthalmology, Univ of Oklahoma Hlth Sci Ctr, Oklahoma City, OK; 2 Microbiology and Immunology, Univ of Oklahoma Hlth Sci Ctr, Oklahoma City, OK.

**Purpose:** Type I interferon (IFN) production elicited by HSV-1-driven IFN16p204 sensor activation in corneal epithelial cells is critical for viral surveillance. How type I IFN pathways regulate tissue pathology in the cornea is unknown. Previously, we reported mice that lack the type I IFN alpha 1 chain (CD118-/-) were highly susceptible to ocular HSV-1 infection. Here, we hypothesize an aberrant immune cell infiltrate and the loss of viral containment in the cornea contribute to significant corneal pathology in the CD118-/- mice mainly by immune-mediated events.

**Methods:** C57BL/6 wild type (WT) and CD118-/- mice were infected with 1,000 plaque forming units of HSV-1 (McKrae strain). At times post infection (pi), the mice were euthanized, and the corneas were collected and processed for immunofluorescence by flow cytometry, viral titers by plaque assay, leukocyte and HSV-1 antigen expression by immunohistochemistry and confocal microscopy, and MMP9 levels by ELISA. We compared corneal pathology including corneal thickness, opacity, and fluorescein staining by histological techniques and slit lamp imaging.

**Results:** CD118-/- infected corneas had higher viral loads than WT mice, but harbored fewer NK cells, macrophages, and inflammatory monocytes but elevated neutrophils compared to WT mice by day 5 pi. Ly6G+ neutrophils within the stroma of CD118-/- mice co-stained with HSV-1 antigen but this was not evident in WT mice. However, adoptive transfer of neutrophils from the cornea of WT or CD118-/- mice into naïve CD118-/- mice resulted in 50 and 100% mortality respectively of the recipients. Along with elevated viral titer, CD118-/- mice exhibited almost complete loss of corneal epithelial cell layers despite overall thickening of the cornea. The pathology was consistent with slit lamp images that showed a dramatic increase in the areas of epithelial lesions, corneal opacity, and loss of iris details coinciding with a significant increase in MMP9 levels in the CD118-/- mice.

**Conclusions:** The loss of a functional type I IFN pathway pre-disposes the cornea to substantial pathology following HSV-1 infection associated with larger viral loads and a significant increase in neutrophil influx and MMP9 expression. Infiltrating neutrophils from WT and CD118-/- mice endocytose virus that is not destroyed and may serve as an additional source for virus to replicate and disseminate.

**Commercial Relationships:** Ana J. Chucair-Elliott, None; Christopher D. Conrady, None; Min Zheng, None; Daniel J. Carr, None

**Support:** EY 021238

**Program Number:** 2535 **Poster Board Number:** D0140

**Presentation Time:** 2:45 PM - 4:30 PM

**IRF-8 Antagonizes Th17 and Tc17 Expansion and Restraints**

Sung-Hye Kim, Chengrong Yu, Bernadette Marrero, Charles E. Egwuagu. Laboratory of Immunology, NEL Bethesda, MD.

**Purpose:** Interferon Regulatory Factor (IRF) factors play important roles in host immunity. Currently, there are nine known IRFs. They are expressed ubiquitously, except IRF-8 whose expression is restricted to myeloid and B cells. However, when naive T cells are stimulated with Ag, IRF-8 is copiously induced. Nonetheless, the significance of IRF-8 expression in activated T cells is unknown. In this study, we have investigated the function of IRF-8 in activated T cells of mutant mice with targeted deletion of IRF-8 in CD4 and CD8 T cells (CD4/CD8KO).

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Methods: We generated the CD4/CD8 KO mouse strain by breeding IRF-8 floxed mice (IRF-8frf/f) (kind gift from Herbert Morse, NIH) with CD4/Cre mice. Deletion of IRF-8 was confirmed by western blot analysis of TCR-activated CD4+ and CD8+ T cells from the CD4/CD8KO mice. Homozygous CD4/CD8 IRF-8KO mouse strain was established by several cycles of brother-sister mating. Effectors functions of CD4/CD8KO T cells were characterized by Thymidine-incorporation assay, CFSE labeling, RT-PCR, western blot analysis, intracellular cytokine staining assays. In vivo functions of IRF-8 were investigated by active immunization of WT or CD4/CD8KO mice with IRBP/CFA and we examined whether IRF-8 in T cells can influence the development or susceptibility to experimental autoimmune uveitis (EAU). We monitored the progression and severity of EAU by fundoscopy and histology. Results: Compared to WT, CD4/CD8KO mice developed a more severe EAU, which is characterized by massive infiltration of cells into the retina, papilledema, focal retinitis, retinal vasculitis and multifocal-chorioiditis. Compared to WT, CD4/CD8KO T cells produced three-folds and four-folds higher levels of IL-17-expressing Th17 and Tc17 cells, respectively, which may have contributed to the severe ocular pathology. CD4+ and CD8+ T cells from CD4/CD8KO exhibited higher proliferative capacity and were more susceptible to apoptosis.

Conclusions: Our data suggest that IRF-8 expression in activated T cells antagonizes Th17 and Tc17 expansion and restrains excessive Th17 inflammatory responses that occur during autoimmune disease. On the other hand, enhanced apoptosis manifested by CD4/CD8KO T cells suggests that IRF-8 may enhance productive immune responses against pathogens (e.g. S.aureus, S. Pneumoniae, Candida albicans and other fungi) by conferring stability to the Th17 and Tc17 T cell subsets.

Commercial Relationships: Sung-Hye Kim, None; Chengrong Yu, None; Bernadette Marrero, None; Charles E. Egwuagu, None

Program Number: 2536 Poster Board Number: D0141 Presentation Time: 2:45 PM - 4:30 PM Evaluation of JAK inhibition with topical tofacitinib in an experimental autoimmune uveitis model (EAU) Jing-Feng Huang1, Yi Zhang2, Brad Hirakawa2, 1La Jolla BioConsulting, San Diego, CA; 2bioTheranostics, Inc., San Diego, CA; 3Pfizer Inc., San Diego, CA. Purpose: To evaluate the therapeutic efficacy of topical Janus kinase (JAK) inhibitor, tofacitinib, in intraocular inflammation in EAU. Methods: Albino Lewis rats were randomly divided into four groups (N=15 each) on Day 1, before injected in the footpad with S-Antigen (100 µg in 100 µl) in Freud’s complete adjuvant to induce EAU. From Day 7 to the end of the study (Day 16), animals were treated with either one of the four: no treatment, systemic cyclosporine A (CsA, 20 mg/kg) once per day, topical tofacitinib (0.03%) three times per day (3x) or once per day (1x). Severity of inflammation was evaluated with a slit-lamp every two days from Day 10 to Day 16. On Day 16, images from day 10 animals were prepared for histological evaluation (retinal thickness and cell infiltration). From the rest of animals in each group, vitreous fluid were collected for analysis of cytokine expression levels. Histology analyses (mean percentage of infiltrated leukocytes, mean clinical score on Day 16 was 2.6 ± 3.0 compared with 3.7 ± 2.9 in the non-treated group). No significant effect was observed in tofacitinib (1x) group, while systemic administration of CsA completely blocked intraocular inflammation in EAU. Compared with non-treated group, tofacitinib (3x) group had significantly lower level of inflammatory chemokine (TNFalpha, lymphotoxin, CCL2, CCL5 and CXCL10) in the vitreous, and markedly lower gene expression level of inflammatory chemokine, immune mediators and receptors (CCL2, CCL5, CXCL10, S100A8, CCR5 and IL-2Receptor gamma) in both ICB and retina/choroid (P < 0.05).

Conclusions: JAK inhibition with topical tofacitinib three times a day reduced intraocular inflammation in autoimmune uveitis. It markedly suppressed the expression of many inflammatory chemokine and chemokine receptors in ocular tissues, and reduced infiltration of immune cells and subsequent tissue damage.

Commercial Relationships: Jing-Feng Huang, Pfizer Inc. (E); Yi Zhang, None; Brad Hirakawa, Pfizer (E)

Program Number: 2537 Poster Board Number: D0142 Presentation Time: 2:45 PM - 4:30 PM Characteristics of patients who attain remission of inflammatory eye disease following treatment and discontinuation of methotrexate Kevin Lai, Tiffany Truong, Travis Jenkins, Zvi A. Kresch, Sanjay Kedhar, Vicente Diaz, John V. Mauro, C. Michael Samson. Ophthalmology, New York Eye and Ear Infirmary, New York, NY. Purpose: Immunomodulatory therapy (IMT) is becoming an increasingly used treatment for inflammatory eye disease. This study investigated the clinical course of patients with inflammatory eye diseases who were treated with methotrexate, obtained remission and were subsequently followed after discontinuation of the medication. The purpose of the study was to determine the characteristics of patients who stay in remission versus develop recurrences.

Methods: A retrospective chart review was conducted on a cohort of 50 patients with inflammatory eye disease treated with methotrexate at a tertiary care center. Patients who were treated with methotrexate until their inflammatory eye disease was inactive were reviewed for up to two years following the discontinuation of methotrexate for recurrence. Factors examined included age, sex, length of methotrexate treatment and concurrent or serial use of other IMT.

Results: 80% of the patients reviewed were female; 20% were male. Average age of patients was 39 years old (range 4-74). Average length of methotrexate treatment was 2.2 years (range 0.6 to 5.1). 16% of patients were treated with concurrent or serial IMT, 65% of female patients and 5% of male patients attained remission at two years. Average age of patients who attained remission at two years was 39 years old (range 4-74). Average age of patients who developed recurrent disease within two years was 40 years old (range 6-72). Average length of methotrexate treatment in patients who attained remission at two years was 1.9 years (range 0.8-4.3). Average length of methotrexate treatment in those who developed recurrent disease within two years was 2.6 years (0.6-5.1). 66% of patients on concurrent or serial use of other IMT and 64% of patients on methotrexate alone attained remission at two years.

Conclusions: Female patients were more likely than male patients to attain remission at two years. The average length of methotrexate treatment in patients who developed recurrence within two years was greater than those who attained remission at two years, but this difference was not statistically significant and may be related to cases that were more difficult to control. Age and concurrent or serial use of other IMT were not associated with remission or recurrence. These results may help ophthalmologists in their treatment and counseling of patients with inflammatory eye disease.
Switching Tumor Necrosis Factor Alpha Antagonists in Patients with Scleritis

Kourtney Houser, Heather B. Leisy, Stephen M. Huddleston, R Christopher Walton. Ophthalmology, Hamilton Eye Institute, University of Tennessee Health Science Center, Memphis, TN.

Purpose: To describe the outcomes of patients with noninfectious scleritis after switching from one TNF-α antagonist (TNFA) to a second TNFA after failure or intolerance of the first.

Methods: Retrospective, interventional, consecutive case series of all patients with non-necrotizing scleritis treated with at least two TNFA between 2000 and 2010 at a single institution. Information regarding associated systemic diseases, scleritis severity, systemic immunosuppressive therapy, duration of treatment of the initial TNFA and reason for discontinuation was collected. Outcome measures were time to resolution of inflammation after initiation of the second TNFA, presence of inflammation after 6 and 12 months, medication side effects and ocular complications.

Results: 11 patients (4 male, 7 female) with mean follow-up 33.1 months (median 22 months, range 6-113 months) were included in the study. Initial TNFA was etanercept (n=7), infliximab (n=3) or golimumab (n=1), with median duration of therapy of 10 months (range 2-74 months). Reasons for discontinuation of initial TNFA were secondary failure (n=7), primary failure (n=3), and intractable headache (n=1). Patients were switched to adalimumab (n=8) or infliximab (n=3) for their second TNFA. Median time to resolution of inflammation after initiation of second TNFA was 4 months (range 1.5-11 months). After 6 months, 9 patients were in remission, 1 patient had less severe scleritis, and 1 patient had persistent, active scleritis. After 12 months, 10 patients had achieved remission, and 1 patient had persistent active scleritis. Of the 10 patients who achieved remission, one patient discontinued the second TNFA following 10 months of successful therapy due to a severe infusion reaction. The remaining 9 patients maintained complete remission of inflammation for at least 12 months with the second TNFA. Complications occurring during treatment with the second TNFA included new or worsening posterior subcapsular cataract (n=2), ocular hypertension (n=1), and severe infusion reaction secondary to infliximab (n=2).

Conclusions: Our results suggest that failure of one TNFA in patients with scleritis does not preclude successful treatment with a second TNFA. Thus, switching to a second TNFA after failure of a first may be a useful treatment option in patients with refractory scleritis.

Commercial Relationships: Kourtney Houser. None; Heather B. Leisy. None; Stephen M. Huddleston. None; R Christopher Walton. None

Support: Unrestricted Grant from Research to Prevent Blindness

Program Number: 2538 Poster Board Number: D0143
Presentation Time: 2:45 PM - 4:30 PM

Anti-Tumor Necrosis Factor Agents in Infectious Inflammatory Eye Disease
Careen Y. Lowder1, Maria M. Choudhary2, Rula hajj-alı3, Sunil K. Srivastava1. 1Cole Eye Institute, Cleveland Clinic, Cleveland, OH; 2Internal Medicine, Cleveland Clinic, Cleveland, OH; 3Orthopedics and Rheumatologic Institute, Cleveland Clinic, Cleveland, OH.

Purpose: To compare the effectiveness of anti-tumor necrosis factor (anti-TNF) agents in adults with non-infectious inflammatory eye disease.

Methods: This was a retrospective chart review of patients with non-infectious uveitis and scleritis treated with anti-TNF agents between 2003 and 2011. Patients >18 years with non-infectious inflammatory eye disease on anti-TNF agents were included. Primary outcomes were time to first remission and time to sustained remission (absence of inflammation and prednisone dose of <10 mg). Proportional hazards regression model was used to adjust for subjects who used more than one anti-TNF agent. Multivariate analysis with backward variable selection was performed to adjust for significant covariates.

Results: Ninety four patients were included: mean age 45.1 (range 19 - 79 years), 69% (65) women, median follow up 40 months; 43 (45.7%) had anterior uveitis, 23 (24.5%) pan, 9 (9.6%) posterior, 3 (3.2%) intermediate uveitis and 16 (17%) had scleritis. 41 patients were on infliximab, 31 on etanercept and 22 on adalimumab. Median survival time to first remission was 1.41 (SD: 7.99) months for infliximab, 1.94 (SD: 8.94) for adalimumab and 3.42 (SD: 14.74) for etanercept (p = 0.048) (Figure 1). Median time to sustained remission was 2.04 months (SD: 6.37) for infliximab, 4.37 (SD: 8.67) for adalimumab and 4.34 (SD: 24.74) for etanercept (p = 0.046) (Figure 2). Patients with psoriatic arthritis had better time to first remission (p = 0.0136, HR = 2.685, 95% CI = 1.225 - 5.882) and time to sustained remission (p = 0.003, HR = 3.379, 95% CI = 1.425 - 8.010). Dose regimens used to achieve sustained remission: etanercept 50 mg once a week, adalimumab 40 mg every 2 weeks and infliximab, mean dose 4.9 mg/kg (median 5.2) with interval range of 1.1 - 7.9 weeks. 95.3% of patients on infliximab, 69% on etanercept and 66.7% on adalimumab achieved sustained remission (p =0.0018).

Conclusions: Patients on infliximab achieve their first remission and sustained remission earlier compared to those on etanercept and adalimumab. The association with psoriasis carried a good prognosis.

Kaplan Meier curve showing time to first remission

Kaplan Meier curve showing time to sustained remission

Commercial Relationships: Careen Y. Lowder. None; Maria M. Choudhary. None; Rula hajj-alı. None; Sunil K. Srivastava. None; Travis Jenkins, None; Zvi A. Kresch. None; Sunil K. Srivastava. None; Maria M. Choudhary. None; John V. Mauro. None; C. Michael Samson. CLS Pharmaceuticals (I), PCAsso (I)
Purpose: To determine the effectiveness and steroid-sparing abilities of TNF-α inhibitors (TNFαI) in the treatment of chronic, non-infectious, non-necrotizing scleritis.

Methods: We conducted a retrospective chart review of patients treated at our institutions for non-infectious, non-necrotizing scleritis between April 2002 and November 2012. Only patients taking TNFαI for > 2 months were eligible. Outcome measures included inflammation grading, TNFαI dosing, concurrent corticosteroid (CS) and/or other immunomodulatory therapy dosing, visual acuity (VA), and adverse effects. Three TNFαIs were included: infliximab (Remicade), adalimumab (Humira), and etanercept (Enbrel).

Results: Twenty-one patients (17 females [81%]) with a total of 33 affected eyes were included. Mean ± SD age at start of TNFαI use was 59.2 ± 13.4 years. All patients had an associated autoimmune disease. Mean duration of TNFαI use was 23.7 months. Inflammation control was achieved in 20 (95%) patients. Three (14%) patients with recurrent scleritis started out with no inflammation but were able prevent flare-ups for a mean duration of 13.6 months while on a TNFαI. Thirteen (62%) patients achieved control of active inflammation on their first trial of TNFαI after a mean duration of 5.3 months. Eight (61%) of these patients maintained quiet scleritis on the same TNFαI for a mean duration of 26.6 months, four (31%) of these patients experienced recurrences of inflammation, and one (8%) switched to a different TNFαI but maintained control of inflammation. Three (14%) patients failed their first trial of Humira and were switched to Remicade, achieving inflammation control after a mean duration of 1.0 month. One (5%) patient had an allergic reaction Remicade and was switched to Humira, achieving inflammation control after 1.1 month. Successful CS sparing was achieved in 13/14 (93%) patients on concurrent CS therapy. Of the 14 patients on concurrent methotrexate therapy, seven (50%) were able to lower their dose. VA improved or stayed the same in 22 (67%) eyes. Aside from one allergic reaction, no other patients experienced adverse effects from TNFαI use.

Conclusions: TNF-α inhibitors are effective and well-tolerated therapy for non-infectious, non-necrotizing scleritis. They can successfully reduce inflammation as well as decrease concurrent corticosteroid and methotrexate doses.

Commercial Relationships: Ashwinee Ragam, None; Anton M. Kolomeyer, None; Christina Fang, None; Yinfei Xu, None; David S. Chu, Abbott (F), Novartis (F), Santen (F), Eyegate (F), Lux Biosciences (F), Bausch & Lomb (R)

Program Number: 2542 Poster Board Number: D0147
Presentation Time: 2:45 PM - 4:30 PM
Rituiximab in the treatment of refractory scleritis in patients with granulomatosis with polyangiitis ( Wegener’s granulomatosis): The Mexican experience

Juan Carlos Serna-Ojeda1, Claudia Recillas-Gispert2, Luis F. Flores-Suarez3. 1Institute of Ophthalmology “Conde de Valenciana”, Mexico City, Mexico; 2Ophthalmology, I.N.C.M.N.S.Z, Mexico City, Mexico; 3Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico.

Purpose: The purpose of the study is to evaluate the clinical response to rituximab in patients with scleritis due to granulomatosis with polyangiitis (GPA) refractory to treatment with steroids and immunosuppressive agents, mainly cyclophosphamide and methotrexate.

Methods: We performed a retrospective chart review of all the patients with scleritis secondary to GPA that were refractory to conventional treatment and that received rituximab as therapy for remission induction. Rituximab was administered using 2 doses of 1 gram, 15 days apart. Patient follow-up was clinical (systemic and ophthalmic) evaluations.

Results: Twenty-five (25) GPA patients (19 females [76%]) with a total of 33 affected eyes were included. The mean ± SD age at diagnosis was 51.9 ± 17.4 years. All patients had an associated autoimmune disease. Mean duration of GPA was 35.8 ± 12.8 months. Inflammation control was achieved in 17 (68%) affected eyes. Of the 15 patients on concurrent CS therapy, 7 (47%) achieved control of inflammation. Three (20%) patients failed their first trial of Rituximab but maintained control of inflammation while on a different TNFαI. Six (40%) patients were able to reduce their CS dose after Rituximab therapy.

Conclusions: Rituximab is an effective and well-tolerated therapy for refractory GPA. It can successfully reduce inflammation as well as decrease concurrent corticosteroid and methotrexate doses.

Commercial Relationships: None; None; None; David S. Chu, Abbott (F), Novartis (F), Santen (F), Eyegate (F), Lux Biosciences (F), Bausch & Lomb (R)
Treatment Outcomes in Uveitic Macular Edema with Serous Retinal Detachment

Stephen M. Huddleston, Kourtney Houser, Daniel K. Bennett, R Christopher Walton. Ophthalmology, University of Tennessee, Memphis, TN.

Purpose: To compare the outcomes of patients with uveitic macular edema with subfoveal serous retinal detachment (MESRD) to those without serous retinal detachment (ME).

Methods: Retrospective analysis of consecutive patients with uveitic macular edema treated with subtenon triamcinolone acetonide. Macular edema was defined as retinal thickness > 315 μm utilizing spectral domain optical coherence tomography (SDOCT). Serous retinal detachment was defined as the presence of fluid between the retina and the retinal pigment epithelium (RPE) as demonstrated by a hyporeflective space between the retina and RPE with SDOCT. Patients with diabetic macular edema, vitreomacular traction, epiretinal membrane, cataract surgery within 3 months of the diagnosis of macular edema, or severe inflammation preventing measurement of the retinal thickness were excluded. All patients received 1-3 subtenon triamcinolone acetonide injections at 2-3 week intervals until resolution of macular edema. Outcome measures were time to resolution of macular edema, resolution of serous retinal detachment, and cumulative number of subtenon triamcinolone acetonide injections.

Results: Thirty-seven patients with uveitic macular edema were included in the study. Twenty eyes had ME and 17 eyes had MESRD. There were no differences in age, gender, race, topical corticosteroid therapy, systemic immunosuppressive therapy, or anatomic type of uveitis between the groups. Using Kaplan Meier survival analysis, the mean time to resolution of macular edema was 6.2 weeks for the ME group and 6.1 weeks for the MESRD group. The log rank test indicated no significant difference in time to resolution of macular edema between the two groups (p=0.89). The mean number of subtenon triamcinolone acetonide injections was 1.9 and 1.6 in the ME and MESRD groups respectively (p=0.18).

Conclusions: In our sample, there was no difference in time to resolution of uveitic macular edema in patients with and without a serous retinal detachment. These results suggest that the presence of a subfoveal serous detachment does not delay the resolution of uveitic macular edema treated with subtenon triamcinolone acetonide.
considered.

**Results:** Median age was 13.1 years (range 6-20.8) and sex ratio (F/M) was 3. Median duration before adalimumab therapy was 82.6 months (range 16-262). The mean follow-up was 35.5 months (range 4-63) and the final median laser flare photometry value was significantly reduced from 149.5 ph/ms (range 24-355) to 85.4 ph/ms (range 4-224) p<0.005. Median oral prednisone decreased from 10.3 mg/day (range 0-30) to 3.2 mg/day (range 0-15) p<0.05. Uveitis was controlled in 11 cases (73.3%). Relapses occurred in one case (6.7%) and was stopped in a patient who had excellent control of inflammation. Three children (20%) discontinued treatment due to severe side effects: one due to intolerance with an allergic reaction, one due to neurologic side-effects (optic neuropathy, polyneuropathy), one because of behavioral disorders.

**Conclusions:** Adalimumab appears to be an effective and well tolerated treatment for refractory pediatric uveitis, with prolonged control of inflammation over several years, even after failure of other anti-TNF alpha agents. A prospective randomized double blind study is currently ongoing in order to confirm these retrospective results.

**Commercial Relationships:** Benjamina Penaud, None; Emmanuelle Champion, None; Christine Fardeau, None; Phuc Le Hoang, allergan (C), bausch Lomb (R), santen (C); Pierre Quartier, Abbott (F); Bahram Bodaghi, None

**Program Number:** 2546 Poster Board Number: D0151

**Presentation Time:** 2:45 PM - 4:30 PM

**Cytosine Arabinoside for the treatment of Ocular Inflammatory disorders: a pilot study**

Mehrine Shaikh1,2, Sana S. Siddique2, Mark S. Dacey2, C. Stephen Foster2. 1Ophthalmology, George Washington University Hospital, Washington, DC; 2Ophthalmology, Ocular immunology and uveitis foundation, Cambridge, MA.

**Purpose:** To ascertain the outcomes of Arabinoside Cytarabine as either monotherapy or combination therapy in the treatment of severe ocular inflammatory disease refractory to multiple traditional chemotherapies. This paper represents the first published cases examining the efficacy and tolerance of treatment with cytosine arabinoside in patients with ocular inflammatory disease.

**Methods:** This is a single-center, retrospective interventional case series. The charts of 7 patients with noninfectious uveitis who treated with Arabinoside Cytarabine (Ara-C) after failing treatment with conventional immunomodulatory therapy were reviewed. Primary outcome measure was inflammatory status while on Ara C therapy. Secondary outcome measure was adverse effects.

**Results:** In this small retrospective study of seven patients, Ara-C has been shown to be effective in inducing durable steroid-free remission from noninfectious ocular inflammatory conditions in 3 of 7 patients(42.9%), with well-tolerated treatment regimens lasting 1-2 years. In 2 out of 7 patients (28.6%), the inflammation was not adequately controlled with Ara-C and ultimately required intravenous cyclophosphamide. 2 out of 7 patients(28.6%) stopped Ara-C infusions due to systemic side effects; one developed new anemia after the onset of Ara-C infusions, and a second developed a persistent axillary abscess that eventually required surgical incision and drainage. Ara-C was generally well tolerated at doses of 100-200 mg/month as both monotherapy and combination therapy, with two patients noting mild nausea after infusions which did not preclude additional infusions.

**Conclusions:** We present these cases to introduce Arabinoside Cytarabine as another option for immunomodulatory therapy in the treatment of ocular inflammatory disease, particularly for patients with persistently stubborn inflammation that has not been placed into remission with multiple prior chemotherapies. This study reveals that Ara-C is a safe and effective immunomodulatory therapeutic option.

**Commercial Relationships:** Mehrine Shaikh, None; Sana S. Siddique, None; Mark S. Dacey, None; C. Stephen Foster, Abbott Medical Optics (C), Abbott Medical Optics (F), Alcon Laboratories, Inc. (C), Alcon Laboratories, Inc. (F), Allergan, Inc. (C), Allergan, Inc. (F), Eyegate Pharmaceuticals, Inc. (I), Eyegate Pharmaceuticals, Inc. (F), IOP Ophthalmics (C), Ista Pharmaceuticals (C), Lux Biosciences, Inc. (C), Lux Biosciences, Inc. (F), Novartis Pharmaceuticals Corporation (C), Novartis Pharmaceuticals Corporation (F), XOMA Ltd (C)

**Program Number:** 2547 Poster Board Number: D0152

**Presentation Time:** 2:45 PM - 4:30 PM

**Pigment Epithelium-Derived Factor (PEDF) hypersensitivity in CAR & AMD**

Charles E. Thirkill. Ocular Immunology, UC Davis, Davis, CA.

**Purpose:** Three patients with vision loss associated with ovarian cancer were producing antibodies reactive with a 45 kd protein expressed in retina, and retinal pigment epithelium (RPE). Preliminary findings from an earlier proteomic analysis suggest the patients are reacting with RPDF, a multifunctional protein that includes control over vascular proliferations. Further research identified what appears to be the same 45 kd antibody reaction in some patients with Age-Related Macular Degenerations (AMD, a retinopathy typified by uncontrolled vascular proliferations). In light of these more recent findings, and in order to clarify any association of PEDF hypersensitivity with vision loss, a commercially obtained preparation of recombinant PEDF was incorporated into continuing immunologic inquiries.

**Methods:** Western blot reactions confirmed the CAR and AMD patient’s antibody activity with the 45 kd protein expressed in both the retina and RPE. The patient’s antibodies were eluted from the 45 kd retina-RPE reaction site of nitrocellulose blots, and applied to a blot of recombinant RPDF; (Sigma cat # SRP 4988). Serum from normal healthy volunteers, and mouse monoclonal anti-RPDF (Millipore cat # MAB1059) was included as comparison controls.

**Results:** Preliminary proteomic evidence incriminating RPDF as the 45 kd antigen is supported by findings of a corresponding reaction with the recombinant counterpart that interacts with the 45 kd reactive patient’s affinity purified antibodies. Mouse monoclonal anti-RPDF identifies the corresponding antigen in both retina and RPE within the same regions of the blots as that seen with the patient’s antibodies. No comparable anti-45 kd immunologic activity was found in normal healthy comparison controls.

**Conclusions:** Any possible immunologic inhibition of the anti-angiogenic nature of RPDF naturally raises questions concerning the clinical significance of this abnormality. In the experience of this lab the 45 kd antigen/antibody reaction is demonstrable only in some CAR and some AMD patients, but not in normal comparison controls. Findings indicate the need to inquire further into what time might prove to be an autoimmune reaction that simultaneously encourages cancers to proliferate through the provision of a nurturing good supply in some CAR patients, and retinas to degenerate due to a loss of control over excessive pathologic vascular proliferations in some AMD patients.

**Commercial Relationships:** Charles E. Thirkill, None

**Support:** Research to Prevent Blindness & NEI core grant: 1 P30 EY12576-8

**Program Number:** 2548 Poster Board Number: D0153

**Presentation Time:** 2:45 PM - 4:30 PM
PGD2 Promotes Eosinophil Chemotaxis, Degranulation and Syk Phosphorylation

Ellen B. Cook1,2, James L. Stahl1,2, Elizabeth A. Schwantes3, Sameer K. Mathur4, Neal Barney1,2, Ophthalmology and Visual Sciences, University of Wisconsin, Madison, WI; 2McPherson Eye Research Institute, University of Wisconsin, Madison, WI; 3Medicine, Allergy Division, University of Wisconsin, Madison, WI.

Purpose: Eosinophils and their catalytic mediators are major features in the pathogenesis of allergic conjunctivitis, contributing to corneal damage in chronic disease. The prostaglandin, PGD2, which is released from activated mast cells, has recently been shown to promote eosinophil chemotaxis. The purpose of this study was to further examine the effects of PGD2 on activation of eosinophil pro-inflammatory processes (chemotaxis, degranulation, phosphorylation of spleen tyrosine kinase [Syk] and survival/viability).

Methods: Human purified peripheral blood eosinophils were obtained using negative immuno-magnetic bead selection. For most experiments, PGD2 was added at concentrations ranging from 0.1 - 1000 nM. For chemotaxis, eosinophils were added to the top compartment of 5.0 µm Transwell chambers, with either media or PGD2 added to the bottom for 1 hr after which eosinophils migrating to the bottom compartment were counted. For degranulation, eosinophils were stimulated with media or PGD2 for 4 hrs and supernates were harvested and evaluated for eosinophil derived neurotoxin using commercial ELISA. Flow cytometry was used for evaluation of Syk-phosphorylation, and trypan blue exclusion was used for evaluation of survival.

Results: Stimulation of eosinophils with PGD2 promoted chemotaxis (1.0 - 100 nM, n=6 subjects, p<0.05), and tended toward promoting degranulation (10, 100 nm, n=4 subjects, p=0.1) and phosphorylation of Syk (100 nM, n=2 subjects), but not survival or viability (n=3 subjects).

Conclusions: PGD2-mediated activation of eosinophils could play a role in eosinophil mediated processes in allergic conjunctivitis and, thus, presents a potential target for therapeutic intervention.

Commercial Relationships: Ellen B. Cook, Alcon (F); James L. Stahl, Alcon Labs (F); Elizabeth A. Schwantes, None; Sameer K. Mathur, Teva (C); Neal Barney, Alcon Laboratories (F)

Support: Alcon Labs, NIH Program Project Grant HL088584, Research to Prevent Blindness

Program Number: 2549 Poster Board Number: D0154
Presentation Time: 2:45 PM - 4:30 PM

CCR7 Expression Profiles in Conjunctival Biopsies from Seasonal Allergic Conjunctivitis Patients Following Challenge

Rose Mathew1, Amirah Mohd Zaki2, Grażyna Galatowicz3, Virginia L. Calder2, Daniel R. Saban1,2, Ophthalmology, Duke University, School of Medicine, Durham, NC; 2Immunology, Duke University, School of Medicine, Durham, NC; 3Ophthalmology, UCL Institute of Ophthalmology, London, United Kingdom.

Purpose: It has recently been demonstrated that CCR7-expressing dendritic cells contribute to the immunopathogenesis of allergic conjunctivitis in the mouse model. However, whether CCR7 plays a similar role in the human condition is unknown. The aim of this study was to investigate conjunctival expression of CCR7 following allergen challenge in seasonal allergic conjunctivitis (SAC) patients.

Methods: Human anonymised conjunctival tissue biopsies (3 micron) were obtained from SAC donors (n=7, from all males; age: 18-65 years) at 8 hr post allergen challenge. Control, non-inflamed, conjunctival tissues collected from anonymised human donors (n=5 from 3 males; age: 31-57) were also examined. Donor tissues were collected after obtaining informed consent and Local Research Ethics approval in accordance with the Declaration of Helsinki. Tissue sections were stained for anti-human CCR7 (eBioscience), anti-human neutrophil elastase (DAKO), or isotype-type matched control rat IgG2a kappa antibody (eBioscience and visualized with a DAB staining kit ( Vectastain). Two independent, masked observers enumerated positively stained cells per biopsy area (at least three fields).

Results: CCR7-expressing cells were detected mainly within the subepithelium and stromal areas. Over 70% of tissues from SAC patients stained positive for CCR7, compared to only 20% from control patients. The numbers of CCR7-expressing cells did not appear to correlate with neutrophil numbers, as the difference in the number of neutrophils between control (mean: 44.0 ± 14.0 cells per biopsy) and SAC (mean 82.9 ± 117.8 cells per biopsy) patients was not statistically significant.

Conclusions: CCR7-expressing cells are detectable in human conjunctival tissues and appear to be upregulated during acute inflammation in SAC.

Commercial Relationships: Rose Mathew, None; Amirah Mohd Zaki, None; Grażyna Galatowicz, None; Virginia L. Calder, Allergan Inc (C), Allergan Inc (F); Daniel R. Saban, Schepens Eye Res Inst, Mass Eye and Ear, (P), Eleven Biotherapeutics (R)
Support: NEI-R01EY021798

Program Number: 2550 Poster Board Number: D0155
Presentation Time: 2:45 PM - 4:30 PM

Effects of D-β-Hydroxybutyrate (HBA) on eosinophil infiltration in allergic conjunctivitis model rat

Ryuji Hisamura, Shigeru Nakamura, Toshihiro Imada, Kazuo Tsubota, ophthalmology, Keio University, Shinjuku-ku, Japan.

Purpose: HBA is a normal component of human metabolic physiology. Recent findings have shown that HBA provides neuroprotection during hypoxia in vitro, in models of global and focal cerebral ischemia in rats, cardiac ischemia, and in lung hemorrhage. In this study, we investigated the effect of HBA on allergic reaction.

Methods: Wistar rats were sensitized by egg albumin (EA) as described by Minami et al. (Int. Immunopharmacol., 4(1):101, 2004). EA has been instilled every day from day 14 to 28 into bilateral eyes. Selection was performed by choosing rats showing obvious eye scratching behavior at day 28. Rats were randomly assigned to 1% HBA or saline eye drop. Eye drops were applied 5 minutes before EA challenge for 2 continuous weeks. Number of eye scratching behavior was counted for 20 minutes after EA challenge and the total number during application period were compared. Rats were sacrificed 24 hours after last EA challenge and eosinophil infiltration into conjunctiva was assessed by histopathological examination.

Results: Topical applied HBA significantly reduced eye scratching number and suppressed eosinophil infiltration compared to saline eye drop treatment.

Conclusions: These results indicate that the suppressive effect of HBA on allergic reaction

Commercial Relationships: Ryuji Hisamura, Opteacs corporation (E); Shigeru Nakamura, Opteacs Co. Ltd (E); Toshihiro Imada, Yamada bee farm corporation (F); Kazuo Tsubota, AcuFocus, Inc (C), Allergan (F), Bausch Lomb Surgical (C), Functional visual acuity meter (P), JINS (P), Kissei (F), Kowa (F), Santen, Inc. (F), Otsuka (F), Pfizer (C), Thea (C), Echo Denki (P), Nidek (F), Opteacs (F), Wakasa Seikatsu (F), CEPT Company (P)

Program Number: 2551 Poster Board Number: D0156
Presentation Time: 2:45 PM - 4:30 PM

An Evaluation of the Effects of the Repeat Conjunctival Allergen Challenge (CAC) Model in Various Strains of Albino Mice

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**Purpose:** Seasonal allergic conjunctivitis displays significant individual variation in humans due (at least in part) to differences in genetic background. As part of a program to optimize a murine model of the disease, we compared the responses of 6 strains of mice to a controlled allergen challenge in order to identify the most sensitive strain.

**Methods:** Six strains of albino mice (B6 [Cg]-Tyrc2/J, SWR/J, B6 Albino [B6N-Tyrc2/BrdCrCrl], Swiss Webster [Crt:CFW(SW)], Balb/C [BALB/cAnNcr], and CD1 [Crt:CD1(ICR)]) were examined in this study. On Day 0, all were sensitized subcutaneously in both hind hocks with 100µg of short ragweed (SRW) in 650µg of aluminum hydroxide. On Days 21 and 22 mice were topically challenged twice daily with 150µg per eye of SRW. The right eye of each mouse was evaluated at baseline and 18 minutes post topical challenge using a modified Micron III imaging system. Allergic responses were evaluated with Ora’s proprietary scale where each endpoint (hyperemia, discharge, and squinting) was measured on a scale of 0-4.

**Results:** Out of six strains, Balb/C mice were the most affected by topical SRW. The Balb/C mice had a change from baseline redness score of approximately 2 units. No other strain showed an increase in hyperemia after topical challenge. Balb/C mice were statistically higher versus B6(Cg)-Tyrc2/J, SWR/J, and Swiss Webster (p<0.01, p<0.001, and p<0.001, respectively). No significance was noted versus the B6 Albino or CD1 mice for hyperemia. Discharge scores showed little increase from baseline in any strain (about a 0.5 unit increase on average). Balb/C mice showed the most consistent increase in squinting scores vs. baseline; increases with this strain were significantly higher than B6 Albino, Swiss Webster, and CD1 mice (p<0.001, p<0.001, p<0.001, and p<0.001, respectively). No significance was noted versus the B6 (Cg)-Tyrc2/J or SWR/J mice.

**Conclusions:** Out of the 6 strains surveyed, Balb/C mice continue to be the most sensitive strain for evaluating novel drugs to treat allergic conjunctivitis.

**Commercial Relationships:** Kortni Violette, Ora, Inc. (E); Laura Belen, Ora, Inc. (E); Jennifer Brackett, Ora, Inc. (E); Andy Whitlock, Ora, Inc. (E)

Program Number: 2552 Poster Board Number: D0157
Presentation Time: 2:45 PM - 4:30 PM

**Ocular Allergy 2013: A Survey of Current Trends**

Emily Schoenmell, Paulo J. Gomes, Donna L. Welch. Allergy, Ora, Inc, Adover, MA.

**Purpose:** To assess demographics of allergic history and treatment prevalence of allergic conjunctivitis patients.

**Methods:** Subjects who were part of an ocular allergy clinical trial database and agreed to participate in a clinical trial were asked to participate in this IRB-approved questionnaire. Of 230 subjects, a total of 205 completed questionnaires were included in the survey analysis. The population of respondents was generally representative of trial participants in terms of age, racial distribution, and the relative numbers of men and women. Subjects provided information on their disease characteristics, their treatment strategies, and their satisfaction with their current therapeutic regimes.

**Results:** The survey population consisted of 59% women and 41% men; the mean age of respondents was 37.8 years. The overwhelming majority (83.9%) reported experiencing nasal as well as ocular allergy symptoms, while smaller percentages (18-31%) stated that they also suffered from food allergies, skin allergies, or asthma. Approximately 1 in 4 reported some type of allergy to medication. As a group, the respondents reflect recent national trends: 38% experience allergic symptoms year-round, while 62% experience seasonal allergic disease. Surprisingly, the second-most reported complaint (after ocular itching) among all patients was excessive tearing or watery eyes, not ocular redness. Another surprising finding was the low percentage of patients that seek treatments for their allergies; 71% of seasonal allergies and 53% with perennial allergies have not sought treatment from an eye care specialist, and 40% reported that they do not regularly purchase over the counter medications to treat their allergies. Almost 80% of patients that used drops report that they are effective “all or most” of the time.

**Conclusions:** This survey confirms that our study population accurately reflects national and global trends regarding incidence of perennial and seasonal disease, and highlights the need for improved treatments for those with year-round allergy. In addition, results demonstrate that as care providers a vital role is one of education, encouraging our patients to take advantage of existing therapeutic options that are likely to improve their quality of life. As expected, subjects reporting year round symptoms also experience other non-ocular allergic symptoms including nasal, asthma and dermatitis compared to seasonal only patients.

**Commercial Relationships:** Emily Schoenmell, Ora, Inc. (E); Paulo J. Gomes, Ora, Inc. (E); Donna L. Welch, Ora, Inc. (E)

Program Number: 2553 Poster Board Number: D0158
Presentation Time: 2:45 PM - 4:30 PM

**Confounding Factors on Computerized Assessment of Conjunctival Redness Induced by Conjunctival Allergen Challenge (CAC)**

Yesha Raval, John D. Rodriguez, Keith J. Lane, Paulo J. Gomes. Allergy, ORA, Andover, MA.

**Purpose:** The Conjunctival Allergen Challenge model (CAC) is the standard regulatory model for evaluation of ocular therapeutic agents and is responsible for 19 new drug approvals. Itching is subjectively assessed while redness of three vessel beds (ciliary, conjunctival, episcleral) is evaluated by a trained clinician using a slit lamp in accordance with standardized and FDA accepted scales. In an effort to add precision to redness quantification, a computer program originally design to capture injection patterns due to dry eye, was adapted to capture redness levels in allergic conjunctivitis. Allergy subjects frequently present with chemosis. This condition causes optical distortion in a digital image due to swelling of the conjunctiva, producing a blanching and opaque effect over the visualization.

**Methods:** Conjunctival photography was taken as part of a 60 subject phase II clinical trial studying the effects of an alpha-adrenergic agonist. Inclusion criteria incorporated a CAC response ≥ 2 for both ocular itching and clinician graded ocular redness within 10 minutes of instillation. Photography was taken of the conjunctiva immediately following inclusion into the study. The Photography was analyzed for redness with a computer program developed with the OpenCV computer vision library for calculating redness intensity and the output was compared to the clinician grades in an effort to verify the ability of the software to capture injection in an allergic eye.

**Results:** The correlation between the clinician grade and the software output was 0.27. This level of accuracy was insufficient to predict clinical graded scores to less than one unit and thus unable to replicate scores of an expert grader.

**Conclusions:** The unexpected low correlation between these two methods of assessing redness of the conjunctiva is likely due to the chemosis that results from an allergic response. A clinician grading the amount of redness with a standardized scale can overcome the resulting optical distortion with the aid of a slit lamp and the ability to scan all different angles with a slit beam. While this computerized
modified CAC was able to generate a chronic allergic inflammatory condition that was responsive to steroid therapy. This model should be useful for evaluating potential therapies for the treatment of chronic ocular allergy.

Conclusions: The modified CAC was able to generate a chronic allergic inflammatory condition that was responsive to steroid therapy. This model should be useful for evaluating potential therapies for the treatment of chronic ocular allergy.
Clinical Trial: NCT01534195

Program Number: 2556 Poster Board Number: D0161
Presentation Time: 2:45 PM - 4:30 PM

**Evaluation of Brimonidine Tartrate for Prevention of Hyperemia Associated with Allergic Conjunctivitis**

**Purpose:** Ocular hyperemia is most commonly treated with vasoconstrictors that provide minimal short term relief. Alternative agents, with minimal side effects, that provide enhanced efficacy are highly sought after.

**Methods:** Subjects (N=60) with a history of allergic conjunctivitis were enrolled in a randomized, single center, Conjunctival Allergen Challenge (CAC) based study to evaluate the safety and efficacy of brimonidine tartrate (BT) (0.01 and 0.025%) as compared with oxymetazoline (OM) 0.025% and brimonidine vehicle (placebo) for prevention of allergen-induced ocular redness. Subjects underwent screening, informed consent, and ocular assessment of baseline symptoms prior to allergen titration. Following titration, ocular and nasal allergy symptoms, measurement of IOP and dilated fundoscopy were evaluated. Following confirmation challenge (redness ≥ 2), subjects were randomized into 4 groups: BT 0.01%; BT 0.025%; OM 0.025%; and placebo. For allergen challenge, agents were instilled 15±5 minutes post-CAC; subjects were graded at 7, 15, and 20 minutes using a 0-4 redness scale. Secondary measures included ciliary and episcleral redness, itching, and tearing. Safety upon, and 1 and 2 minutes post-instillation was graded using a 10 point scale.

**Results:** Mean conjunctival redness scores in the BT 0.01% and BT 0.025% groups were significantly lower (P < 0.05) than placebo at all 3 post-CAC time points. Mean conjunctival redness scores in the OM 0.025% group were not significant versus placebo at any time point. Mean ciliary and episcleral redness scores in the BT 0.01% and the BT 0.025% groups were also significantly lower (P < 0.05) than the placebo group at all 3 time points. Treatment differences (active minus placebo) were greater than 0.5 units at all time points and greater than 1 unit for the majority of the time points measured for ciliary redness. Mean ciliary redness scores for both BT groups were significantly lower (P < 0.05) than the OM 0.025% group at 7 and 15 minutes post-CAC. Other secondary endpoints showed no significant difference between groups. Both BT solutions were found to be safe and well tolerated as dosed in this study.

**Conclusions:** In this model of allergic redness, BT at both 0.01% and 0.025% demonstrated statistical superiority over placebo and OM in the prevention of conjunctival redness when CAC was performed 15 minutes post drug instillation.

**Commercial Relationships:** Matt J. Chapin, Ora, Inc. (E); Gerald Horn, Alpha Synergy Corp (I), Alpha Synergy Corp (P), Alpha Synergy Corp (S); Paul J. Gomes, Ora, Inc. (E)

Clinical Trial: NCT01275105

Program Number: 2557 Poster Board Number: D0162
Presentation Time: 2:45 PM - 4:30 PM

**Inflammation at the Cellular Level in the Chronic Allergic Conjunctivitis Model Using Confocal Imaging**

**Purpose:** To evaluate chronic allergic inflammation of the eye at the cellular level following the standard micro-Conjunctival Allergen Challenge (micro-CAC) using confocal imaging.

**Methods:** Sixteen (16) subjects allergic to dust mites were enrolled in a 3 visit study. Subjects were titrated to their sensitivity of dust mite allergen at Visit 1. At Visit 2 the subjects received two sessions of micro-CAC 8 hours apart. Assessments included conjunctival injections, chemosis, ocular itching, eyelid swelling, as well as leukocyte infiltration of the vasculature. The Ora Calibra scale of confocal microscopy grades on the density, adherence and extravasation of leukocytes in conjunctival vessels. Subjects were assessed at Visit 3 (16 hours post Visit 2) to explore the level of inflammation that persisted. This was followed by another challenge at Visit 3B (19 hours post V2) to examine the effects of an allergic challenge on a system already in a state of inflammation.

**Results:** Following the first two micro-CAC challenge sessions, injection increased to 2.5 (p<0.001), chemosis increased to 1.3 (p<0.001), itching increased to 2.9 (p<0.001), eyelid swelling increased to 1.4 (p<0.001) and confocal infiltration of cells increased to 3.1 (p<0.001). At visit 3A, injection was 1.7 (p<0.001), chemosis was 0.69 (p<0.001), itching was 0.25 (p<0.018), eyelid swelling was 0.5 (p<0.012), and confocal infiltration of cells was 2.13 (p<0.001). Following the third session of micro-CAC at Visit 3B, chemosis increased to 1.64 (p<0.037), injection to 2.8 (p<0.001), and itching to 3.24 (p<0.060) from Visit 2B.

**Conclusions:** The level of inflammation created with this model produced chronic lingering effects on signs and symptoms of allergic conjunctivitis at 16 hours post CAC. The increase in infiltration of leukocytes at the vasculature level is not only clear following the micro-CAC challenges but is also present at 16 hour post challenge (Visit 3A). Furthermore, the induced state of chronic inflammation following another micro-CAC (Visit 3B) causes an increase in reaction compared to the acute challenge. Confocal imaging and grading of leukocytes in real time allows us to track and the course of chronic inflammation while correlating to signs and symptoms. Currently the anti-inflammatory effect at the cellular level of a topical corticosteroid is being investigated.

**Commercial Relationships:** Endri Angjeli, Ora, Inc. (E); Paul J. Gomes, Ora, Inc. (E); Stephanie Breton, Ora, Inc. (E); Keith J. Lane, Ora, Inc. (E)

Clinical Trial: NCT 01730872

116 Clinical and Translational Studies in Ocular Infection and Immunity

Tuesday, May 07, 2013 8:30 AM-10:15 AM
Exhibit Hall Poster Session

Program #/Board # Range: 2879-2926/B0248-B0295
Organizing Section: Immunology/Microbiology
Contributing Section(s): Clinical/Epidemiologic Research

Program Number: 2879 Poster Board Number: B0248
Presentation Time: 8:30 AM - 10:15 AM

**A High Speed Detection Platform Based on Surface-Enhanced Raman Scattering for Rapid Diagnosis of Bacterial Endophthalmitis**

Ching-Ju Hsieh, Feng-Rong Hu, Da-Wei Wang, Juen-Kai Wang, Yuh-Lin Wang, Chi-Hung Lin, Ophthalmology, Taipei City Hospital, Taipei, Taiwan; Institute of Biophotonics, National Yang Ming University, Taipei, Taiwan; Ophthalmology, National Taiwan University Hospital, Taipei, Taiwan; Institute of Atomic and Molecular Sciences, Academic Sinica, Taipei, Taiwan; Center for Condensed Matter Sciences, National Taiwan University, Taipei, Taiwan; Physics, National Taiwan University, Taipei, Taiwan; Institute of Microbiology and Immunology, School of Life Science, National Yang-Ming University, Taipei, Taiwan.

**Purpose:** Bacterial endophthalmitis (BE) is a vision-threatening disease. Early diagnosis of causative pathogens can be crucial to
optimize final visual prognosis. Conventional culture-based method for diagnosing BE is considered as the gold standard but inevitably takes time ranging from days to weeks or even months. Using the surface-enhanced Raman scattering (SERS) platform developed by our group, the pathogens can be differentiated on the basis of their SERS spectra which are believed related to their surface chemical components. The aim of this study was to develop SERS as a rapid whole-organism fingerprinting method for the characterization of bacteria associated with BE.

Methods: We collected the SERS spectra of Gram-positive bacteria (GPB), and Gram-negative bacteria (GNB), including coagulase-negative Staphylococci, Staphylococcus aureus, Streptococcus viridans, Enterococcus faecalis, Pseudomonas aeroginosa, Klebsiella pneumoniae, and Proteus mirabilis. These samples were obtained from patients at hospital in Taiwan and were believed to represent the real diversity of clinical pathogens. The Raman signals of bacteria were enhanced by silver/aluminum anodic oxide (Ag/AAO) substrate and collected between 400 and 1600 cm\(^{-1}\) by Raman microscope. The multivariate statistical techniques of linear discriminant analysis (LDA), hierarchical cluster analysis (HCA) and support vector machine (SVM) were applied in order to group these organisms based on their spectral fingerprints.

Results: Each of the individual species had its specific SERS spectrum, and the spectra of GNB also differed from those of GNB. By analyzed the specific SERS spectrum of each individual species using HCA and LDA methods, we found that the individual GPB strains have highly similar spectra with each other and the spectra of P. aeroginosa showed most between-strain differences. Using SVM method, the classification accuracy of identifying different bacterial strains can achieve 89% and the mean accuracy of differentiating GPB from GNB was 90%.

Conclusions: The SERS profiles of BE recorded by such a platform are sensitive and stable that could readily reflect different bacterial cell walls found in GPB, GNB, or individual species. We believe this would be the first report showing bacterial discrimination of BE using SERS. This high-speed SERS detection could develop to a novel approach for microbial diagnostics.

Commercial Relationships: Ching-Ju Hsieh, None; Fung-Rong Hu, None; Da-Wei Wang, None; Juen-Kai Wang, None; Yuh-Lin Wang, None; Chi-Hung Lin, None

Support: the National Program for Nanoscience and Nanotechnology of National Science Council, Taiwan (NSC95-3114-P-001-001-

Program Number: 2880 Poster Board Number: B0249
Presentation Time: 8:30 AM - 10:15 AM
Longitudinal Cytokine Analysis of Aqueous Humor in CMV Retinitis - The CMV Retinitis Intravitreal Ganciclovir Singapore Study (CRIGSS)
Jayant V. Iyer\(^1,\) Bijin Au\(^2,\) Suisheng Tang\(^2\), John E. Connolly\(^2\), Rupesh V. Agrawal\(^2\), Tun Kuan Yeo\(^3\), Stephen C. Teoh\(^2\).

\(^1\)Ophthalmology, National Healthcare Group, Tan Tock Seng Hospital, Singapore, Singapore; \(^2\)Singapore Immunology Network, Singapore, Singapore; \(^3\)Ophthalmology, Singapore National Eye Centre, Singapore, Singapore.

Purpose: To perform longitudinal analysis of cytokine, chemokine and growth factor levels in the aqueous humor of patients with cytomegalovirus retinitis (CMVR) through the course of treatment with intravitreal ganciclovir.

Methods: Aqueous humor samples were collected from HIV-positive patients with CMVR scheduled to undergo weekly intravitreal ganciclovir therapy as part of the prospective CMV Retinitis Intravitreal Ganciclovir Singapore Study (CRIGSS) over the course of 1 year. Aqueous humor samples were drawn and analyzed for these patients pre-treatment (0 weeks) and at certain points during course of therapy - 4 weeks, 14 weeks and 18 weeks. Full data across all the time points was obtained and analyzed. Aqueous humor was comprehensively analyzed for 41 cytokine and chemokine factors using real-time PCR with the FlexMAP 3D (LumineX®) platform and assessed using the Milliplex Human Cytokine® kit.

Results: Nine patients have been recruited of which 6 patients have completed the study at all 4 time-points. Longitudinal assessment of samples from 6 patients across the 4 time points using repeated measures ANOVA revealed decreasing levels of MCP-1 (p=0.04) and IL-8 (p=0.04) through the course of intravitreal ganciclovir therapy. Further analysis revealed one of the six subjects to have minimal decrease in these cytokine levels suggestive of possible resistance to ongoing treatment.

Conclusions: This data identifies underlying intraocular immunological response through course of treatment in patients with CMVR. Earlier detailed cytokine analysis as part of CRIGSS had already revealed a unique immunologic signature in aqueous of CMVR. Study of aqueous cytokines through the course of treatment has now identified 2 cytokines as potential markers of treatment response. Further longitudinal analysis of CMVR patients would shed more light on underlying immunological mechanisms in treatment response or resistance, and help in prognostication of the disease. This may lead to development of novel and more targeted treatment strategies for CMVR management in the near future.

Commercial Relationships: Jayant V. Iyer, None; Bijin Au, None; Suisheng Tang, None; John E. Connolly, None; Rupesh V. Agrawal, None; Tun Kuan Yeo, None; Stephen C. Teoh, None
Support: NMRC, Singapore (NMRC/NIG/1046/2011)
Clinical Trial: NMRC/NIG/1046/2011

Program Number: 2881 Poster Board Number: B0250
Presentation Time: 8:30 AM - 10:15 AM
SPECTRAL DOMAIN-OPTICAL COHERENCE TOMOGRAPHY FINDINGS IN ACUTE SYMPHILITIC POSTERIOR PLACOID CHIORIOTRETINOATHPY
Antonio P. Ciardella\(^1\), Francesco Pichi\(^2\), Emmett T. Cunningham\(^3,4\), I. Michael Jumper\(^1\), Janet L. Davis\(^5\), Thomas A. Albin\(^2\), David Sarraf\(^6\), Enrico Bertelli\(^7\), Mariachiara Morara\(^8\), Paolo Nucc\(^2\).

\(^1\)Ophthalmology, Policlinico S Orsola Malpighi, Bologna, Italy; \(^2\)San Giuseppe Hospital, University Eye Clinic, Milan, Italy; \(^3\)& 4 Department of Ophthalmology, California Pacific Medical Center, San Francisco, CA; \(^5\)Department of Ophthalmology, Stanford University School of Medicine, Stanford, CA; \(^6\)West Coast Retina Medical Group, California Pacific Medical Center, San Francisco, CA; \(^7\)Bascom Palmer Eye Institute, University of Miami-Miller School of Medicine, Miami, FL; \(^8\)Jules Stein Eye Institute, UCLA, Los Angeles, CA; \(^9\)Bolzano Central Hospital, Department of Ophthalmology, Bolzano, Italy.

Purpose: To describe the appearance of Acute Syphilitic Posterior Placoid Chorioretinitis (ASPPC), a rare ocular manifestation of syphilis, on Spectral Domain-Optical Coherence Tomography (SD-OCT) both before and after treatment.

Methods: Ophthalmic examination, imaging studies, and SD-OCT scans of 30 eyes of 19 confirmed ASPPC cases were analyzed both at the time of presentation and at each follow-up visit. Standard treatment for neurosyphilis was given to each patient, including 4 million units of penicillin G administered intravenously every 4 hours for 10 days.

Results: Fundus examination and imaging studies were consistent with previous reports, and confirmed the diagnosis ASPPC. In 13 eyes (43.3%), baseline SD-OCT scans were performed within 1 to 2
days of presentation and revealed a small amount of subretinal fluid (SRF), disruption of the inner segment/outer segment (IS/OS) junction, and hyperreflective thickening of the retinal pigment epithelium (RPE). All 30 eyes were again scanned between day 7 and 9 following presentation and revealed loss of the IS/OS and OS/RPE bands, and irregular hyperreflectivity of the RPE with prominent, nodular elevations, but without SRF. Early disruption of the external limiting membrane (ELM) and punctate chorioidal hyperreflectivity were seen in 1/30 (3.3%) and 14/30 (46.6%) eyes, respectively. Vision improved and the outer retinal abnormalities normalized in 28/30 eyes (93.3%) following treatment for neurosyphilis.

**Conclusions:** Patients with ASPPC show characteristic outer retinal abnormalities on SD-OCT imaging, including disruption of the IS/OS band, nodular thickening of the RPE with loss of the linear OS/RPE junction, and, in some cases, loss of the ELM, accumulation of SRF, and punctate hyperreflectivity in the choroid. Vision improved and these abnormalities reversed following treatment for neurosyphilis in the vast majority of patients.

**Commercial Relationships:** Antonio P. Ciardella, None; Francesco Pichi, None; Emmett T. Cunningham, None; J. Michael Jumper, None; Janet L. Davis, None; Thomas A. Albini, Bausch and Lomb (C), Allergan (C), Genentech (F), Eleven Biotherapeutics (C); David Sarraf, Genentech (F), Regeneron (F), Allergan (F), Alcon (F), DORC (F); Enrico Bertelli, None; Mariachiara Morara, None; Paolo Nucci, Alcon (F), Bausch &Lomb (F), Allergan (F), ALFA INTEST (S), VISUFARMA (F), THEA (F), SOOFT (F), SIFI (F), CHICCO (F), FARMIGEA (S), EUFARMED (S)

**Program Number:** 2882 Poster Board Number: B0251
**Presentation Time:** 8:30 AM - 10:15 AM

**Ocular syphilis: case series (2000-2009) from two tertiary care centers in Montreal**

*Kinda Najem1, 2, Laurence Jaworski3, Annie-Claude Labbé3, 4, Claude Fortin5, 6, Éric Fortin6, 7, Marie-Lyne Bélair1, 8, Bouchara Serhir9, Marie-Josée Aubin1, 2, 3, 7, 8, 9*; University of Montreal, Montreal, QC, Canada; 1Ophthalmology, Notre Dame Hospital, CHUM, Montreal, QC, Canada; 2Ophthalmology, Sacré-Cœur Hospital, Montreal, QC, Canada; 3Microbiology, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada; 4Microbiology, Notre Dame Hospital, CHUM, Montreal, QC, Canada; 5Ophthalmology, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada; 6Laboratoire de santé publique du Québec, Sainte-Anne-de-Bellevue, QC, Canada.

**Purpose:** To review ocular syphilis cases diagnosed and treated between 2000 and 2009 at Maisonneuve-Rosemont Hospital and Notre-Dame Hospital, Montreal, and to describe the demographics, clinical presentations, proportion of co-infection with HIV, treatment and outcome.

**Methods:** Medical records of patients who had positive treponemal serologic testing and who visited the ophthalmology department at Maisonneuve-Rosemont Hospital and Notre-Dame Hospital for ocular manifestations related to syphilis between the years 2000 to 2009 were retrospectively reviewed. Several data were compiled and included: patient demographics; clinical presentation and examination; past syphilis history; syphilis serology results and cerebrospinal fluid analysis results; HIV status; ophthalmological diagnosis; medical and surgical treatment; follow-up serology results and final best-corrected visual acuity (BCVA).

**Results:** Ninety-one patients (80% males) were included in the study. The majority of cases were found in men aged 51-60 years old (26%) and 41-50 years old (17%). Around 30% of the patients were men who have sex with men (MSM). Snellen BCVA was converted to logMar notation. Pre-treatment mean was 0.42 (BCVA around 20/50) while post-treatment mean was 0.34 (BCVA around 20/40). The most common ophthalmological diagnoses were all types of uveitis (anterior being the most frequent one with a proportion of 31%). Coinfection with HIV was found in 34% of patients. Lumbar puncture was done in 55% of patients and VDRL serology was positive in 11% of those patients. The mainstay of treatment was intravenous penicillin in 75% of the patients. In about 85% of patients treated, no history of reinfection was noted.

**Conclusions:** Syphilis is known as the great masquerader with a diversified presentation and has seen a significant increase in the past ten years. In this context, it is primordial to keep this diagnosis in mind, especially since the treatment is readily available and has an excellent outcome.

**Commercial Relationships:** Kinda Najem, None; Laurence Jaworski, None; Annie-Claude Labbé, None; Claude Fortin, None; Éric Fortin, Abbott (F); Marie-Lyne Bélair, None; Bouchara Serhir, None; Marie-Josée Aubin, None

**Program Number:** 2883 Poster Board Number: B0252
**Presentation Time:** 8:30 AM - 10:15 AM

**Utility of Lyme Antibody Testing in the Uveitis Workup**

*Lana M. Rifkin, Andrea D. Birnbaum, Anjali S. Parekh, Chaisiri Jumboendlarasame, Dmitry Pyatetsky, Debra A. Goldstein.* Ophthalmology, Northwestern University, Chicago, IL.

**Purpose:** To determine the utility of testing for Lyme disease in patients with ocular inflammation at single center in the Midwestern United States.

**Methods:** A search of the Electronic Data Warehouse at Northwestern University was performed to identify patients with a diagnosis of uveitis or scleritis evaluated between August 2007 and November 2012 who also had available results of Lyme Enzyme-linked immunosorbent assay (ELISA) or Western Blot testing. Data on clinical presentation, results of diagnostic testing, and final diagnosis were collected.

**Results:** 113 patients with a diagnosis of scleritis or uveitis had available results of Lyme testing. Clinical presentations included anterior uveitis (n=56), intermediate uveitis (n=19), posterior uveitis (n=21), panuveitis (n=11) and scleritis (n=6). Only 2 patients (2.65%) had positive ELISA for Lyme; 1 patient had an equivocal result. Of these 4 patients, 2 were diagnosed with syphilis and their positive Lyme titers were felt to be due to cross reaction; the other 2 patients were ultimately diagnosed with juvenile idiopathic arthritis and ocular sarcoidosis. No patient had positive Lyme Western blot testing.

**Conclusions:** Lyme testing is often performed in patients with uveitis and scleritis as part a diagnostic evaluation. In this small series, only 4 of the 113 tested patients had positive ELISA testing, and none were ultimately diagnosed with ocular Lyme disease. No patients had positive Western blot testing. Obtaining Lyme titers in patients with ocular inflammation may have a low yield, at least in non-endemic areas, and should probably be reserved for those patients with a history of tick bite and rash, or other systemic findings suggestive of the diagnosis.

**Commercial Relationships:** Lana M. Rifkin, None; Andrea D. Birnbaum, None; Anjali S. Parekh, None; Chaisiri Jumboendlarasame, None; Dmitry Pyatetsky, None; Debra A. Goldstein. Bausch and Lomb (C), Bausch and Lomb (R)

**Support:** Northwestern Memorial Department of Ophthalmology is supported by an unrestricted grant from the Research to Prevent Blindness Foundation (NY)

**Program Number:** 2884 Poster Board Number: B0253
**Presentation Time:** 8:30 AM - 10:15 AM

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A Comparison of the Etiology of Infectious Corneal Ulcers and Bacterial Susceptibility to Antibiotics in Non-Contact Lens and Contact Lens Wearing Patients

Krishna Patel1, Michael A. Saidel2. 1University of Missouri Kansas City, Kansas City, MO; 2University of Chicago, Chicago, IL.

Purpose: To compare the laboratory results of central corneal ulcers in contact lens and non-contact lens wearers seen at the University of Chicago between 2002 and 2009 to determine the relative frequencies of pathogens causing bacterial ulcers and their susceptibility to antibiotic treatment.

Methods: A retrospective chart review was done for patients identified as having a bacterial central corneal ulcer between the years 2002 and 2009. The culture results of the central ulcers and the bacterial susceptibility to different antibiotics were analyzed for each subset.

Results: 314 charts were identified by ICD-9 coding of “ulcer” and reviewed. A total of 128 central bacterial ulcers were identified, 52 in non-contact lens wearers and 76 in contact lens wearers. 111 (86.7%) of these ulcers were cultured and 65 (58.5%) had positive cultures. The most common organism isolated in non-contact lens wearers was coagulase negative Staphylococci (n=10) whereas the most common organism in contact lens wearers was Pseudomonas aeruginosa (n=22). Some of the organisms common to both subsets include Pseudomonas aeruginosa, coagulase negative Staphylococci, alpha hemolytic Streptococci, Staphylococcus aureus, Serratia, Corynebacterium, MRSA, and Escherichia coli. The organisms that exclusively grew in contact lens wearers include Klebsiella (n=2), Achromobacter (n=2), non-alpha hemolytic Streptococcus (n=1), Actinobacter (n=1) and Micrococcus (n=1). The organisms isolated in non-contact lens wearers only include Moraxella (n=5), MSSA (n=3), gram negative bacilli (n=2), Streptococcus pneumoniae (n=1) and gram positive bacilli (n=1). Of antibiotics available in a topical ophthalmic formulation, only vancomycin demonstrated no resistance among bacteria tested in non-contact lens wearers. Gentamicin, levofloxacin, tobramycin, trimethoprim-sulfamethoxazole and vancomycin demonstrated no resistance among bacteria tested in contact lens wearers.

Conclusions: Our study showed that the most common organism in non-contact lens associated central ulcers was coagulase negative Staph which was consistently susceptible to vancomycin, and Pseudomonas in contact lens wearers which was susceptible to ciprofloxacin, gentamicin, levofloxacin, and tobramycin. With this information, empirical treatment of corneal ulcers can be more specific.

In a separate study, we investigated antibiotic susceptibility among clinical isolates of P. aeruginosa.

Elizabeth Shen1, Fung-Rong Hu2. 1Department of Ophthalmology, Buddhist Tzu Chi General Hospital Taipei Branch, Xindian, Taipei, Taiwan; 2Department of Ophthalmology, National Taiwan University Hospital, Taipei, Taiwan.

Purpose: To determine the association between serotypes, antibiotic susceptibility, and the Type III secretion system (T3SS) genotype among ocular isolates of P. aeruginosa.

Methods: Clinical ocular isolates of P. aeruginosa collected from 2001-2011 were serotyped and analyzed with multiplex PCR for exoS, exoU, and exoT genes. Minimum inhibitory concentrations (MIC) were determined using agar dilutions containing gentamicin, amikacin, ceftazidime, piperacillin, ciprofloxacin, gatifloxacin, and moxifloxacin. The Fisher Exact Test was used to compare the antibiotic susceptibility between different serotypes and genotypes of P. aeruginosa.

Results: Among a total of 119 ocular isolates collected, 37 isolates were from contact lens-related microbial keratitis (CLMK) and 48 were from non-CLMK. Genotype distribution found 62.2% of CLMK strains were cytotoxic (expressing exoU) while only 18.8% of non-CLMK strains were cytotoxic (P<0.01). Among CLMK isolates, serotypes 11(51%), 2(16%), and 6(11%) were most commonly found. Serotypes 2(40%), 11(23%), and 6(15%) were more prevalent in non-CLMK isolates. Among the antibiotics tested, 13% of the invasive strains were resistant to gentamicin and next highest was to moxifloxacin at 10%. Cytotoxic strains comparatively had lower resistance rate to all the antibiotics tested compared to invasive strains although not reaching statistically significant difference. Invasive strains with serotype 2 from CLMK isolates had 50% sensitivity to fluoroquinolones. There were five multiple drug resistant (MDR) strains: 2 of cytotoxic genotype and 3 of invasive genotype.

Conclusions: Contrary to previously reports, our cytotoxic strains did not show a statistical significant increase in antibiotic resistance compared to invasive strains. Invasive strains with serotype 2, in particular, showed a higher resistance to fluoroquinolone antibiotics.
Clinician should be aware of increased fluoroquinolone resistance especially among contact lens associated corneal infections.

**Commercial Relationships:** Elizabeth Shen, None; Fung-Rong Hu, None

**Program Number:** 2886 **Poster Board Number:** B0255  
**Presentation Time:** 8:30 AM - 10:15 AM  
**Prevalence and characteristics of MRSA from clinical conjunctivitis trials versus ocular surveillance studies**  
**Purpose:** Methicillin-resistant Staphylococcus aureus (MRSA) strains can cause severe infections of the eye. Typical hospital-acquired (HA) MRSA strains contain the SCCmec II resistance cassette and are multi-drug resistant, while community-acquired (CA) MRSA isolates generally possess the SCCmec IV cassette and are usually considered to be more virulent. Both subgroups also tend to be genetically distinct. This study determined the proportion of HA and CA MRSA among isolates from clinical conjunctivitis trials compared to those from ocular surveillance studies.  
**Methods:** 27 MRSA isolates from three conjunctivitis trials conducted between 2004-2007, and 298 MRSA isolates from various types of ocular infections from the 2009-2011 ARMOR surveillance studies were characterized and compared. Antibiotic susceptibility testing against oxacillin (OXA), ciprofloxacin (CIP), and azithromycin (AZI) was performed according to current CLSI guidelines. PCR amplification and DNA sequence analysis were used to determine the spa and SCCmec types and ascertain the presence of the Panton-Valentine Leukocidin (PVL) toxin.  
**Results:** Of the ocular MRSA isolates studied here, both the conjunctivitis trial isolates and the surveillance study isolates contained similar ratios of strains that met the criteria for HA-MRSA (51.9% and 48.1%, respectively) and for CA-MRSA (46.0% and 50.7%, respectively). Overall, the conjunctivitis trial isolates showed a higher diversity in genetic lineages (spa types) compared to the surveillance study isolates, which were dominated by spa types t002 and t008. Twelve (44.4%) different spa types were identified among the 27 conjunctivitis trial isolates, compared to 52 (17.4%) different spa types identified among the 298 surveillance study isolates (3 of which were novel and not found in the Ridom SpaServer database).  
**Conclusions:** The data from analysis of conjunctivitis trial and surveillance study MRSA isolates indicate that both HA and CA strains are fully capable of infecting the eye. The results further suggest that, while the surveillance study MRSA isolates include a higher percentage of isolates that are of concern due to drug resistance and virulence, the conjunctivitis trial group also contains a notable number of these isolates.

<table>
<thead>
<tr>
<th>Conjugative Trials</th>
<th>Surveillance Studies</th>
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<tr>
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<td>Resistant to OXA, CIP, &amp; AZI</td>
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**Commercial Relationships:** Timothy W. Morris, Bausch & Lomb, Inc. (E); Christine M. Sanfilippo, Bausch & Lomb, Inc. (E); Christine K. Hejje, Bausch & Lomb, Inc. (E); Matthew E. MacGillray, Bausch and Lomb (E); Wolfgang Haas, Bausch & Lomb, Inc. (E)

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antibiotic/steroid drops had ocular cultures. Positive external or intraocular cultures were identified in 36 of the 50 patients. The culture sites included corneal scraping (38%), conjunctiva (18%), vitreous (8%), anterior chamber (6%), lid (6%), socket (2%), and contact lens (2%). Overall, 38 individual organisms were identified comprising 20 different species. The following gram-positive organisms comprised 20 of 38 (53%) isolates: coagulase negative Staphylococcus (CNS, 28.9%), S. aureus (10.5%), Streptococcus pneumonia (5.3%), S. sanguinis (2.6%), Diphtheroids (2.6%), and Bacillus cereus (2.6%). S. epidermidis accounted for four of the 11 CNS organisms. The following gram-negative organisms comprised 13 of 38 (34%) isolates: Pseudomonas aeruginosa (18.4%), Enterobacter aerogenes (5.3%), Serratia marcescens (5.3%), Klebsiella oxytoca (2.6%), and Moraxella osloensis (2.6%). Five of 38 (13%) isolates were non-bacterial, each identified once, representing 2.6% of isolates: Acanthamoeba, Candida parapsilosis, Fusarium, Nocardia, and one non-specified fungus. Two cultures (5.5%) were polymicrobial. The most common organisms cultured in patients using steroid drops were CNS (33.3%) and P. aeruginosa (19.4%). The most common organisms identified in patients using combination steroid/antibiotic drops were CNS (25%), S. aureus (25%), and P. aeruginosa (18.8%). In the current study, methicillin-resistance was found in 2 of 4 S. epidermidis and 1 of 4 S. aureus isolates.

Conclusions: The most common organisms identified in the current study were CNS and P. aeruginosa. Corneal scraping in patients with keratitis was the most common site of positive cultures. Broad spectrum antimicrobial coverage is necessary until laboratory data are available. The use of steroid and combination steroid/antibiotic drops may predispose to the occurrence of more virulent and non-bacterial organisms.

Commercial Relationships: Peter Belin, None; Darlene Miller, None; Ajay E. Kuriyan, None; Harry W. Flynn, None

Support: NIH Center Core Grant P30EY014801, Research to Prevent Blindness Unrestricted Grant, Department of Defense (DOD-Grant#W81XWH-09-1-0675)

Program Number: 2889 Poster Board Number: B0258

Presentation Time: 8:30 AM - 10:15 AM

Interleukin-6 Gene Polymorphisms in Patients with Keratitis

venkata nagaraju konda, None; Mark D. Wilcox, Allergan Inc (C), Allergan Inc (R), Brien Holden Vision Institute (P), Bausch + Lomb (C), Bausch + Lomb (R); Inderjeet Kaur, None; Preeji M. Sudharaman, None; Prashant Garg, None; Subhbrata Chakraborti, None

Program Number: 2890 Poster Board Number: B0259

Presentation Time: 8:30 AM - 10:15 AM

Demographics and Bacterial Contamination Influences in Antibacterial Effectiveness of Human Milk


Purpose: Human milk is used for ocular surface disease in developing countries. A study by our group using an agar well diffusion assay found that human milk has significant antibacterial effect against Neisseria gonorrhoeae, Moraxella catarrhalis, and Streptococcus viridans. One explanation of this effect is competitive inhibition by commensal bacteria. The purpose of this study is to investigate whether maternal or infant age and bacterial contamination in the samples are associated with the antibacterial effect of human milk.

Methods: We used an agar well diffusion assay to determine the susceptibility of various bacteria to human milk. For each milk sample, we also measured the amount of bacterial contamination in the specimen by inoculating dilutions of 20 μL of human milk onto blood agar, incubating the plates for 18-24 hours at 35°C in 5% CO2, and counting the number of colony forming units per mL. We assessed for associations between bacterial inhibition and (1) infant age, (2) maternal age, and (3) amount of bacterial contamination using the Spearman’s correlation coefficient.

Results: Twenty-three samples were tested. Maternal age ranged from 22 to 43 years, median 32 years. Infant age ranged from 0.58 to 36 months, median 4 months. The association between infant age and bacterial inhibition was variable: M. catarrhalis (Spearman’s ρ=0.52, p-value=0.01), N. gonorrhoeae (ρ=0.09, p-value=0.70), and S. viridans (ρ=0.19, p-value=0.39). The association between maternal age and inhibition was also dependent on the organism: N. gonorrhoeae (ρ=0.48, p-value=0.03), M. catarrhalis (ρ=0.15, p-value=0.53), and S. viridans (ρ=0.08, p-value=0.72). The results for amount of bacterial contamination and inhibition were: N. gonorrhoeae (ρ=0.42, p-value=0.05), M. catarrhalis (ρ=0.07, p-value=0.05), and S. viridans (ρ=0.39, p-value=0.29).
Conclusions: Significantly, 16S sequences and additionally identified a number of phage species indicating multibacterial type of infection. BRiSK corroborated deep Pseudomonas aeruginosa homeostatic and the onset of stable pathological b.

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previously showed that P. aeruginosa biofilms on contact lenses removed from infected rat eyes caused faster corneal infections than naive P. aeruginosa. In addition, exposure of P. aeruginosa biofilms on contact lenses to human tear fluid and human corneal epithelial cells in vitro, allowed bacteria to penetrate the corneal epithelium in vivo whereas lens-grown biofilms alone did not. Since the type three secretion system (T3SS) is a major virulence mechanism of P. aeruginosa, we tested the hypothesis that exposure to tear fluid triggers T3SS expression in lens-grown P. aeruginosa biofilms.

**Methods:** To form biofilms, human contact lenses were incubated in P. aeruginosa PAO1 (~1000 cfu/mL) in tryptic soy broth (TSB) at 37°C in stationary conditions for seven days. Controls included P. aeruginosa grown in shaken tryptic soy broth (TSB) overnight (first comparison), or a sub-set of biofilm-coated contact lenses further exposed to PBS (second comparison) or human tear fluid overnight. T3SS gene expression (exsA and exoS) was quantified by real time q-PCR to compare planktonic/biofilm and biofilm exposed to PBS/tears. The internal control for q-PCR was ribosomal 16s.

**Results:** T3SS gene expression in biofilms was upregulated compared to planktonic cells; for exsA 5.46 ± 0.24 fold increase (95% CI: 4.43 - 6.50, p = 0.02); for exoS 3.76 ± 0.36-fold increase, (95% CI: 2.61 - 4.90, p = 0.01). For biofilms exposed to tears overnight, expression of both exsA and exoS was further upregulated compared to biofilms without tear exposure. For exsA 2.10 ± 0.38 fold higher (95 % CI: 1.03 - 3.15) (p < 0.04); for exoS 1.90 ± 0.26 fold (95 % CI: 1.47 - 2.31) (p < 0.001).

**Conclusions:** These data show that biofilm growth on a contact lens, and subsequent exposure to human tear fluid, upregulate expression of the T3SS in P. aeruginosa. Since this pathogen can form biofilms on contact lenses in vivo, and T3SS is a known virulence determinant in the cornea, these data suggest a potential mechanism for initiation of contact lens-related infectious keratitis.

**Commercial Relationships:** Yvonne Wu, None; Connie Tam, "Antimicrobial Peptides and Methods of Use Thereof" (P); David J. Evans, U.S. Provisional Patent Application No. 61/479,507, (P), U.S. Issued Patent 7,332,470 B2 (P); Suzanne M. Fleischig, Allergan (C), Allergan (F), New methods for preventing infection (P) Support: American Optometric Foundation; NHMRC CJ Martin Fellowship; School of Optometry and Vision Science, The University of New South Wales, Australia

**Program Number:** 2894 Poster Board Number: B0263

**Efficacy of photodynamic antimicrobial chemotherapy on gram negative bacteria**

Takaihito Chikama1, Miftahul Akhyar Latief2, Monoko N. Shihasaki3, Ji-Ae Ko1, Takaaki Sasaki1, Yoshiaki Kiuchi1, Takemasa Sakaguchi2, Akira Obana1. 1Ophthalmology, Hiroshima Univ Grad Sch of Biomed Sci, Hiroshima, Japan; 2Virology, Hiroshima Univ Grad Sch of Biomed Sci, Hiroshima, Japan; 3Ophthalmology, Seirei Hamamatsu General Hospital, Hamamatsu, Japan.

**Purpose:** To evaluate the effectiveness of photodynamic antimicrobial chemotherapy (PACT) on gram negative bacteria.

**Methods:** A derivative porphyrin (TONS504; Porphyrin Lab., Okayama) with light emitting diode (LED) exposure by the LED device (CCS Inc., Kyoto) was applied in vitro onto 10 colony forming unit of Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli) and Serratia marcescens (S. marcescens) with an additional agent of ethylene diamine tetra acetic acid (EDTA). The LED lighting device which can supply a single wavelength (660 nm) was applied with 10, 20 and 30 J/cm². After applying TONS504 with various concentrations (0.5 to 20μg/mL) mixed with 0.05M of EDTA and followed by LED exposure, bacterial suspensions on 24 wells plate were incubated overnight in 37°C. The growth activity was evaluated by cultured on standard agar, and confirmed by counting bacterial colony formation after 30 minutes and 24 hours incubation.

**Results:** TONS504-PACT showed a dose dependent inhibition on bacterial growth of gram negative bacteria. After 30 minutes LED exposure, there is no complete inhibitory effect on bacterial growth from this treatment. The completely inhibitory effect on bacterial growth was founded after 24 hours incubation with the concentration of TONS504 from 10μg/mL with 10J/cm², and from 1μg/mL with 20 and 30J/cm² of LED exposure. However, TONS504 in the concentration between 0.5 to 20μg/mL without LED exposure had no inhibition on gram negative bacteria.

**Conclusions:** TONS504-PACT had a bactericidal effect in vitro on gram negative bacteria with the presence of EDTA.

**Commercial Relationships:** Takaihito Chikama, None; Miftahul Akhyar Latief, None; Monoko N. Shihasaki, None; Ji-Ae Ko, None; Takaaki Sasaki, None; Yoshiaki Kiuchi, None; Takemasa Sakaguchi, None; Akira Obana, None

**Support:** JST AS232201652F

**Program Number:** 2895 Poster Board Number: B0264

**Presentation Time:** 8:30 AM - 10:15 AM

**Increasing coverage of a vaccine against herpes zoster at New York University Langone Medical Center and Bellevue Hospital**

Ilyse D. Haberman1, Elisabeth J. Cohen1, Zachary Elkin1, Judith D. Goldberg2, Xiao-chun Li3, Euliana Castano1, Lisa Park2, Michael H. Perskin1. 1Department of Ophthalmology, New York University School of Medicine, New York, NY; 2Division of Biostatistics, New York University School of Medicine, New York, NY; 3Division of General Internal Medicine, Department of Medicine, New York University School of Medicine, New York, NY; 4New York University School of Medicine, New York, NY.

**Purpose:** To increase coverage following recommended guidelines of the vaccine against herpes zoster at New York University Langone Medical Center (NYULMC) by studying barriers to vaccination before and after introducing interventions to facilitate usage.

**Methods:** An IRB approved follow-up survey of internal medicine physicians was administered to assess knowledge, attitudes, practices, and perceived barriers regarding the vaccine against herpes zoster. This survey was administered 1 year after the baseline survey as reported at ARVO 2012. Interventions included education, increased availability at the outpatient pharmacy with the option for administration by a nurse, and implementation of electronic medical record alerts and reminders. The survey began October 1, 2012 and was extended due to flood damage from Hurricane Sandy from November 30 to December 21, 2012. Pharmacy data for vaccine administration was reported for the months prior to and following the interventions.

**Results:** Results from the follow-up survey of 262 internal medicine physicians and documented changes in vaccine use at the NYULMC pharmacy from the period prior to the baseline survey will be presented. To date, the response rate to the survey is 23.6% (62/262). After interventions, the percentage of physicians who prescribe to fewer than 50% declined 15.4% (70.1% to 54.7%). The percentage of physicians who believe zoster vaccination is a clinical priority remained stable (69.3% to 72.2%). Of the perceived barriers identified from the baseline survey, physicians who reported cost decreased 9.3% (77.8% to 68.5%), and those who reported competing clinical focuses decreased 23.1% (50.9% to 27.8%). Following increased vaccine availability at the outpatient pharmacy, along with a physician and patient education campaign, vaccine prescriptions per
month at the outpatient pharmacy increased from an average of 47 (range 33 to 59) in the three months prior to the baseline survey to an average of 134.4 (103 to 169) during the 10 months following the survey (286% increase in vaccines administered per month).

**Conclusions:** Understanding and addressing the barriers to the use of the herpes zoster vaccine can increase its usage in accordance with existing national recommendations.

**Commercial Relationships:** Ilyse D. Haberman, None; Elisabeth J. Cohen, Merck (F); Zachary Elkin, None; Judith D. Goldberg, None; Xiaochun Li, None; Eliana Castano, None; Lisa Park, None; Michael H. Perskin, None

**Support:** This study was supported in part by a research grant from Investigator-Initiated Studies Program of Merck Sharp & Dohme Corp. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Sharp & Dohme Corp.

**Clinical Trial:** NCT01483378

**Program Number:** 2896 Poster Board Number: B0265
**Presentation Time:** 8:30 AM - 10:15 AM

**Rare and newer Non Sporulating Moulds emerging as corneal pathogens identified by molecular techniques in a Tertiary Eye Care Centre**

Gayathri Ramasubban, Lily Therese, Bagyalakshmi Radhakrishnan, Hajib N. Madhavan. L&T Microbiology Research Centre, Vision Research Foundation, Chennai, India.

**Purpose:** To report on the identification of Non Sporulating Moulds (NSM) isolated from corneal ulcers / keratitis by PCR based DNA sequencing targeting ITS region in a tertiary eye care centre.

**Methods:** A total of 151 fungal isolates from 813 corneal specimens (608 corneal scraping and 155 corneal buttons) processed for Microbiological investigations from suspected cases of corneal ulcer/ keratitis during January 2012 to November 2012 were included in the study. Among the 151 fungal isolates, 21 (13.9%) were Non Sporulating Moulds (NSM). In order to identify the NSM to species level, standardised PCR based DNA sequencing targeting Internal transcribed spacer region (ITS region) was applied on DNA extracted from the 21 NSM followed by BLAST analysis.

**Results:** Out of the 21 NSM, 14 were from corneal scrapings and 7 were from corneal buttons. PCR based DNA sequencing targeting ITS region resulted in identification of 9 NSM as Pythium insidiosum (Corneal scraping-5, Corneal button-4), 5 as Lasiodiplodia theobromae (Corneal scraping-3, Corneal button-2), 2 as Mortierella wofliffi (from 1 corneal scraping and 1 corneal button of the same patient) and 1 each of Lasiodiplodia pseudotheobromae, Colletotrichum species, Humicola fusca and Chaetomium species in 5 corneal scrapings. The NSM identification to species level was possible within 36-48 hours by PCR based DNA sequencing.

**Conclusions:** PCR based DNA sequencing is a rapid, reliable tool to identify the NSM to species level. Pythium insidiosum was the most common fungus identified followed by Lasiodiplodia theobromae in this study. To the best our knowledge this is the first report on Colletotrichum species as the causative agent of keratitis and Mortierella wofliffi as the human pathogen.

**Commercial Relationships:** Gayathri Ramasubban, None; Lily Therese, None; Bagyalakshmi Radhakrishnan, None; Hajib N. Madhavan, None

**Program Number:** 2897 Poster Board Number: B0266
**Presentation Time:** 8:30 AM - 10:15 AM

**Efficacy and Safety of Voriconazole and Amphotericin B as Additives in Optisol-GS Corneal Storage Media Against Candida Species**

Noelle Layer1, Vicky Cevallos2, Andrew J. Maxwell2, Caroline Ulrickson1, Jeremy D. Keenan3, Bennie H. Jeng1, 2.

1 Ophthalmology, University of California, San Francisco, San Francisco, CA; 2 Francis I. Proctor Foundation, San Francisco, CA; 3 SightLife, Seattle, WA.

**Purpose:** Voriconazole is the most commonly used corneal storage medium in the United States; however, it currently does not include an antifungal additive. The purpose of this study was to assess the efficacy and safety of voriconazole and amphotericin B in reducing Candida contamination of Optisol-GS under normal storage conditions.

**Methods:** Vials of Optisol-GS were supplemented with either voriconazole at 1x, 10x, or 50x minimum inhibitory concentration (MIC) or amphotericin B at 0.25x, 0.5x, 1x, or 10x MIC. Unsupplemented control groups were also used. Isolates of C. albicans and C. glabrata were each added to a set of vials, which were refrigerated at 4 degrees C. On days 2, 7, and 14, samples were taken to determine viable colony counts immediately after removal from refrigeration and after warming to room temperature for 2 hours. Safety studies were performed by separating 15 pairs of donor corneas into unsupplemented Optisol-GS or Optisol-GS plus voriconazole at 50x MIC, or Optisol-GS plus amphotericin B at 0.25x, 0.5x, 1x, or 10x MIC. Corneal thickness via pachymetry and endothelial cell density (ECD) via specular microscopy were determined at days 0 and 7.

**Results:** Growth of C. albicans and C. glabrata in Optisol-GS was observed at each concentration of voriconazole. In contrast, with supplementation of amphotericin B, there was no growth of C. albicans by day 2 or C. glabrata by day 7 at all concentrations. Viable counts of C. glabrata were reduced by 99% and 96% with amphotericin B supplementation at 0.25x and 0.5x MIC, respectively, on day 2. Compared to paired controls, there was a significant reduction in ECD with Optisol-GS plus amphotericin B at 10x MIC (P = 0.04), a similar trend with amphotericin B at 1x (P = 0.07), and no significant difference at other concentrations.

**Conclusions:** The addition of amphotericin B, but not voriconazole, to Optisol-GS may significantly improve activity against contamination with Candida spp., a major cause of fungal endophthalmitis after corneal transplantation. While there appears to be toxicity to the corneal endothelium at the maximal concentration of amphotericin B studied, there is no evidence for toxicity at the lower doses. A larger study is warranted to confirm these findings.

**Commercial Relationships:** Noelle Layer, None; Vicky Cevallos, None; Andrew J. Maxwell, None; Caroline Ulrickson, None; Jeremy D. Keenan, None; Bennie H. Jeng, None

**Support:** Richard Lindstrom/Eye Bank Association of America Research Fund, That Man May See, Research to Prevent Blindness, Inc, SightLife

**Program Number:** 2898 Poster Board Number: B0267
**Presentation Time:** 8:30 AM - 10:15 AM

**Rapid and sensitive diagnosis of Acanthamoeba keratitis by loop- mediated isothermal amplification**

Ge Zhao, Qing Yuan. Shandong Eye Institution, Qingdao, China.

**Purpose:** To develop a loop-mediated isothermal amplification (LAMP) assay for the detection of Acanthamoeba.

**Methods:** The sensitivity of the LAMP assay was tested using different copies of positive DNA. The specificity of the assay was tested using DNA extracted from Acanthamoeba, Pseudomonas aeruginosa, Candida albicans, herpes simplex virus-1 and human corneal epithelial cells. The effectiveness of the LAMP assay was evaluated and compared with culture, corneal smear examination and real-time PCR in the corneal samples of Acanthamoeba keratitis.

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(AK) mice. We also tested 3 corneal samples from patients with suspected Acanthamoeba or fungal infection using LAMP.

**Results:** LAMP was confirmed to be very sensitive, with the lowest detection limit being 10 copies/tube of Acanthamoeba DNA. The LAMP primers only amplified Acanthamoeba DNA. During the development of AK in mice, almost all of the positive rates of LAMP at each time point post-infection were higher than those of culture or corneal smear examination. The total positive rate of LAMP was significantly higher than those of culture and corneal smear examination (P<0.05), while the sensitivity of LAMP and real-time PCR was comparable. However, the trends of positive change in these different test methods were generally similar. Of the three clinical corneal specimens, two suspected AK were tested positive for Acanthamoeba using LAMP along with culture or corneal smear examination, while the other suspected fungal keratitis was tested negative.

**Conclusions:** The LAMP assay is a simple, rapid, highly specific and sensitive method for the diagnosis of keratitis caused by Acanthamoeba.

**Predictors of outcome in fungal keratitis using data from the Mycotic Ulcer Treatment Trial I**

**Purpose:** To determine baseline factors that are predictive of outcome in fungal keratitis using data collected in the Mycotic Ulcer Treatment Trial I (MUTT I).

**Methods:** MUTT I was a multicenter, randomized, double-masked, NEI-funded clinical trial comparing clinical outcomes in 323 patients with fungal keratitis receiving 5% topical natamycin or 1% topical voriconazole. Demographics, ocular medical history and clinical characteristics were collected at the enrollment visit for all patients, and were used as predictors in our analysis. Pre-specified outcomes included best spectacle-corrected visual acuity (BSCVA) at 3 months, infiltrate/scar size at 3 months, corneal perforation or transplant and re-epithelialization time. Separate univariate and multivariable analyses were performed for all predictors with each of the above four outcome variables. We adjusted for multiple comparisons using the Bonferroni correction.

**Results:** In our multivariable model, significant predictors of worse 3-month visual acuity were worse presentation visual acuity (P<0.001), larger epithelial defect size at presentation (P<0.001) and randomization to voriconazole instead of natamycin in the trial (P=0.007). For 3-month infiltrate/scar size, significant predictors at presentation include larger infiltrate size (P<0.001), larger epithelial defect size (P<0.001), worse presentation visual acuity (P=0.003) and use of topical antifungals prior to trial enrollment (P<0.001). Worse presentation visual acuity (P<0.001), older age (P=0.011) and randomization to voriconazole instead of natamycin (P=0.008) were predictive of perforation. Epithelial defect size (P=0.001) and presentation ulcer depth (P=0.012) were significant predictors of longer time to re-epithelialization.

**Conclusions:** Ulcer severity at presentation is highly predictive of worse outcomes. In our analysis, we found that clinical characteristics at presentation, such as epithelial defect size and visual acuity, seem to provide more information about prognosis than visual outcome.

**Commercial Relationships:** Ge Zhao, None; Qing Yuan, None

**Support:** National Natural Science Foundation of China (81100651)
Acanthamoeba Keratitis

Purpose: To investigate in vivo corneal changes of keratoneuritis in early stage Acanthamoeba keratitis (AK) using in vivo laser confocal microscopy.

Methods: Thirteen eyes (twelve patients, five men, seven women; mean age, 22.3 ± 4.2 years) with keratoneuritis due to early stage AK participated in this study.

Results: In all patients, Acanthamoeba cysts were clearly observed in the basal epithelial cell layer as highly reflective round-shaped particles with a diameter of 10 to 20 µm. Bowman’s layer infiltration of Acanthamoeba cysts was observed in only one case, and no cases showed stromal and/or nerve infiltration of Acanthamoeba cysts. In the stroma, all cases showed highly reflective activated keratocytes forming a honeycomb pattern; these changes were significant around the keratoneuritis. Infiltration of inflammatory cells, possibly polymorphonuclear cells, was observed along with keratocyte bodies in all cases. Numerous highly reflective spindle-shaped materials were observed around the keratoneuritis. Most notably, highly reflective patchy lesions were observed around the keratoneuritis in eleven cases (84.6%). Inflammatory cells were also observed in the endothelial cell layer in four cases (30.8%).

Conclusions: In vivo laser confocal microscopy identified consistent corneal abnormalities around keratoneuritis in early stage AK patients, of which highly reflective patchy lesions may be characteristic to keratoneuritis. Further morphological studies of corneas with early stage AK in a larger number of patients may elucidate the clinical significance of radial keratoneuritis and may help us to understand the interaction between Acanthamoeba organisms and host corneal cells or nerves.

Commercial Relationships: Natsuko Yamazaki, None; Akira Kobayashi, None; Hideaki Yokogawa, None; Kazuhisa Sugiyama, None

Program Number: 2902 Poster Board Number: B0272
Presentation Time: 8:30 AM - 10:15 AM

Antibiotic resistance and molecular characterization of ocular isolates of Acinetobacter baumannii

Deepa Talreja1,2, Chithra Muraleedharan2, Keith Kaye1, Satish K. Walia1, Ashok Kumar1, 4

1Ophthalmology, Kresge Eye Institute, Detroit, MI; 2Biological Sciences, Oakland University, Auburn Hills, MI; 3Internal Medicine, Detroit Medical Centre Wayne state University, Detroit, MI; 4Anatomy and Cell Biology, Wayne State University, Detroit, MI.

Purpose: Acinetobacter baumannii is an opportunistic pathogen that most frequently causes nosocomial infections. Although few clinical studies have documented A. baumannii as a causative agent of keratitis and endophthalmitis, the detailed characterization of ocular isolates remains to be determined. In this study, we assessed the antibiotic-resistant pattern, genetic relatedness and plasmid profiles of ocular isolates from South East Michigan.

Methods: Ocular A. baumannii isolates (n=12) were taken from the clinical microbiology laboratory of Detroit Medical Center. The minimum inhibitory concentration (MIC) of various antibiotics was determined against each isolate using Micro scan. Plasmid profiling
Results: Majority of the isolates were multidrug resistant. However, none of them were β-lactamase producers. All isolates harbored multiple plasmids and ten distinct plasmid profiles were observed. Multiple antibiotic resistance genes transferred from donor resistant bacteria to recipient E. coli. J53. PFGE analysis of Apa I-digested genomic DNA showed the presence of distinct genotypes among all isolates. Biofilm formation assay revealed that 10 isolates were strong and two were moderate biofilm producers. Moreover, all isolates induced cytotoxicity and showed strong affinity towards adherence and internalization in HCECs. Virulence genes *ompA* and *bap* were constitutively expressed whereas the expression of *PhoC* and *PhoD* varied among the isolates.

Conclusions: This study highlights an importance of *A. baumannii* as a potential ocular pathogen which can cause aggressive infections in the eye. Considering the highly resistant nature and their presence on ocular surface warrants further investigation to assess the pathogenesis of *A. baumannii* ocular infections.

Commercial Relationships: Deepa Talreja, None; Chithra Muraleedharan, None; Keith Kaye, Pfizer (C), pfizer (F), Cubist (C), cubist (F), forest (F), forest (C), merck (F), merck (C); Satish K. Wala, None; Ashok Kumar, None

Support: NH Grant EY19888, Research to Prevent Blindness

Program Number: 2904 Poster Board Number: B0273

Presentation Time: 8:30 AM - 10:15 AM

Antibiotic Resistance Surveillance of Ocular Pathogens - four years of ARMOR Study Results


Purpose: Antibiotic resistance surveillance data can guide clinicians in the empiric treatment of ocular infections. Here we report the study results to date for the 2012 ARMOR (Antibiotic Resistance Monitoring in Ocular MicRoorganisms) surveillance study and compare the results to those from the previous three years.

Methods: To date, 456 isolates of *Streptococcus pneumoniae*, *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Pseudomonas aeruginosa*, and *Haemophilus influenzae* from 25 sites were subjected to antibiotic susceptibility testing. Minimum inhibitory concentrations were determined by broth microdilution for up to 16 representative antibiotics per Clinical and Laboratory Standards Institute methods. Systemic breakpoints (where available) were used to categorize isolates as susceptible or non-susceptible (intermediate and resistant).

Results: While drug resistance among *H. influenzae* isolates was not observed, non-susceptibility among *P. aeruginosa* isolates was noted for ciprofloxacin (9.1%), imipenem (11.4%), tobramycin (4.5%), and polymyxin B (4.5%). *S. pneumoniae* isolates were non-susceptible to imipenem (13.1%), penicillin (4.9%), chloramphenicol (3.3%), and azithromycin (41.0%). *S. aureus* and CoNS isolates were non-susceptible to oxacillin/methicillin (37.3-41.9%), ciprofloxacin (33.8-36.6%), clindamycin (18.3-31.3%), azithromycin (58.8-60.1%), and other antibiotics. More than 33% of *S. aureus* and CoNS isolates were resistant to 3 or more antibiotics. Methicillin-resistant isolates of *S. aureus* (MRSA) and CoNS (MRCoNS) were predominantly multi-drug resistant (>73%). Compared to the three previous years, non-susceptibility rates were similar and the new data set reduced some of the fluctuation seen over the previous years.

Conclusions: After accounting for annual fluctuations, overall resistance rates generally did not show substantial changes over the four year study period. However, a number of isolates were resistant to commonly used ophthalmic antibiotics. Multi-drug resistance was especially prevalent among the MRSA and MRCoNS isolates. Therefore, continued vigilance is warranted to monitor the contribution of resistant isolates to the pathogen population.

Commercial Relationships: Wolfgang Haas, Bausch & Lomb, Inc. (E); Jennifer Deane, None; Timothy W. Morris, Bausch & Lomb, Inc. (E); Daniel F. Sahm, None

Program Number: 2905 Poster Board Number: B0274

Presentation Time: 8:30 AM - 10:15 AM

Case Control Study of Herpes Zoster Ophthalmicus in the Bronx Using Population-based and Clinic-based Controls

David M. Poulsen1,2, Grace Honik1,2, David C. Gritz1,2.

1Ophthalmology and Visual Sciences, Albert Einstein College of Medicine, Bronx, NY; 2Ophthalmology and Visual Sciences, Montefiore Medical Center, Bronx, NY.

Purpose: Herpes zoster ophthalmicus (HZO) has an estimated incidence of 1.5 to 6.4 cases per 10,000 persons per year in the general population. As far as the authors are aware, no previous study has reported the epidemiology of HZO in the Bronx, or estimated the risk of developing HZO among persons with HIV. HIV is 3.6 times more prevalent in the Bronx than in the U.S. overall, allowing examination of the association between HIV and HZO. Montefiore Medical Center is the largest healthcare provider in the Bronx, caring for about one-third of the Bronx population each year.

Methods: This is a retrospective case-control study of the risk of developing herpes zoster ophthalmicus among patients that are HIV-positive and living in the Bronx. Cases were Bronx residents diagnosed with new-onset HZO during the ten year study period (May 1, 2002 to April 30, 2012) at Montefiore Medical Center. The study utilizes two different case control approaches: 1. A population-based control group taken from the 2010 U.S. Census and 2010 New York City HIV/AIDS Annual Surveillance Statistics, matched to the Zip codes of cases; 2. Clinic-based controls chosen randomly on the same appointment date as cases in a 20:1 ratio. The data were used to calculate odds ratios of HIV infection and HZO. The data was analyzed using STATA 12 (College Station, TX) and statistically significant relationships were assessed at p<0.05.

Results: During the study period, 106 incident cases of herpes zoster ophthalmicus met inclusion criteria. Compared to the population-based controls (n=1,405,127), infection with HIV showed an increased association of having HZO relative to those not known to be HIV-positive by an odds ratio of 11.027 (95% CI: 6.324-19.229; p<0.001), using multivariate logistic regression to control for age group, zip code, and gender. Compared to clinic-based controls (n=2,120), HIV infection was shown to increase the odds of HZO by 10.281 (95% CI 5.422-19.493) using multivariate regression to control for age, gender, and race.

Conclusions: Infection with HIV is a significant risk factor for having an incident case of herpes zoster ophthalmicus. This is particularly significant in the Bronx where the prevalence of HIV infection is relatively high. Patients, particularly young patients, who develop HZO should be tested for HIV, especially if other risk factors for HIV are present.

Commercial Relationships: David M. Poulsen, None; Grace Honik, None; David C. Gritz, None

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Clinical and Microbiological profile of Paediatric Keratitis in a tertiary care hospital in Hong Kong

Vishal Jhanji1, 2, Alvin L. Young1, K. S. Leung1, Nicole Tsim1, Mamie Hui2, Lulu Cheng2. Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, Hong Kong; 1Ophthalmology and Visual Sciences, Prince of Wales Hospital, Shatin, Hong Kong, Hong Kong; 2Microbiology, The Chinese University of Hong Kong, Hong Kong, Hong Kong.

Purpose: Microbial keratitis is a serious cause of ocular morbidity in pediatric age group. In this study, we retrospectively evaluated the clinical and microbiological profile of cases of pediatric keratitis presenting to a tertiary care hospital in Hong Kong.

Methods: All cases of microbial keratitis in pediatric age group (< 18 years) who had presented to our hospital between January 2000 and December 2010 were identified. A retrospective review of medical records was conducted. The medical records were reviewed for associated risk factors, microbiological profile, pre- and post-treatment visual acuity, treatment modalities, and, final outcomes.

Results: Overall, 18 patients (male: female; 5: 13) were recruited. The mean age was 12.4 years (range: 3-17 years). The most common associated risk factor was contact lens wear, identified in 15 (83.3%) patients. 7/15 eyes were associated with orthokeratology lens overnight wear. 2 cases were related to intrinsic keratopathy (exposure keratopathy and vernal keratitis). Only 1 eye was infected secondary to trauma. 6 out of 18 eyes showed positive smear results (5 gram negative bacilli, 1 gram positive cocci). 16 out of 18 eyes showed positive cultures. On microbiological culture, 14 cases had a single pathogen identified and 2 cases had a co-infection. Pseudomonas sp. was the most commonly isolated organism (10 eyes, 62.5%), followed by coagulase -ve staphylococci (5 eyes, 31.2%) and Corynebacterium sp. (2 eyes, 12.5%). All Pseudomonas infections were related to contact lens wear. 14 eyes were treated with fortified antibiotics and 4 were treated with intensive topical levofl oxacin. One case with trauma required multiple surgeries including tectonic penetrating keratoplasty followed by lens aspiration and retinal detachment repair. At the last follow-up, 13 out of 17 eyes (76.5%) had best-corrected visual acuity (BCVA) 20/40 or better, 3 (17.6%) had BCVA between 20/50 and 20/100, and 1 (5.9%) had BCVA < 20/200. Corneal scarring was documented in 13 eyes after treatment. One case required optical deep anterior lamellar keratoplasty and achieved 20/20 with the aid of rigid gas permeable contact lens.

Conclusions: Microbial keratitis in pediatric age group was mostly associated with contact lens wear in our cohort. Prompt diagnosis and treatment of these cases resulted in good visual outcomes without the need of invasive surgery in most patients.

Commercial Relationships: Vishal Jhanji, None; Alvin L. Young, None; K. S. Leung, None; Nicole Tsim, None; Mamie Hui, None; Lulu Cheng, None

Assessment of Risk Factors for Oxacillin-Resistant Ocular Flora from Patients Undergoing Cataract Surgery

Hugo Y. Hsu1, John T. Lind2, Darlene Miller1. Ophthalmology, Doheny Eye Institute, Los Angeles, CA; 1Ophthalmology, Saint Louis University, Saint Louis, MO; 2Ophthalmology, Bascom Palmer Eye Institute, Miami, FL.

Purpose: To assess the risk factors for harboring oxacillin-resistant Staphylococcus species on the ocular surface in a cohort of patients undergoing cataract surgery.

Methods: Conjunctival cultures were obtained from patients undergoing cataract surgery on the day of surgery before the instillation of any ophthalmic medications. Patients also answered a questionnaire about risk factors that might lead to having oxacillin-resistant Staphylococcus organisms. The demographic and questionnaire risk factors tested against having oxacillin-resistant organisms were: 1) age, 2) gender, 3) race, 4) recent antibiotic usage, 5) recent hospitalization, and 6) exposure to health-care or institutional settings. Multivariate logistic regression analysis was performed.

Results: 183 eyes were cultured. 27 eyes showed no growth. 128 eyes revealed Staphylococcus organisms of which 70 eyes (54.7%) had oxacillin-resistant organisms. Of these 128 subjects, 19 had incomplete questionnaires; therefore a total of 109 subjects were utilized for risk-factor analysis. Of the six risk factors, only prior antibiotic usage was significantly associated with having oxacillin-resistant organisms (OR 8.2; 95% CI 2.2—30.5; p = 0.002). The rest of the risk factors were not significantly associated: age (p = 0.06), gender (p = 0.33), race (p = 0.34), hospitalization (p = 0.94), and institutional settings (p = 0.10).

Conclusions: While the non-ophthalmic literature has put forth various risk factors for patients to harbor oxacillin-resistant organisms, in our cohort of patients undergoing cataract surgery, only antibiotic usage in the preceding 30 days prior to surgery was significantly associated with having oxacillin-resistant organisms on the ocular surface. This finding is of importance to ophthalmic surgeons when considering peri-operative antibiotic prophylaxis.

Commercial Relationships: Hugo Y. Hsu, Bausch & Lomb (R); John T. Lind, Allergan (C), Allergan (R); Darlene Miller, None

Support: Research to Prevent Blindness, Inc.
ulceration in 58% specimens (37/64), and stromal fibrosis consistent with scarring in 13% (8/64). In this group, repeat biopsies revealed bacteria in only 2 of 6 patients.

**Conclusions:** Histopathology and laboratory culture provide complementary information to ophthalmologists in cases of suspected infectious keratitis. Bacteria were frequently identified by culture even when not visible in histopathologic sections, while some cases of fungi and Acanthamoeba were only seen in the histopathologic examination.

### Table 1. Microbiologic and histopathologic results for 67 specimens with microorganisms identified.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Culture only</th>
<th>Culture and histopathology</th>
<th>Histopathology only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of specimens (25%)</td>
<td>No. of specimens (6/22)</td>
<td>No. of specimens (6/17)</td>
</tr>
<tr>
<td><strong>Bacterial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. staph.</td>
<td>22</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><strong>fungi</strong></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*3 specimens had polymicrobial infections. In 2 cases, cultures were positive for bacteria whereas histology was positive for fungi. In one case, culture was positive for bacteria whereas histology revealed Acanthamoeba. *Mycobacteria identified in two specimens, one according to culture results and the second according to histologic findings.

**Commercial Relationships:** Yousef J. Cruz-Inigo, None; Sanjay V. Patel, None; Leo Maguire, None; Joaquin J. Garcia, None; Diva R. Salomao, None

**Support:** “Research to Prevent Blindness; and Mayo Foundation”

**Program Number:** 2909 Poster Board Number: B0278

**Presentation Time:** 8:30 AM - 10:15 AM

**Clinical features of anterior uveitis caused by three different types of herpes virus**

Jan Suzuki, Junichi Sakai, Yoshikiko Usui, Takeshi Kezuka, Hiroshi Goto. Ophthalmology, Tokyo Medical University Hospital, Tokyo, Japan.

**Purpose:** To compare clinical findings in patients with anterior uveitis caused by herpes simplex virus (HSV), varicella zoster virus (VZV) and cytomegalovirus (CMV).

**Methods:** Forty-three patients (45 eyes) with viral anterior uveitis diagnosed by polymerase chain reaction using aqueous humor were studied. Clinical profiles of HSV iritis (HSV-I: 10 cases), VZV iritis (ZSH-I: 17 cases) and CMV iritis (CMV-I: 16 cases) were compared.

**Results:** Only 2 patients with CMV-I had bilateral disease. Intraocular pressure was elevated in all patients. Keratic precipitate was seen in all patients and mutton-fat type was observed mostly in HSV-I (100%) and ZSH-I (82%). Iris atrophy was seen in HSV-I (67%) and ZSH-I (71%), which was typically round and sector shaped, respectively. Anterior chamber inflammation was severer in patients with HSV-I and ZSH-I compared to CMV-I. The number of viral copies in aqueous humor was high in patients with ZSH-I. In CMV-I, characteristic keratic precipitates (coin-shaped lesion) was seen in 50% and reduced number of corneal endothelial cell was detected in 93% of the patients. Viral DNA was detected in iris and trabecular meshwork in two patients with CMV-I who underwent trabeculectomy.

**Conclusions:** Anterior uveitis caused by various types of herpes virus share common features, but has some characteristic features for each type.

**Commercial Relationships:** Jun Suzuki, None; Junichi Sakai, None; Yoshikiko Usui, None; Takeshi Kezuka, None; Hiroshi Goto, None

**Support:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) 2010/17350-6

**Clinical Trial:** NCT01739920

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**Comparative efficacy of two different regimens of povidone-iodine 5% eye drops instillation in reducing conjunctival bacterial flora - a preliminary report**

Leticia F. Barroso, Antonio Brunno Nepomuceno, Sarah P. Cazella, Jefferson A. Ribeiro, Lilianes Castilho, Andre Messias, Rodrigo Jorge. Ophthalmology, School of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil.

**Purpose:** To investigate efficacy of 1 or 3 drops of povidone-iodine 5% eye instillation in reducing conjunctival bacterial flora culture positivity.

**Methods:** A total of 63 patients (n=63 eyes) were included, and randomly divided to receive 1 (group PVPI; n=32) or 3 (group PVPIplus; n=31) eye-drops. Conjunctival swab was obtained five minutes before and 30 minutes after the first povidone iodine drop was instilled into the conjunctival sac of study eye. Corneal pachymetry was performed before and after procedure using OcuScan Alcon RXP (Alcon, Fortworth, Texas). Conjunctival swabs were incubated aerobically in enriched Thioglycolate liquid medium (meat broth) and in three solid culture media (Agar Chocolate, Trypticase Soy Agar with 5% sheep blood, and Agar Sabouraud).

**Results:** The proportion of patients that had microorganisms isolated from the conjunctival swab before and after treatment was 14/32 (43%), and 9/32 (28%) for PVPI group, and 8/31 (26%), and 3/32 (10%) for PVPIplus group. A trend towards significant difference was found for the number of positive cultures after treatment between groups (p=0.05). Staphylococcus epidermidis were the microorganisms more frequently isolated in cultures from both groups. Mean corneal thickness increased significantly in 5.7 ± 2.5 μm after treatment in the PVPI group (p=0.033; t-Test), but did not for PVPIplus (-1.2 ± 2.3 μm; P=0.300), and this change was significantly different between groups (P=0.025). There was no significant difference in keratoconjunctivitis scores between groups.

**Conclusions:** This preliminary data evaluation indicates a trend towards better conjunctival bacterial flora reduction if 3 instead of 1 PVPI drops are used, without additional corneal toxicity. Larger samples are warranted to confirm these preliminary findings.

**Commercial Relationships:** Leticia F. Barroso, None; Antonio Brunno Nepomuceno, None; Sarah P. Cazella, None; Jefferson A. Ribeiro, None; Lilianes Castilho, None; Andre Messias, None; Rodrigo Jorge, None

**Support:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) 2010/17350-6

**Clinical Trial:** NCT01739920

**Program Number:** 2911 Poster Board Number: B0280

**Presentation Time:** 8:30 AM - 10:15 AM

**Ocular straylight and contrast sensitivity in HIV-positive patients compared with normal subjects**

Nazli Demirkaya¹, Thomas J. Van Den Berg², Stanley Darma², Reinner O. Schlingemann², Frank D. Verbraak², ¹Ophthalmology, Academic Medical Center Amsterdam, Amsterdam, Netherlands; ²Biomedical Engineering and Physics, Academic Medical Center Amsterdam, Amsterdam, Netherlands; ³Ocular Signal Transduction, Netherlands Institute for Neuroscience, Amsterdam, Netherlands.

**Purpose:** To assess ocular straylight values and spatial and temporal contrast sensitivity in HIV-positive patients compared with normal subjects.

**Methods:** 75 HIV-positive patients, well controlled using cART, and 50 healthy, age and socio-economically matched controls were enrolled in this study. Participants with a history of retinitis or any...
Molecular Quantification of Herpes Simplex Virus (HSV) by Real-Time PCR in Tear Film and Correlation with Human Disease

Nora L. Cothran, Kacy Ramirez, Rebecca Thompson, Anami Patel, Daniel G. Fuller, John DeVencenzo

Purpose: To describe the main clinical ocular manifestations and the immunosuppressive medication used in Granulomatosis with Polyangiitis in a tertiary eye care center (Institute of Ophthalmology Conde de Valenciana). The purpose of this publication is to introduce a new way of effective treatment for the management of opthalmic acute Herpes Zoster.

Methods: A real-time Taqman probe-based qPCR assay, amplifying HSV DNA polymerase, was created with conserved primers for, and melting point discrimination between, HSV-1 & 2. Standard curves of known copy number were made from ultra-purified whole HSV-1 & 2, allowing harmonization of all qPCR HSV assays. LOD was determined by probit statistical analysis of >360 assays, ranging over >9 Log copies/ml. Normal saline, spiked with known quantity of HSV-1, was absorbed onto Schirmer strips, mimicking clinical tear collection. Standard volume tear samples, from the following three groups, were evaluated: A) healthy adults devoid of historical or current ocular surface disease and no history of herpetic eye disease, B) patients presenting to an eye care clinic with symptomatic red eye, C) patients with active dendritic corneal disease (HSV quantity evaluated over time, following therapy initiation).

Results: The LOD for HSV-1 & 2 were 111.24 copies/ml (95% CI, 30.74-250.09) and 453.24 copies/ml (95% CI, 192.13-741.27), respectively. One strip type exhibited qPCR inhibition. Another type was further tested, demonstrating consistent volumes of absorption between strips. Mimicked tear collection and storage of strips were evaluated under various defined conditions including storage of dry strips at room temperature for 7 days before processing. Recovery of HSV-1 from strips was 98.89% of the 4.88 Log copies/ml spiked into the saline. Descriptive statistics, including mean (SD) viral load, were determined for the three study populations. Comparisons of viral loads between groups were made. Sensitivity, specificity, and positive and negative predictive values for dendritic corneal disease, were determined for various viral load thresholds.

Conclusions: A molecular quantification method for HSV-1 & 2 in human tears has been developed, allowing clinical diagnostic trials of herpetic corneal disease and offering potential for improved detection and early therapy.

Commercial Relationships: Nora L. Cothran, None; Kacy Ramirez, None; Rebecca Thompson, None; Anami Patel, None; Daniel G. Fuller, Ciba Vision (ALCON) (F), Allergan (F); John DeVencenzo, None

Program Number: 2914 Poster Board Number: B0283
Presentation Time: 8:30 AM - 10:15 AM

Effective treatment of Herpes Zoster Ophthalmicus Acute with a single injection preauricular

Eduardo Arenas, Fernando Aliviz. Dept 0630, Santa Fe Foundation, Miami, FL.

Purpose: The purpose of this publication is to introduce a new way of effective treatment for the management of opthalmic acute Herpes Zoster.

Program Number: 2912 Poster Board Number: B0281
Presentation Time: 8:30 AM - 10:15 AM

Ocular manifestations of Granulomatosis with Polyangiitis from the “Institute of Ophthalmology Conde de Valenciana”


Purpose: To describe the main clinical ocular manifestations and the immunosuppressive medication used in Granulomatosis with Polyangiitis in a tertiary eye care center (Institute of Ophthalmology Conde de Valenciana).

Methods: Medical records of Granulomatosis with Polyangiitis patients were compiled and reviewed from September 2007 until August 2012. A database was made combining all the ocular clinical manifestations listed. Percentage of occurrence for each ocular disease as a first manifestation in Granulomatosis with Polyangiitis were obtained and categorized. The immunosuppressive treatment was reviewed too.

Results: Among the 34 patients identified, 53% have uniocular disease and 47% have binocular disease. Anterior diffuse scleritis was the most frequent ocular manifestation affecting 32% of the population. Necrotizing scleritis was present in 29% of the patients. UK was the first manifestation in 15% of our population. Orbital disease appeared in 9%. Nodular scleritis were identified in 6%. Retinal vasculitis was infrequent affecting only 3% also anterior uveitis (3%). The main medication administered were oral cyclophosphamide and oral methotrexate in 41% of the patients each one, intravenous cyclophosphamide and azathioprine were used in 26.5%. Mycophenolate mofetilo was used in 6%. Only one patient was taking Anti TNF drugs.

Conclusions: The main ocular manifestation of Granulomatosis with Polyangiitis in our hospital was scleritis and the most common treatment used was oral cyclophosphamide and oral methotrexate.

Commercial Relationships: Miguel Pedroza-Seres, None; Edson J. Robles, None; Diana A. González, None

Program Number: 2913 Poster Board Number: B0282
Presentation Time: 8:30 AM - 10:15 AM

other disease known to influence the retina were excluded. After exclusion, 63 HIV-positive patients and 39 controls were evaluated. Spatial contrast sensitivity was examined using the Pelli Robson (PR) chart. Ocular straylight was assessed with the Oculus C-Quan device. Temporal contrast sensitivity (TCS), as measure for retinal sensitivity without confounding by optics, was assessed with custom made software, implemented using the C-Quan hardware. Only one eye of each participant was selected for analysis.

Results: The patient group had a significantly lower PR score compared with the controls (1.89±0.1 vs 1.94±0.04 logCS; p=0.017) but no difference in TCS was found between both groups (2.13±0.16 vs 2.14±0.16 logTCS; p=0.753). Straylight values were significant higher in patients (1.92±0.18 vs 1.11±0.15 log(s); p=0.032)

Conclusions: The higher straylight value might be attributed to media opacities, e.g. development of cataract, since HIV-positive patients have been reported to have a higher incidence of cataract surgery, caused possibly by accelerated aging. The discrepancy in PR scores and TCS values could be ascribed to the fact that PR outcome is influenced by both optical and retinal components, while TCS assesses purely retinal function.

Commercial Relationships: Nazli Demirkaya, None; Thomas J. Van Den Berg, Oculus GmbH (P); Stanley Darma, None; Reinier O. Schlingemann, None; Frank D. Verbraak, None

Support: Aids Fonds Netherlands
Methods: We present three consecutive cases where a single injection of a combination of acyclovir and Bethametasone Dipropionate/ sodium phosphate placed deep in the preauricular affected zone. The procedure starts with the placement of 3 cc of lidocaine to avoid pain, wait 3 minutes and then throughout the same needle which is kept in place. Inject a mixture of 1cc of Acyclovir and 1 cc of Bethametasone depot.

Results: The dose applied was enough to stop the clinical evolution of the disease in all three cases. Pain and skin lesions slowed next day after the treatment and all signs and symptoms disappeared completely two weeks after. None of the cases developed post herpetic neuralgia.

Conclusions: We believe that the physiopathological fast response of this method may be explained, by the well known anterograde and retrograde axonal flux of the peripheral trigeminal nerves that in this disease compromise the trigeminal ganglia.

Commercial Relationships: Eduardo Arenas. None; Fernando Alvizu. None

Program Number: 2915 Poster Board Number: B0284
Presentation Time: 8:30 AM - 10:15 AM
Yield of Ophthalmology Consults in Fungemic Patients in a Tertiary Care Hospital Setting
Ankoor R. Shah, Devon Ghodasra, Brian VanderBeek.
Ophthalmology, University of Pennsylvania Scheie Eye Institute, Philadelphia, PA.
Purpose: Fungemia is a common affliction in patients who have undergone a transplant, are immunocompromised, or are critically ill. As it is standard practice is to consult ophthalmology when a patient becomes fungemic, we aim to evaluate the yield of these consults.

Methods: A retrospective case series of all inpatient ophthalmology consultations between September 1st, 2010 and August 31st, 2011 at the Hospital of the University of Pennsylvania, a tertiary care center.

Results: A total of 619 new consults were performed during the study period. Among these 619 consults, 69 (11%) were to evaluate for fungal eye involvement and were triggered by positive blood, infected line, or wound cultures. 21 (30%) of these patients were seen in an intensive care unit (ICU) setting and 19 (27%) had lesions consistent with chorioretinal involvement. Only 1 (1.5%) patient had vitreous involvement requiring intravitreal injection of anti-fungal medication.

Conclusions: Consults to rule out ocular involvement in fungemic patients are a significant portion of the total consults performed by the ophthalmology service in our tertiary care setting. The number of patients in which management was changed (addition of intravitreal to systemic antifungal therapy) due to the ophthalmic consult was low. Since these consults represent a substantial portion of total inpatient consults performed by the ophthalmology service in our tertiary care setting, further research is needed to examine the utility of ocular screening for all fungemic patients.

Commercial Relationships: Ankoor R. Shah. None; Devon Ghodasra. None; Brian VanderBeek. None

Program Number: 2916 Poster Board Number: B0285
Presentation Time: 8:30 AM - 10:15 AM
Interferon Alpha in the Treatment of Chronic Cystoid Macular Edema Following Cataract Surgery (Irvine-Gass Syndrome)
Christoph M. Deuter, Faik Gelisken, Manfred Zierhut, Deshka Doycheva. Centre for Ophthalmology, University of Tuebingen, Tuebingen, Germany.
Purpose: Cystoid macular edema (CME) is a common cause of visual impairment after cataract surgery. The aim of this retrospective analysis was to evaluate the long-term effects of interferon (IFN) alpha in the treatment of chronic CME following cataract surgery (Irvine-Gass syndrome).

Methods: Treatment with IFN alpha-2a was started at an initial dose of 3 million IU per day subcutaneously for approximately four weeks. Afterwards, the dose of IFN alpha-2a was tapered stepwise to the lowest possible dose that keeps CME in remission, and was finally discontinued if possible. Treatment efficacy has been assessed by optical coherence tomography (OCT).

Results: Seven patients (3 male, 4 female; mean age 67.7 years, range 49-76 years) with 8 affected eyes have been analyzed. Mean follow-up was 29.1 months (range 27-34 months). Mean duration of treatment with IFN alpha-2a was calculated for 23.3 months (range 4-34 months). Ineffective pre-treatment included systemic corticosteroids (6 patients), acetazolamide (3 patients), and intravitreal triamcinolone and/or bevacizumab (5 patients). In 7 eyes (87.5%), IFN alpha-2a led to complete resolution of CME. Within 3 months, mean central foveal thickness (CFT) decreased from 543.8 μm (range 340-920 μm) to 249.1 μm (range 110-690 μm). An improvement of visual acuity of at least two lines could be observed in 5 eyes (62.5%). In 2 patients IFN alpha-2a could be discontinued after 21 and 24 months, respectively, in complete remission of CME. During further follow-up of 6 and 7 months, respectively, no recurrence of CME occurred in these two patients. Treatment with IFN alpha was generally well tolerated. Only 1 patient discontinued treatment after 21 months due to side effects (persistent fatigue; CME in remission).

Conclusions: For patients, in whom pseudophakic CME does not respond to conventional treatment, IFN alpha represents a promising therapeutic approach. It is our impression that the proportion of patients, in whom IFN alpha treatment could be discontinued without further relapses of CME, is higher in chronic pseudophakic CME compared with chronic uveitic CME. Further studies are necessary to define the role of IFN alpha in chronic CME following cataract surgery.

Commercial Relationships: Christoph M. Deuter, Novartis (F); Faik Gelisken. None; Manfred Zierhut. None; Deshka Doycheva. None
ARVO 2013 Annual Meeting Abstracts by Scientific Section/Group – Immunology/Microbiology
taps) was performed in this series. The mean duration of intravenous treatment was 20 days for VZV, 14 d for CMV, 13 d for HSV-2, and 21 d for HSV1. Combined IV antiviral treatment was not more effective on viral load decrease than IV monotherapy. HSV-2 group was followed by qualitative PCR. Immunosuppression was associated with a persistent high viral load. The decrease in viral load was relatively well correlated with the clinical response regardless of the causative virus.

No modeling of viral kinetics could be made for HSV2 virus, nor in case of unfavorable evolution with viral load stagnation. In case of viral decrease and regardless of the clinical course, we have modeled the viral kinetics by exponential curves with a coefficient of determination $R^2$ of 0.9.

Complications and severity are depending on the virus type.

**Conclusions:** Severity and evolution of necrotizing herpetic retinitis vary depending on the type of virus involved, possible immunocompromised status and antiviral treatment. Monitoring the viral load kinetics by AC taps optimizes antiviral therapy, thus potentially improving the final visual outcome.

**Commercial Relationships:** Anne Sikorav, None; Phuc Le Hoang, allergan (C), bausch Lomb (R), santen (C); Flore Rozenberg, None; Bahram Bodaghi, None

**Program Number:** 2918 Poster Board Number: B0287
**Presentation Time:** 8:30 AM - 10:15 AM

**Yellowish dots in the retina: a new finding of ocular syphilis?**
Renan Rodrigues, Gustavo Salomao, Heloisa Nascimento, Cristina Muccioli. Ophthalmology, Federal University of Sao Paulo, Sao Paulo, Brazil.

**Purpose:** To report the occurrence of pale-yellowish perivascular preretinal dots in twelve patients with ocular syphilis

**Methods:** Prospective study of twenty eyes from twelve patients with syphilitic uveitis. All of them were examined at the Uveitis Sector of the Department of Ophthalmology, between March, 2011 and October, 2012. After confirmation of Syphilis diagnosis, Fundus photographs and OCT were performed to identify the localization of the yellowish dots

**Results:** Demographic data comprised eleven males (91,6%), mean age of presentation was 38,1 years, 4 patients had bilateral panuveitis (30%), 1 had unilateral retinitis (8,3%), 3 had unilateral panuveitis (25%), 1 had anterior uveitis (8,3%), 1 had bilateral optic neuritis (8,3%), 1 had bilateral posterior uveitis (8,3%), 1 had asymetric bilateral uveitis (8,3%). Cerebral Spinal Fluid was positive in 2 patients (16,6%), negative in 7 patients (58,3%), and not collected in 2 cases. Blood VDRL was positive in 8 cases (66,6%) and negative in 4 patients (33,3%), whereas all patients had positive blood FTA-Abs. The majority of patients had improvement of visual acuity with the treatment (66,6%) whereas 4 patients had no improvement. From all patients, 8 patients were HIV positive (66,6%)

**Conclusions:** Although not yet recognized in the literature as a typical manifestation of ocular syphilis, these findings are very common in clinical practice. We believe that these dots are caused by the development of perivasculitis secondary to the infection by the treponema. Other authors suggest that they can be granulomas, but more studies, especially pathologic studies, are needed. It is important to recognize these findings and remember that syphilis can present with several forms. To conclude, the ophthalmologist must always consider syphilis in the differential diagnosis of uveitis.

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Nine patients (41%) experienced a one or more line decrease in vision throughout their course. **Conclusions:** Ocular syphilis in our population affected primarily African American Females, presenting most commonly as anterior uveitis. A concurrent diagnosis of HIV is not always present, and lab work -- while helpful, is not always conclusive. Even with appropriate treatment with systemic penicillin, patients may have recurrent disease and require repeat treatment. With the prevalence of systemic syphilis increasing worldwide, the need to recognize and appropriately treat ocular syphilis becomes increasingly more important.

**Commercial Relationships:** Archana T. Seethala, None; Nicole H. Siegel, None; Steven D. Ness, None; Deeba Husain, None

**Program Number:** 2920 Poster Board Number: B0289  
**Presentation Time:** 8:30 AM - 10:15 AM  
**The Efficacy and Outcomes of combination Intravitreal Foscarnet and Ganciclovir in Herpetic Retinitis**  
Rajiv E. Shah, Sunir J. Garg, Ophthalmology, Wills Eye Institute, Philadelphia, PA.  
**Purpose:** To evaluate the long-term outcomes and safety for combination intravitreal foscarnet and ganciclovir in the treatment of herpetic retinitis.  
**Methods:** All cases of herpetic retinitis (Acute Retinal Necrosis, Progressive Outer Retinal Necrosis, and CMV retinitis) were identified from 2008-2012 that were treated with combination intravitreal foscarnet and ganciclovir in addition to standard systemic antiviral therapy. Outcomes of time to retinal detachment, visual acuity, and safety of dual simultaneous intravitreal antiviral therapy were evaluated.  
**Results:** 7 eyes were identified with herpetic retinitis that were treated with dual injection therapy. There were no cases of increased injection related endophthalmitis nor complications related to the increased injection. Dual injection therapy was found safe and no retinal detachments were seen several months after dual injection therapy in all herpetic retinitis eyes.  
**Conclusions:** Injection of Intravitreal foscarnet and ganciclovir is safe and efficacious in the treatment of herpetic retinitis. Dual injection therapy may lead to a lower rate of retinal detachment in herpetic retinitis eyes.

**Commercial Relationships:** Rajiv E. Shah, None; Sunir J. Garg, Lux (F), EyeGate (F), Regeneron (F), Genentech (F), Allergan (C)

**Program Number:** 2921 Poster Board Number: B0290  
**Presentation Time:** 8:30 AM - 10:15 AM  
**Novel Minimum Inhibitory Concentration (MIC) Assay to Measure the Effectiveness of Antimicrobial Treatments Against Acanthamoeba Trophozoites and Cysts**  
Christopher Kovacs, Shawn C. Lynch, Joseph G. Carr, Christine M. Sanfilippo, Wolfgang Haas, Jenille Kilbury, Kimberly A. Millard, Timothy W. Morris, Bausch & Lomb, Inc. (E); Shawn C. Lynch, Bausch & Lomb, Inc. (E); Joseph G. Carr, Bausch & Lomb, Inc. (E); Christine M. Sanfilippo, Bausch & Lomb, Inc. (E); Wolfgang Haas, Bausch & Lomb, Inc. (E); Jenille Kilbury, Bausch + Lomb (E); Kimberly A. Millard, Bausch & Lomb (E); Timothy W. Morris, Bausch & Lomb, Inc. (E)

**Purpose:** Acanthamoeba keratitis (AK) is a severe condition with sight-threatening potential. Although the genus Acanthamoeba has been classified into 16 different genotypes based on rDNA sequence analyses (T1-T16), T4 genotype has been most related with ocular infection. In order to provide an accurate discriminative tool to differentiate Acanthamoeba T4 and non-T4 genotypes, the 18S ribosomal RNA gene (18S rDNA) was studied and a fingerprinting profile was developed  
**Methods:** The research was conducted in accordance with the tenets of the Declaration of Helsinki. Approval of the study was obtained from the local institutional review boards. Clinical isolates of Acanthamoeba were obtained from corneal scraping of different patients, while A. castellanii strain (T4 genotype) was obtained from American Type Culture Collection (ATCC 30011). All amoebae were grown without shaking, at room temperature, in 5 ml of Neff medium for 72 hours. The nuclear DNA was extracted and a specific-fragment within 18S rDNA gene of Acanthamoeba spp, denominated ASA.S1, was amplified by PCR reaction.  
**Results:** A PCR-amplified 18S rDNA Acanthamoeba-specific product followed by restriction analysis were able to provide a discriminative profile between Acanthamoeba T4 and non-T4 genotypes. In addition, two Acanthamoeba isolates investigated were classified as non-T4 genotype.

**Commercial Relationships:** Christopher Kovacs, Bausch & Lomb, Inc. (E); Shawn C. Lynch, Bausch & Lomb, Inc. (E); Joseph G. Carr, Bausch & Lomb, Inc. (E); Christine M. Sanfilippo, Bausch & Lomb, Inc. (E); Wolfgang Haas, Bausch & Lomb, Inc. (E); Jenille Kilbury, Bausch + Lomb (E); Kimberly A. Millard, Bausch & Lomb (E); Timothy W. Morris, Bausch & Lomb, Inc. (E)
Conclusions: Since the earlier detection of Acanthamoeba species/genotypes followed by a specific therapeutic procedure could avoid the spread of infection to deep stroma and decrease the severity of infection, the present study showed a reproducibility method based on molecular biology tool to differentiate Acanthamoeba T4 genotype, which is most related with AK cases, and non-T4 genotypes. Thus, the application of 18S rDNA gene fingerprint procedure could be used as useful molecular marker in the differentiation of Acanthamoeba genotypes and open perspectives to an earlier detection of pathogenic Acanthamoeba strains in corneal infections.

Support: FAPESP (Grant 08/53969-0), CAPES (PNPD Scientific Program)

Commercial Relationships: Denise Freitas, None; Felipe Marques de Carvalho Taguchi, None; Linda C. Carrijo-Carvalho, None; Viviane Peracini, None; Annette Foronda, None; Fábio Ramos, None

Program Number: 2923 Poster Board Number: B0292

Presentation Time: 8:30 AM - 10:15 AM

The Preventive Effect by the Drug Released Soft Contact Lens against Bacterial Endophthalmitis Shinichiro Kobayakawa, Toru Matsumagana, 1 1St Dept of Ophthalmology, Toho University, Tokyo, Japan; 2Seed Co., Ltd., Kounosu, Japan.

Purpose: We developed a drug released soft contact lens (DR-SCL) of a hydrogel material that releases antibiotics in a sustained manner. The purpose of this study was to investigate the preventive effect of the DR-SCL to bacterial endophthalmitis in a rabbit model.

Methods: Gatifloxacin (GFLX) 0.3% eye drops were used. Staphylococcus aureus (MRSA, ATCC 43300) was used to induce experimental endophthalmitis. Each DR-SCL was soaked in GFLX 0.3% eye drop solutions and uptake of the antibiotic using an ion ligand mechanism. Forty-five Japanese albino rabbits were divided into three groups post-inoculation of S.aureus to anterior chamber. DR-SCL group, SCLs presoaked with GFLX were administered to eyes (n=15); control group, SCLs without antibiotics administered to eyes (n=15); eye drop group, topical GFLX was administered to eyes twice or three times every four hours for three days (n=15). At 24, 48, and 72 hr post-inoculation, five rabbits from each group were euthanized, and those inoculation eyes enumerated for the enumeration of bacteria. Moreover, those eyes were evaluated by slit-lamp biomicroscopy examination at each time-point.

Results: All rabbits in the control group developed severe intraocular infection. The bacterial populations were significantly smaller in DR-SCL (0 log_{10} CFU/ml) and eye drops group (3.54-4.17 log_{10}CFU/ml) than in the control group (4.82-7.3 log_{10} CFU/ml) throughout 72 hours after inoculation (p<0.01). Most importantly, bacterial populations were undetectable in the DR-SCL group throughout the experimental periods. The DR-SCL group populations were significantly smaller than those in the eye drop group at 24 hours. However, there were no significant differences at 48 hours and in the eye drop group, and the populations were undetectable at 72 hours. Inflammation scores of the DR-SCL group were significantly smaller than of the eye drops group, and of the control group (p<0.05) at 48 and 72 hours.

Conclusions: DR-SCL contained with GFLX prevented to the growth of S.aureus through 72 hours. It was suggested that DR-SCL have a preventive effect against acute postoperative endophthalmitis.

Commercial Relationships: Shinichiro Kobayakawa, None; Toru Matsumagana, SEED Co., Ltd. (E), JPS132958 (P)

Program Number: 2924 Poster Board Number: B0293

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the local ethical review committee.

Results: We reviewed 11,567 elective cataract surgeries and identified 173 cases where ruptures of the posterior capsule occurred. The risk for a rupture of the posterior capsule was 1.50%. In all patients meticulous preoperative flush-irrigation of the cul-de-sac had been performed using 10ml 1% povidone iodine (PVI). Median postoperative follow-up was 4 days. No cases of postoperative endophthalmitis occurred in the study population. Mean preoperative BCVA was 0.37 in the right (OD) and 0.35 in the left eye (OS) while mean postoperative BCVA was 0.40 OD an 0.35 OS. Mean intraocular pressure was 14.9 mmHg OD and 14.3 mmHg and 13.9 mmHg OD and 13.8 mmHg OS after surgery. Vitreous loss occurred in 80% of patients with posterior capsular rupture and agitating occurred in 7.5% of cases.

The rate of ruptures of the posterior capsule in other studies with larger study population was between 4.09% and 2.09%. Our incidence rate was lower (1.50%). At the same time, there was no case of postoperative endophthalmitis in our study group, while other studies published rates of postoperative endophthalmitis after cataract surgery with intraoperative rupture of the posterior capsule (0.16% respectively 0.18%).

Conclusions: The preoperative prophylaxis including flush-irrigation of the cul-de-sac with 10ml of PVI may have contributed to the low rates of postoperative endophthalmitis after complicated cataract surgery. Therefore we recommend to strictly adhering to a preoperative prophylaxis protocol including flush-irrigation of the conjunctiva with copious amounts of PVI.

Commercial Relationships: Amos Aranowicz, None; Karsten U. Kortuem, None; Martin M. Nentwich, None; Yasmín Yactayo Miranda, None; Anselm Kampik, None; Herminia Mino de Kaspar, None

Program Number: 2926 Poster Board Number: B0295
Presentation Time: 8:30 AM - 10:15 AM
Prevalence of Staphylococcal Cassette Chromosome Mec (SCCmec Cassette) Types and Panton-Valentine Leukocidin (PVL) Toxin Among Staphylococcus aureus Vitreous and Anterior Chamber Isolates
Noy Ashkenazy1, Jorge Maestre2, Ashkan M. Abbey2, Darlene Miller2, Harry W. Flynn2
1University of Miami Miller School of Medicine, Miami, FL; 2Bascom Palmer Eye Institute, Miami, FL.
Purpose: To determine the presence of SCCmec types and Panton-Valentine Leukocidin toxin among random vitreous and anterior chamber Staphylococcus aureus isolates.

Methods: A multiplex PCR assay with six primer sets was used to characterize SCCmec Types I-IV among random vitreous (Vit) and anterior chamber (A/C) Staphylococcus aureus isolates (N=36, MRSA-8, and MSSA-28) collected between 1990 and 2012. Separate PCR assays were run to confirm the presence of the mecA gene and detect the PVL gene locus. SCCmec types and PVL genes were correlated with isolate origin (CA-MRSA v. HA-MRSA) and ocular source.

Results: Both healthcare-acquired (HA-MRSA) SCCmec types (I, II, III, N= 22, 25%) and community-acquired (CA-MRSA) SCCmec types (IV, N= 1, 12.5%) were documented among the MRSA isolates. 5/8 (62.5%) of the MRSA isolates were nontypeable for the SCCmec types. The PVL toxin was documented in 15/36 (41.7%) of the total isolates; it was found in 3/8 (37.5%) of MRSA isolates and in 12/28 (48.9%) of MSSA isolates. Of the typeable MRSA isolates, the PVL toxin was documented in 2/3 (66.7%), 1/2 (50%) of type II SCCmec and 1/1 (100%) of SCCmec type IV. Among MRSA isolates, the PVL toxin was most frequently associated with SCCmec type II (2/8, 25%), followed by SCCmec type IV (1/8, 12.5%).

Ocular sources included vitreous (Vit, 11/36, 30.6%) and anterior chamber (A/C, 25/36, 69.4%). 2/8 (25%) of MRSA isolates originated from A/C, while 6/8 (75%) of MRSA isolates originated from Vit. Of the MRSA typeable isolates (N=3, 83.3%), 2/2 (100%) of the HA-MRSA isolates (type II) originated from A/C and 1/1 (100%) of the CA-MRSA (types IV) originated from Vit.

Conclusions: The predominant profile for ocular MRSA isolates among this group was SCCmec type II (HA-MRSA). While the majority of the typeable MRSA isolates harbored the PVL toxin, a conclusion about which SCCmec subtypes predominantly harbor the PVL toxin cannot be made from this population of isolates. Understanding the profile of ocular MRSA will provide the opportunity to further explore the presence of the PVL toxin in MRSA subtypes correlating with both healthcare-acquired and community-acquired MRSA infection. Future study involves assessing the level of various antibiotic resistances in order to aid in the prevention and management of intraocular MRSA infection.

Commercial Relationships: Noy Ashkenazy, None; Jorge Maestre, None; Ashkan M. Abbey, None; Darlene Miller, None; Harry W. Flynn, None

317 Posterior Segment Inflammation I

Evaluation of Suprachoroidal Microinjection of Triamcinolone Acetonide in a Model of Panuveitis in Albino Rabbis
Samirkumar R. Patel1, Ricardo Carvalho2, Karen E. Mandwiler2, Carol Meschter1, Rozemarijn S. Verhoeven1
1Clearside Biomedical, Atlanta, GA; 2Biological Testing Center, Irvine, CA; 3Comparative Biosciences, Sunnyvale, CA.
Purpose: To evaluate the effects of pretreatment with suprachoroidal or intravitreal triamcinolone acetonide (TA) in a subretinal endotoxin-induced model of posterior segment uveitis in New Zealand white rabbits.

Methods: On Day 1, female rabbits (4/group) received a single unilateral injection of vehicle or 4 mg TA (Triesence®) into the suprachoroidal space (SCS) using a 33g 750µm microneedle, or a 4 mg TA IVT injection using a standard 30g needle. Intraocular pressure (IOP) was assessed prior to uveitis induction. On Day 6, each animal received a single unilateral subretinal injection of lipopolysaccharide (LPS) to induce ocular inflammation in the treated eye. Animals were monitored for 22 days following dose administration. Endpoints included body weights, ocular observations, slit lamp biomicroscopy with McDonald-Shadduck scoring and photography, indirect ophthalmoscopy, fundus photography, and histopathology.

Results: There were no test article- or administration-related effects on mortality, body weights, ocular observations, or IOP. Following LPS injection in this endotoxin-induced uveitis model, eyes developed acute anterior and posterior segment inflammation with extensive fibrin formation in the anterior chamber and vitritis. Twenty-four hours following LPS injection, eyes that were administered either SCS vehicle or IVT TA displayed greater panuveitis than SCS TA eyes. Vitritis, aqueous flare, and cellularity were substantially less severe in both SCS and IVT TA groups of eyes compared to SCS vehicle eyes. Iris vessel dilation and tortuosity
was reduced in SCS TA animals and reduced to a lesser extent in IVT TA animals when compared with the SCS vehicle group. SCS TA caused a significant reduction in inflammatory endpoints when compared with the vehicle group throughout the study. There was a marked reduction in inflammation as assessed histopathologically in eyes administered either SCS or IVT TA when compared with the vehicle group.

**Conclusions:** SCS administration of 4 mg TA using a Clearside Biomedical proprietary microneedle was as effective as 4 mg IVT TA in reducing the inflammatory response in this subretinal endotoxin-induced model of panuveitis in the albino rabbit.

**Commercial Relationships:** Samir Kumar R. Patel, Clearside Biomedical (E), Clearside Biomedical (I), Clearside Biomedical (P); Ricardo Carvalho, Clearside Biomedical (F); Karen E. Mundwiler, None; Carol Meschter, None; Rozemarijn S. Verhoeven, Clearside Biomedical (E)

**Program Number:** 2928 Poster Board Number: B0297

**Presentation Time:** 8:30 AM - 10:15 AM

**Beta-glucogallin Suppresses Lipopolysaccharide-induced Inflammatory Markers by Aldose Reductase Inhibition in Murine Macrophages and Ocular Tissues**

**KUN-CHE CHANG**,1,2 Brian Laffin1, Jessica Ponder2, Anna Enzoly1, Janos Nemeth1, Daniel V. LaBarbera1, Jonathan M. Petrash2,1 Ophthalmology, University of Colorado, Aurora, CO; 2Pharmaceutical Sciences, University of Colorado, Aurora, CO; 3Ophthalmology, Semmelweis University, Budapest, Hungary.

**Purpose:** Uveitis is a chronic inflammatory disease of the eye and can be induced in experimental mice by exposure to endotoxins such as lipopolysaccharide (LPS). Among other effectors, aldose reductase (AR) has been linked to ocular inflammation in the endotoxin-induced uveitis (EIU) model. We recently discovered β-glucogallin (BGG) as a novel AR inhibitor from extracts of the Indian gooseberry (Emblica officinalis). The purpose of this study is to investigate whether BGG is effective against various inflammatory markers in the EIU model.

**Methods:** Cytotoxicity of BGG was determined by cell viability assay. AR activity in cells was estimated by measuring sorbitol accumulation with an enzyme-linked assay. The detection of inflammatory markers was investigated by ELISA assay and western blotting. The presence and severity of uveitis was estimated by counting inflammatory cells in histological sections. The morphology of macrophage cells was observed by fluorescence microscopy. Cell migration was measured using a transwell assay. Active MMP-9 was detected by gelatin zymography.

**Results:** BGG showed low cytotoxicity in Raw264.7 murine macrophages (5% cell growth inhibition in the presence of 50 μM) and effectively inhibited AR activity as measured by suppression of sorbitol accumulation by approximately 50% compared to control. In addition, BGG prevented LPS-induced release of TNF-α and IL-1β, activation of JNK, p38 and lowered ROS levels. We also demonstrated that BGG suppresses the infiltration of inflammatory cells into the ocular media of mice with experimental uveitis. In Raw264.7 macrophages, BGG attenuated LPS-induced morphological changes and migration, and inhibited activation of MMP-9.

**Conclusions:** These results suggest that BGG may be useful as a therapeutic agent against inflammatory diseases in the eye.

**Commercial Relationships:** KUN-CHE CHANG, None; Brian Laffin, Flagship Biosciences (E); Jessica Ponder, None; Anna Enzoly, None; Janos Nemeth, None; Daniel V. LaBarbera, None; Jonathan M. Petrash, University of Colorado (P)

**Support:** NIH Grant EY005856 and EY021498

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Laboratory of Ophthalmology, Chongqing Eye Institute, Chongqing, China.

**Purpose:** Uveitis is a common cause of vision loss. Renin Angiotensin System (RAS) which plays a vital role in cardiovascular system is also a potent mediator of inflammation and has been implicated in pathogenesis of uveitis. The new established axis of RAS: ACE2/Ang1-7, has emerged as a novel target by counteracting the deleterious effect of Angiotensin II. The purpose of this study is to investigate the effect of ACE2 activation in protecting endotoxin induced uveitis (EUU) in mice.

**Methods:** ACE2 activator DIZE (Diminazene Aceturate) was administered both systemically and locally. For systemic administration, female Balb/c mice received intraperitoneal injection of DIZE (60mg/kg BW) for two days prior to LPS intravitreal injection (125ng) to induce uveitis. For local study, DIZE was given at 0.5, 0.1, and 0 mg/ml as eyedrops 6 times before LPS injection followed by 6 times in the second day. The anterior segment of the mice was examined at 12 and 24 hours after LPS injection and clinical scores were determined at the same time. Morphology and infiltrating inflammatory cells were evaluated after 24 hours. The mRNA levels of inflammatory cytokines in the retinas were analyzed by RT-PCR. ACE2 activity was determined using self-quenching fluorescent substrate.

**Results:** At the 24th hour, the clinical score of mice treated with DIZE systemically was significantly lower (mean: ~1.75) than the saline vehicle group (mean: ~4) (P <0.001). Histological examination showed ~40% reduction of total infiltrating inflammatory cells in DIZE treated eyes. The CD45+ inflammatory cells in vitreous of DIZE treated group were decreased (~35%) compared to the vehicle group (P <0.05). The mRNA level of IL-6 was significantly reduced in DIZE treated group (P <0.01). The infiltrating inflammatory cells were also significantly reduced in eyes received topical administration of DIZE: 77% reduction in 0.5mg/ml group and 55% reduction in 0.1mg/ml group compared to the control group. ACE2 activity was reduced in LPS injected eyes. DIZE treatment resulted in significantly increased ACE2 activity, even more increase in wildtype eyes without LPS injection (P <0.001).

**Conclusions:** DIZE has protective effect on LPS induced ocular inflammation in EUU mouse model. These results support the notion that RAS plays a role in modulating ocular immune response and that enhancing ACE2 provides a novel therapeutic strategy for uveitis.

**Commercial Relationships:** Yiguo Qiu, None; Pollob K. Shil, None; Ping Zhu, None; Hongxia Yang, None; Bo Lei, None; Qiuhong Li, None

**Support:** American Diabetes Association, American Heart Association, Research to Prevent Blindness, NIH Grant EY021752 and NIH Grant EY021721

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**Program Number:** 2932 Poster Board Number: B0301
**Presentation Time:** 8:30 AM - 10:15 AM
**A national survey of Canadian ophthalmologists' knowledge and application of uveitis management guidelines**

Crystal Cheung1,2, Nima Noordelch, Chloe Gottlieb3. 1Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada; 2Ophthalmology, University of Ottawa Eye Institute, Ottawa, ON, Canada.

**Purpose:** To assess current awareness and clinical practice among Canadian ophthalmologists of published uveitis treatment guidelines. Also assessed was frequency of applications to the public health system for immunomodulatory therapy (IMT), and identification of primary prescribers.

**Methods:** A 25-item questionnaire with clinical practice-related patient scenarios was sent to 759 practicing Canadian ophthalmologists. The published guidelines referenced are of an international panel of uveitis experts (Jabs et al., 2000). Six questions assessed demographics, including year of residency completion, presence of uveitis specialists during residency, and fellowship training in uveitis or a related sub-specialty. Seven questions assessed application of the guidelines to clinical scenarios, and twelve assessed referral patterns and success of obtaining coverage for IMT.

**Results:** Of 144 respondents, twelve (8.3%) were uveitis specialists. Uveitis specialists were present during residency for 45.1% of respondents. Correct responses reporting 1) awareness and 2) utilization of the guidelines was 60.4% in both cases. 75.1% appropriately identified instances where referral to a specialist for IMT is needed. Recent graduates (completed residency between 2001 and 2012) referred patients to uveitis specialists (55.3%) less frequently than earlier graduates. Recent graduates also managed uveitis patients more frequently with intravitreal or pericocular steroids (48.4%) than those graduated before 1980 (10.5%), who reported more usage of systemic therapy. 88.9% of respondents...
Intravitreal dexamethasone implant for the treatment of persistent uveitic Macular Edema

Alfredo Adan Civera1, Victor Llorens1, Marina Mesquida1, Blanca Molins2, Ana Isabel de Rocha Cardoso3, Mariana R. Santos de Almeida4, Laura Pelegrin5. 1Ophthalmology, Hospital Clinic, Barcelona, Spain; 2Ophthalmology, Fundació Clinic Recerca Biomèdica, IDIBAPS, Barcelona, Spain; 3Ophthalmology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; 4Ophthalmology, Centro Hospitalar Leiria, Pombal, Portugal.

**Purpose:** To evaluate the effects of intravitreal dexamethasone (DEX) implant (Ozurdex®; Allergan, Inc., Irvine, CA) for the treatment of persistent uveitic macular edema (ME).

**Methods:** Medical records of 37 patients with persistent uveitic ME treated with intravitreal DEX implant were reviewed. Patients had an inadequate control of ME despite different therapies. The main outcome measure was reduction in central retinal thickness (CRT). Secondary outcomes were: improvement in BCVA and decrease in uveitis activity. Tolerability of the implant was assessed.

**Results:** Forty-seven eyes of 37 patients were included in the analysis. Mean postoperative follow-up was 11.9 months (range 2-24). At the time of intravitreal injection 21 eyes (44.7%) were vitrectomized and 26 eyes (55.3%) were non-vitrectomized. In 5 eyes combined treatment with pars plana vitrectomy and DEX implant injection and the end of the surgery was performed. The mean baseline CRT was 519.89 μm, decreased to 331.79 μm at 3 months (p=0.0001), and then reached 200.79 μm at 14 months (p=0.009). The mean BCVA improved to 0.46 ± 0.30 logMAR (p=0.0001) at 6 months. Uveitis activity decreased in all patients after the implant. Similar CRT reductions with DEX implant treatment for uveitic ME in vitrectomized and non-vitrectomized eyes were found. Eleven eyes received 2 DEX implants and 4 eyes received 3 DEX implants. All eyes with a 2nd or 3rd implant improved CRT. An increase in intraocular pressure (IOP) of 10 mm Hg or more was seen in 21.2% (10/47) of eyes. Cataract surgery was performed in 2 eyes. Anterior chamber displacement of the implant occurred in 3 eyes.

**Conclusions:** Intravitreal DEX implant seems to be a safe and effective treatment for patients with persistent uveitic ME. Our results suggest that efficacy of the implant in difficult-to-treat vitrectomized eyes with uveitic ME. Repeated intravitreal DEX implant may produce long-term clinically meaningful benefits.
**Results:** Eight eyes of 7 patients were included. Mean age at implant placement was 35.3 years (range, 17-42 years). Two eyes were pseudophakic at the time of surgery, and 5 eyes had cataract operation at the time of surgery. Mean follow-up duration was 43.5 months (range, 12-57.6 months). Postoperative visual acuity improved over 3 lines in 5 eyes (62.5%). Inflammation was well controlled postoperatively in all study eyes, with decreased medication for inflammatory control in all patients. 3 patients were able to discontinue all systemic medications, 2 others were able to decrease to less than 10mg of steroid, but the other 3 patients required systemic medications due to inflammation in the fellow eye. During the follow up period, the mean number of recurrence of uveitis was 0.33 in the implanted eye and 1.5 in the fellow eye. Six eyes (75%) had postoperative intraocular pressure spikes over 30 mmHg, two eyes (25%) having over 40 mmHg despite medication. All patients required glaucoma shunting surgery postoperatively for intraocular pressure control. No recurrence of inflammation after implant surgery was observed in any of the patients. The single phakic eye developed a visually significant posterior subcapsular lens opacification requiring cataract extraction. There was one case of postoperative cytomegalovirus endothelitis, resulting in corneal endothelial decompensation. Infection was controlled with oral valganciclovir and the patient did not require implant removal. There were no postoperative wound leakage or implant dislocation.

**Conclusions:** The fluorocinolone implant was effective in the control of intractable inflammation of Behcet's uveitis. Elevation of IOP still remains a major potential complication, and the possibility of infection should also be considered.

**Commercial Relationships:** Eun kyu Oh, None; Hyeong Gon Yu, None

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**Program Number:** 2935 Poster Board Number: B0304

**Presentation Time:** 8:30 AM - 10:15 AM

**Long-term results of Intravitreal Bevacizumab for the Treatment of Choroidal Neovascular Membranes associated with Presumed Ocular Histoplasmosis Syndrome**

Sarah Escott, Susie Chang, Ahmad Tarabishy, Mark Barsamian, John B. Christoforidis, Frederick Davidorf, Alan Letson, Ophthalmology, The Ohio State University Wexner Medical Center, Columbus, OH.

**Purpose:** To assess the long-term efficacy of intravitreal bevacizumab therapy for choroidal neovascularization (CNV) in patients with presumed ocular histoplasmosis syndrome (POHS).

**Methods:** All patients diagnosed with CNV associated with POHS and treated with intravitreal bevacizumab (1.25 mg) between December 2005 and September 2012 were included. Baseline best-corrected visual acuity (BCVA) was compared with first post-treatment visit BCVA and final visit BCVA. Fluorescein angiography confirmed the presence of CNV prior to initial treatment.

**Results:** Seventeen eyes of 12 patients received intravitreal bevacizumab. Nine eyes were newly diagnosed; the remaining 8 previously treated eyes received prior therapy for CNV more than 1 year before initiation of intravitreal bevacizumab treatment. Mean follow-up was 45.24 months (3.77 years) with a range of 2.4 to 6.4 years. Overall there was a statistically significant improvement in mean logMAR VA after 1 treatment (0.16, Snellen equivalent of 20/29) when compared to mean baseline logMAR VA (0.35, Snellen equivalent of 20/45). At final visit, treatment effect was sustained in 15 of 17 study eyes. The improvement between baseline and final BCVA outcome in both the treatment naive and previously treated groups was not statistically significant; however the overall improvement in vision was greater in those naive to treatment (-0.16 versus -0.04 logMAR improvement). Eyes with subfoveal lesions (n=13) as a group demonstrated a statistically significant improvement of BCVA from baseline to both first post-treatment visit and final visit.

**Conclusions:** Long-term visual outcomes are favorable for patients treated with intravitreal bevacizumab for CNV associated with POHS. In patients with POHS, intravitreal bevacizumab may be more effective as initial therapy for new onset CNV and without other previous treatment. Furthermore, we demonstrate that in the majority of treatment naive eyes, the visual acuity is sustained over a period of at least four years. These findings suggest intravitreal bevacizumab is an appropriate first-line therapy for the management of CNV in patients with POHS.

**Commercial Relationships:** Sarah Escott, None; Susie Chang, None; Ahmad Tarabishy, None; Mark Barsamian, None; John B. Christoforidis, None; Frederick Davidorf, None; Alan Letson, Genentech (F)

**Program Number:** 2936 Poster Board Number: B0305

**Presentation Time:** 8:30 AM - 10:15 AM

**Trimethoprim-sulfamethoxazole versus placebo to reduce the risk of recurrences of Toxoplasma gondii retinochoroiditis: randomized controlled clinical trial (ISROT)**

Joaop Felix, Rafael S. Zacchia, Jaqueline M. Toribio, Mauricio A. Nascimento, Carlos E. Arieta, Heitor Panetta, Valdir Balarin, Rodrigo P. Lira, Oftalmologia, Unicamp, Campinas, Brazil.

**Purpose:** To compare the effects of trimethoprim-sulfamethoxazole versus placebo in reducing the risk of recurrences of Toxoplasma gondii retinochoroiditis.

**Methods:** This study was a single-center, prospective randomized, double-masked, clinical trial. 81 patients from Campinas, Brazil, with active recurrent Toxoplasma gondii retinochoroiditis were included (a new focus of necrotizing retinochoroiditis with active inflammation either adjacent to or remote from preexisting retinochoroidal scars, with positive IgG for Toxoplasmosis). All patients were successfully treated with 1 tablet of trimethoprim-sulfamethoxazole (800mg/160mg) two times daily for 45 days. After that, two patients drop out of the study. The remaining patients were randomized to group 1 (Trimethoprim-sulfamethoxazole tablet every two days) or group 2 (identical placebo tablet every two days). Block randomization was performed (blocks of 4 patients, 2 in each group) with stratification by gender. The primary outcome was 6-months incidence of recurrent Toxoplasmosis retinochoroiditis, and the secondary outcome was 6-months change of best correct visual acuity/BCVA (ETDRS chart).

**Results:** A total of 79 patients completed the 6-months follow-up (Figure 1). Demographic data were comparable in the 2 groups (Table 1). The incidence of recurrent Toxoplasmosis retinochoroiditis was 0/40 (0%) at group 1 and 5/39 (12.8%) at group 2 (P = .026). The changes of BCVA were 19 SD 21 letters in group 1 and 20 SD 16 letters (P = .833). No significant adverse events (drug reactions) were registered.

**Conclusions:** Trimethoprim-sulfamethoxazole compared with placebo may reduce the recurrences of Toxoplasma gondii retinochoroiditis.
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Purpose: To evaluate the prevalence, incidence, and risk factors associated with evedative retinal detachment (ERD) in patients with uveitis.

Methods: We performed a retrospective chart review of a large multi-center cohort (Systemic Immunosuppressive Therapy for Eye Disease Cohort Study) of patients with ocular inflammation at four tertiary care academic ocular inflammation centers in the US between 1978 and 2007, evaluating the prevalence, incidence and risk factors for ERD.

Results: 138 of 8478 uveitic patients presented with prevalent ERD (1.6%). Risk factors for prevalent ERD were Vogt-Koyanagi-Harada syndrome (VKH) (odds ratio (OR) 80.92, p<0.0001), sympathetic ophthalma (SO) (OR 8.01, p=0.0064), retinocochroioditis (OR 8.92, p<0.0001), panuveitis (OR 6.14, p<0.0001), posterior uveitis (OR 12.36, p<0.0001), posterior scleritis (OR 30.33, p<0.0001), and necrotizing scleritis(OR 5.75, p=0.0213). Clinical features predictive of ERD were choroidal neovascularization (OR 2.61, p=0.0219) and band keratopathy (OR 2.89, p=0.0007).

Among the 5409 uveitic patients with follow-up and initially free of ERD, 105 incident ERD cases were observed (incidence rate=0.65%/person-year). Among the ocular inflammatory diagnoses, only retinocochroioditis (hazard ratio (HR) 7.13, p=0.0014) was significantly associated with incident ERD. VKH (HR=3.88, p=0.0367), SO (HR=3.27, p=0.32), and posterior scleritis (HR=4.46, p=0.14) had increased incidence, but not to a statistically significant degree. Systemic diagnoses of rheumatoid arthritis (HR 2.39, p=0.00367) and juvenile idiopathic arthritis (HR 2.35, p=0.0402) had higher incidence of ERD.

In both the prevalence (OR=4.36, p<0.0001) and incidence analyses (HR=2.80, p<0.0001), active inflammation was associated with higher risk of ERD. In the incidence analysis, a dose-response relationship with ERD incidence was observed for time-updated anterior chamber cell grade, and for vitreous cell grade, as well as a higher risk among cases with vitreous haze.

Conclusions: ERD is a rare finding strongly, not only associated at presentation with VKH and posterior scleritis, but also with active inflammation, other forms of chorioretal or scleral inflammation. These results are informative in indicating that an important number of ERD cases are unrelated to VKH or posterior scleritis, especially following initial presentation, and confirm the importance of controlling inflammation to avoid ERD.

Commercial Relationships: Deepika Shah, None; Craig Newcomb, None; Grace Levy-Claire, Johnson and Johnson Vision Care (E); Robert B. Nussenblatt, None; James T. Rosenbaum, Genentech (C), Abbott (F), Xoma (C), Eyegate (F), Bristol Myers (F), Novartis (C), Regeneron (C), Teva (C), Therakine (F), Mitotech (F), Aquinox (F), Allergan (C), Santen (C); Eric B. Suhler, Abbott (F), Abbott (C), Bristol-Myers-Squibb (F), EyeGate (F), Genentech (F), Luxbio (F), LuxBio (C), Eleven Biotherapeutics (C), XOMA (F); Jennifer E. Thorne, Allergan (C), XOMA (C), Santen (C); C. Stephen Foster, Abbott Medical Optics (C), Abbott Medical Optics (F), Alcon Laboratories, Inc. (C), Alcon Laboratories, Inc. (F), Allergan, Inc. (C), Allergan, Inc. (F), Eyegate Pharmaceuticals, Inc. (I), Eyegate Pharmaceuticals, Inc. (F), IOP Ophthalmics (C), Ista Pharmaceuticals (C), Lux Biosciences, Inc. (C), Lux Biosciences, Inc. (F), Novartis Pharmaceuticals Corporation (C), Novartis Pharmaceuticals Corporation (F), XOMA Ltd (C); Douglas A. Jabs, Alcon (C), Allergan Uveitis Board (C), Abbott Laboratories (C), Genzyme Corporation (C), Novartis (C), Applied Genetic Technologies Corporation (C), Roche (C), GLaxoSmithKline (C), Genentech (C), Coept (C), Regeneron (C); John H. Kempen, Alcon (C), Allergan (C), Clearside (C), Can-Fite (C), Lux Biosciences (C), Xoma (C), NEI/NIH (F), FDA (F), Research to Prevent Blindness (F), Mackall Foundation (F), EyeGate Pharma (F), University of Pennsylvania (E); Support: Grant EY014943 from NEI/NIH (J.H. Kempen)

Program Number: 2940 Poster Board Number: B0309
Presentation Time: 8:30 AM - 10:15 AM

Imaging of paravascular infiltrates and epiretinal proliferation in posterior uveitis using adaptive optics

Michel Paques, Marie-Hélène Errera. Clinical Investigation Center 503, Quinze-Vingts Hospital, INSERM, Paris, France.

Purpose: Posterior uveitis may present with a variety of fundus features. Retinal vasculitis is characterized by the presence of paravascular inflammatory infiltrates, which are often difficult to document with precision with currently available imaging systems. Epiretinal proliferation is also a common complication of posterior uveitis. Here, we report the findings of adaptive optics (AO) near infrared imaging in patients with posterior uveitis of various etiologies.

Methods: Ten patients with posterior uveitis seen in our department were included. The diagnoses associated with uveitis were 2 cases of Lyme's disease, two cases of multiple sclerosis, one case of idiopathic retinal vasculitis, aneurysm and neuroretinitis (IRVAN), and five idiopathic cases. All underwent routine ophthalmological and general workup in addition to AO NIR flood imaging (rtx1, ImagineEye, Orsay, France) within a IRB-approved protocol.

Results: AO imaging showed foci of grayish linear infiltrates (0.5-3 mm in length, up to 50 μm in width) alongside veins (see figure) in 7 patients. They were more often detected in areas where there was irregularity of the vessel lumen. The case of IRVAN had infiltrates around veins as well as around arteries, especially around macroaneurysms. These infiltrates were not or very faintly visible by funduscopy, fluorescein angiography or optical coherence tomography. Follow-up demonstrated that there was slow, continuous remodelling of both the infiltrate and of the underlying venous stenosis. In the three other cases, small foci of epiretinal membranes were disseminated along vessels.

Conclusions: AO imaging is of interest to identify the presence of paravascular inflammatory infiltrates and of early stages of epiretinal membranes. These features appear more prevalent than previously thought since they can be observed in funduscopically and angiographically normal fundi. We found that irregularity of the venous lumen is a strong indicator of the presence of inflammatory infiltrates. Detection of paravascular infiltrates may be of interest for establishing a diagnosis of vasculitis in patients with inflammatory syndromes, either ocular or general. The significance of epiretinal
Commercial Relationships: Michel Paques, MerckSerono (C), Roche (C), Sanofi (C); Marie-Hélène Errera, None
Support: ANR_09_TECS_009_01_iPhot
Clinical Trial: C10-03

Program Number: 2941 Poster Board Number: B0310
Presentation Time: 8:30 AM - 10:15 AM
OCT characteristics of patients with uveitis with epiretinal membranes, cystoid macular edema, or both

Ghazala A. Datoo O’Keefe1, Hossein Nazari Khanamiri1,2, Narsing A. Rao1, 1Ophthalmology, USC Doheny Eye Institute, Los Angeles, CA; 2Department of Ophthalmology, Rassoul Akram Hospital, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.

Purpose: To investigate whether quantitative and qualitative characteristics on spectral domain optical coherence tomography (SD-OCT) correlates with visual acuity and complications of uveitis, i.e. cystoid macular edema (CME), epiretinal membrane (ERM) or both.

Methods: We retrospectively selected all patients with uveitis who were followed at our institute from July 2009 to July 2012 and had CME, ERM or both CME and ERM. Patients were grouped to three groups: 1- Eyes that had CME; 2- Eyes that had ERM; and 3- Eyes with both CME and ERM. Retinal changes as defined by SD-OCT and central foveal thickness were compared among the groups by chi square and Kruskal-Wallis tests respectively. Spearman correlation analysis was used to explore whether there is an association between SD-OCT features, visual acuity, and the above mentioned uveitis complications. The level of significance was considered as less than 0.05.

Results: Of 35 eyes included in the analysis, 4 eyes had only CME, 13 eyes had only ERM, and 18 eyes had both CME and ERM. The central foveal thickness was thinnest in patients with ERM (292 microns±46.1) and thickest in patients with both CME and ERM (409.5 microns±197.3) (p=0.0029). Visual acuity followed the same trend with best visual acuity in the ERM group (0.15±0.29 LogMAR) compared to the CME group (0.35±0.22 LogMAR) and the cystoid macular CME and ERM group (0.38±0.49 LogMAR) but was not statistically significant (p=0.1303). Visual acuity and central foveal thickness did not correlate with each other in the different groups except for patients with both CME and ERM where there was an association if one outlier in the group was removed (p=0.0064).

Conclusions: Simultaneous presence of both CME and ERM trends towards worse VA in patients with uveitis. Eyes with both ERM and CME had the thickest central foveal thickness and lowest visual acuities. Presence of ERM in patients with uveitis is associated with a thinner fovea compared to the eyes complicated with both ERM and CME and eyes with only CME.
patients were included: 7 patients with acute VKH, 2 patients at recurrence of VKH, and 3 patients in the convalescent stage. Macular sensitivities were measured with the Microperimeter-1 (MP1, Nidek, Japan). The association between best corrected visual acuities (BCVA) and mean central 10 degrees retinal sensitivities was evaluated with Spearman correlation analysis. A subgroup analysis of patients with acute VKH was performed, evaluating BCVA and micropereimetric retinal sensitivities at baseline and at 1 week, 1 month and 6 months after treatment.

**Results:** The mean age was 43.5 years (SD±13.5). 58.3% were female and 83.3% were Chinese. In acute VKH, 86.6% of the patients (n=5) presented with serous exudative retinal detachments, 57.1% (n=4) had optic disc swelling and 28.6% (n=2) had choroidal folds. In the convalescent phases, all patients had sunset glow fundus with concentric peripapillary atrophy. The mean LogMAR BCVA was 0.80 ± 0.822 in acute VKH, 0.47 ± 0.077 in convalescent phase, and 0.17 ± 0.945 in recurrent disease. Patients with convalescent VKH had better LogMAR BCVA compared to the active group (p=0.082). The mean retinal sensitivities within the central 10 degrees in acute, convalescent, recurrent stages of VKH were 7.19 ± 7.470 decibels (dB), 18.13 ± 0.818 dB, and 12.80 ± 3.747 dB respectively. The mean retinal sensitivities were lower in eyes with acute VKH than those with convalescent VKH (p=0.004). The mean retinal sensitivity correlated significantly with visual acuity (r=−0.713, p<0.001).

In the subgroup analysis of patients with acute VKH, there was a statistically significant upward trend for mean retinal sensitivities (Spearman’s rho = 1.000) and downward trend for LogMAR BCVA (Spearman’s rho = -0.900, p = 0.037) over the course of treatment. However interestingly, there was a significant correlation between mean retinal sensitivity and BCVA only at baseline (p =0.001), but not at Week 1, Month 1 or Month 6.

**Conclusions:** Microperimetry provides a noninvasive indicator of disease severity and dynamic changes in macular pathology, reflecting the effect of treatment in association with improvement in visual acuity. This may help to guide tapering of systemic corticosteroid treatment.

**Commercial Relationships:** Xi-Ling Tan. None; Stephen C. Teoh. None; Su Ling Ho. None

**Program Number:** 2944 Poster Board Number: B0313

**Presentation Time:** 8:30 AM - 10:15 AM

**DEMOGRAPHICS AND CLINICAL FEATURES IN SERPIGNOUS CHOROIDOPATHY AND ACUTE MULTIFOCAL PLACOID PIGMENTED EPITHELIOPATHY (AMPPE)**

**Ester Carreno**1, Dawn A. Sim1,2, Pearson A. Keane1,2, Javier Zarranz-Ventura1,2, Guillermo Fernandez Sanz1, Dhanes Thomas1, Mark C. Westcott1, Adnan Tufail2, Carlos E. Pavesio1. 1Medical Retina, Moorfields Eye Hospital, London, United Kingdom; 2National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom; Vitreo-Retinal Service, Gloucestershire NHS Foundation Trust, Cheltenham, United Kingdom.

**Purpose:** To describe the clinical features and outcomes of a large group of patients with a spectrum of clinical appearances and diagnosed as having Serpiginous Choroidopathy (SC) or Acute Multifocal Placoid Pigmented Epitheliopathy (AMPPE).

**Methods:** In a retrospective consecutive case series, patients seen during a 12-year period at Moorfields Eye Hospital who were diagnosed as having SC or AMPPE were included. Main outcome measures included initial and final visual acuity, laterality, sparing of the peripapillary area, development of choroidal neovascularization (CNV), number of flare ups during follow up. Statistical significance for intergroup differences was assessed by Mann-Whitney’s U test and Fisher’s test.

**Results:** A total of 43 patients (86 eyes), 16 (32 eyes) with serpiginous choroidopathy and 27 (54 eyes) with AMPPE were included. The average age was 37.7 years: 49.3 years in the SC group, and 30.9 in the AMPPE group (p=0.001). 24/43 patients (55.8%) were male; 7/16 (46.67%) males in the SC group and 17/27 (60.71%) males in the AMPPE group (p=0.341). The overall mean follow-up was 3.79 years. Thirty-two patients had bilateral disease, 13 bilateral SC, 19 bilateral AMPPE (p=0.494). Mean initial visual acuity 20/30 in all patients (20/40 SC, 20/25 AMPPE) (p=0.003). The mean final visual acuity was 20/25 (20/40 SC, 20/20 AMPPE) (p=0.000). 4 patients with SC, compared to non with AMPPE developed CNV (4.7%) (p=0.017). The mean number of flare ups during the follow up period was 0.67 in all patients, 0.88 for SC, and 0.55 for AMPPE (p=0.003).

**Conclusions:** A large group of patients with serpiginous choroidopathy and AMPPE were reviewed. Different clinical findings and visual outcomes were found between both groups. AMPPE has a better visual outcome, affecting younger patients, less flare ups during the follow-up and lack of associated CNV. These finding suggest a different underlying etiopathology for SC and AMPPE.

**Commercial Relationships:** Ester Carreno. None; Dawn A. Sim. None; Pearson A. Keane. None; Javier Zarranz-Ventura. None; Guillermo Fernandez Sanz. None; Dhanes Thomas. None; Mark C. Westcott. None; Adnan Tufail. Allergan (C), Bayer (C), GSK (C), Oculogics (C), Pfizer (C), Thrombogenics (C), Amakem (C), Heidelberg Engineering (R), Novartis/Alcon (C), Sanofi/Genzyme (C); Carlos E. Pavesio. None

**Program Number:** 2945 Poster Board Number: B0314

**Presentation Time:** 8:30 AM - 10:15 AM

**Masquerade Syndromes**

Amr Kouchouk1, 2, Monica D. Dalal1, H Nida Sen1, 2, Robert B. Nussenblatt1, Landon Grange2, Mahdi Rostamizadeh1, 2, George Washington University, Arlington, VA; 2National Eye Institute, Bethesda, MD.

**Purpose:** Masquerade syndromes are neoplastic or nonneoplastic conditions that present as uveitis. The goal of our study is to identify the proportion of patients with masquerade syndrome in a tertiary uveitis clinic and determine the baseline clinical characteristics of patients with masquerade syndromes.

**Methods:** All patients that presented with uveitis were identified by electronic medical record database search from 2004-2012. Nonmalignant masquerade syndromes such as retinal detachment, trauma or infection were not included in our search. Demographic and clinical data such as age at onset of disease, initial and final diagnosis, intraocular inflammation and visual acuity were collected on all patients. Clinical characteristics at presentation were then compared between the masquerade syndrome group and the uveitis group.

**Results:** A total of 855 patients with “uveitis” as the presenting diagnosis were identified. Of these 23 (2.69%) were determined to be neoplastic masquerade syndromes. The most common masquerade syndrome was primary intraocular lymphoma (PIOL). 772 of the 832 non-masquerade uveitis patients had non-infectious autoimmune uveitis (92.8%) whereas 60 (7.2%) had infectious uveitis. The average age of patients with masquerade syndromes at presentation was 57.5 (range 41-77) whereas the average age of uveitis cases was 46.2 (range 4-98). The M:F ratio was 1:1.1 among patients with...
masquerade syndrome and 0.7:1 amongst uveitis patients. Average visual acuity (logMAR) on presentation was 0.51 in the masquerade syndrome group, and 0.37 in the uveitis group. On initial visit 15/19 (65.2%) of masquerade syndrome patients had active anterior chamber, vitreous or inflammation of both.

**Conclusions:** Our data suggests that there is a difference between the presenting characteristics of patients with masquerade syndromes and those with uveitis. Patients with masquerade syndromes tend to be older, with lower visual acuity on presentation. Diagnosis of masquerade syndromes is difficult, but clinical characteristics may help raise suspicion for differentiating neoplastic masquerade syndromes from uveitis.

**Commercial Relationships:** Amr Kouchouk, None; Monica D. Dalal, None; H Nida Sen, None; Robert B. Nussenblatt, None; Landon Grange, None; Mahdi Rostamizadeh, None

**Support:** NEI Intramural Research Program

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**Spontaneous Dislocation of the Pellet from the Strut in the Fluocinolone Acetonide Sustained Release Implant (Retisert®)**

**Purpose:** To characterize the spontaneous dissociation of the pellet from the strut of the Fluocinolone Acetonide Sustained Release Implant (Retisert; Bausch and Lomb), describe a bimanual technique for pellet removal, and report clinical outcomes.

**Methods:** The medical records of 74 Retisert implantations in 47 patients by a single surgeon between 1998 and 2012 were reviewed for evidence of posterior segment pellet dislocation, either in the clinic or at the time of implant replacement.

**Results:** Four (5.4%) of 74 implants had spontaneous pellet dislocations identified on preoperative examination at a mean of 71.1 months (range 41-127 months) after implantation, with a mean follow up of 42 months (range 1-126 months). All pellets were removed using a bimanual technique utilizing an infusion chandelier and a silicone soft-tip aspiration cannula to safety engage the implant on the surface of the retina and elevate it to the mid-vitreous cavity where it could be grasped with diamond-dusted foreign body forceps and removed through an enlarged scleral wound. No posterior segment complications occurred, and vision returned to pre-operative levels or better in all four patients at 3 months. Of the 30 Retisert implants that were exchanged in this series, an additional eight (26.7%) pellets were identified to be separated from the strut at the time of implant removal and not during preoperative exams.

**Conclusions:** Pellet separation from the strut can spontaneously occur in Retisert implants usually as a late complication after 3 years. Removal of the dislocated pellet can be achieved safely without a significant decline in vision. Dislocated pellets may also not be significantly dislodged from the strut and may only be diagnosed at the time of surgical replacement.

**Commercial Relationships:** Sujit Itty, None; Joseph Martel, Glenn J. Jaffe. Dept of Ophthalmology, Duke Eye Center, Durham, NC.

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**Detection of Progression of Visual Field Loss in Serial Humphrey Visual Fields in Birdshot Chorioretinopathy by Pointwise Linear Regression Analysis**

**Purpose:** To evaluate the effect of treatment modality on vision-related quality of life (VR-QOL) and health-related quality of life (HR-QOL) in patients with non-infectious uveitis.

**Methods:** Eligible patients had a diagnosis of non-infectious anterior, intermediate, posterior, or panuveitis. VR-QOL was assessed by the 25-Item Visual Function Questionnaire (VFQ-25) and HR-QOL was assessed by the Short Form 12-Item Health Survey (SF-12). Treatment modality groups were observation with treatment as needed, local, systemic, and implant. Fisher’s exact test and t-test were used to evaluate associations between categorical and continuous variables, respectively. Multivariate regression was used to evaluate associations between treatment modality and VR-QOL or HR-QOL.

**Results:** Among the 63 patients, the mean age was 48.4 years with 20 males (31.7%) and median best-corrected visual acuity (BCVA) of 20/25 in better-seeing and 20/40 in worse-seeing eyes. VR-QOL scores were lower in all subscores of VFQ-25 in females compared to males (p=0.0008-0.036) except in general vision, social functioning, color vision and peripheral vision. HR-QOL scores were statistically different between males and females but not statistically different in physical component summary (PCS) but were lower in females in mental component summary (MCS) (p=0.014). Between males and females, BCVA and uveitis location and activity were not statistically different; however, treatment modality was statistically different with females more likely to receive systemic or implant therapy compared to males (p=0.011). In males, the effect of treatment modality on VR-QOL was not statistically different in all subscores of VFQ-25 except in role difficulties (p=0.024) and dependency (p=0.047), adjusted for BCVA, PCS and MCS, and the effect on HR-QOL was not statistically different in PCS and MCS of SF-12, adjusted for BCVA and presence of medical co-morbidities. In females, the adjusted effect of treatment modality on VR-QOL or HR-QOL was not statistically different in all subscores of VFQ-25 or PCS and MCS of SF-12.

**Conclusions:** Female patients with non-infectious uveitis reported lower VR-QOL and mental health HR-QOL scores and were more likely to receive systemic or implant therapy compared to males, suggesting more severe disease or more flare-ups. Treatment with systemic or implant therapy did not compromise VR-QOL or HR-QOL compared to observation with treatment as needed or local therapy.

**Commercial Relationships:** Wei Gui, None; Matthew Dombrow, None; Inna Marcus, None; Baylah Tessier-Sherman, None; Meredith H. Stowe, None; John Huang, None

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**Effect of Treatment Modality on Quality of Life in Patients with Non-Infectious Uveitis**

**Purpose:** To study progression of visual field loss in serial Humphrey Visual Fields (HVF) by pointwise linear regression analysis of luminance sensitivity against time, over long term follow up in a large series of patients with Birdshot chorioretinopathy (BCR).

**Methods:** Retrospective review of charts and HVF of all HLA A29
positive BCR patients, diagnosed in accordance with the diagnostic criteria laid out by the International Consensus Conference on BCR and seen at Moorfields Eye Hospital from 1999 to 2012. All reliable white on white SITA Standard/ SITA Fast 24-2/ 30-2 HVF available for eyes with 3 or more reliable field tests were uploaded on the progressor software for analysis. Studies were excluded from analysis if they failed reliability criteria of < 33% fixation losses and < 20% false positive and < 20% false negative responses or if the patient had coexistent pathology in the visual pathway that may have contributed to visual field defects. Progression was defined as a significant regression slope (p<0.05) showing >/= 1 dB per year sensitivity loss for non edge points and >/= 2 dB per year for edge points (beyond 15 degrees eccentricity).

**Results:** 133 HVF from 31 eyes of 17 patients were included in the analysis. 10 patients were female. All were white. Mean time interval between the first and the last field test in each series was 54.47 months. Median time interval was 44 months (Interquartile range 27-61months). 23 eyes (74%) had more than 3 reliable fields.

There was evidence of progressive field loss in 5 eyes of 3 patients (18% patients, 16% eyes) of which 2 patients had received and one had declined systemic treatment for BCR. The only other untreated patient maintained stable visual fields over an 85 month follow up. The mean slope of global sensitivity change in eyes with stable fields was 0.59+/- 1.80 db/yr, range -0.23 to 3.32 db/yr.

**Conclusions:** In this series of patients with BCR, there was good concordance between the progression status of a subject’s left and right eyes. Non-progressing eyes had a positive mean slope which we interpret as learning effect. Only a small percentage of patients showed significant white on white progressive visual field loss, suggesting that with treatment, most visual fields can be preserved over the long term. However, not all untreated patients will necessarily suffer progressive field loss.

### Study 1: TSLP mRNA and protein were largely induced in DCs from BALB/c mice by lipopolysaccharide (LPS) and flagellin, ligands to TLR4 and TLR5 respectively. LPS and flagellin promoted DC maturation with enhanced expression of CD40, CD80, CD86 and MHC class II, as evaluated by flow cytometry. The expression of MyD88, NFKB1, and RelA, the nuclear translocation of NFKB p65, and the induction of TSLP mRNA and protein were significantly stimulated in DCs by flagellin, but blocked by TLR5 antibody or NFkB inhibitor, as analyzed by RT-qPCR, immunofluorescence staining, ELISA and Western blotting. These stimulatory effects of flagellin were also observed in DCs from MyD88+/- but not in MyD88-/- mice. TSLP was found to promote expression of CD40, CD80, OX40 ligand, IL-13 and CCL17 by DCs. TSLP-producing DCs were further identified in vivo in mouse ocular surface and draining cervical lymph nodes by topically challenged LPS or flagellin. TSLP/OX40L signaling by DCs was also observed in ocular surface and cervical lymph nodes of BALB/c mice with experimental allergic conjunctivitis induced by short ragweed pollen.

**Conclusions:** Our findings demonstrated that DCs not only respond to TSLP, but also produce TSLP via TLR4/MyD88/NFkB pathways in response to microbial pathogens, suggesting local allergic inflammation may be amplified by DC-produced TSLP through a potential autocrine mechanism.

### Study 2: Correlation of anti-IgG/anti-IgM antibody response to microbial pathogens, suggesting local allergic inflammation may be amplified by DC-produced TSLP through a potential autocrine mechanism.

### Commercial Relationships:
- **Commercial Relationships:** De-Quan Li, None; Zhitao Su, None; Lili Zhang, None; Jing Lin, None; Cintia S. De Paiva, Glaxo Smith Kline (C), Baylor College of Medicine (P); Stephen C. Pflugfelder, Allergan (C), Glaxo Smith Kline (C), Bausch and Lomb (C), Baylor College of Medicine (P)
- **Support:** NIH NEI grants EY11915 (SCP) and Core Grant for Vision Research EY002520, Alkek Foundation (DQL), an unrestricted grant from Research to Prevent Blindness, the Oshman Foundation and the William Stamps Farish Fund.

### Program Number: 3176
**Presentation Time:** 11:15 AM - 11:30 AM
**Autoimmune component in glaucoma: IgG autoantibody accumulation, plasma cells and microglia under pro-inflammatory conditions**

*Oliver W. Gramlich, Sabine Beck, Anika Ziegler, Norbert Pfeiffer, Franz H. Grus.* Experimental Ophthalmology, Department of Ophthalmology, University Medical Center, Mainz, Germany.

**Purpose:** An autoimmune component is discussed in the pathology of glaucoma due to the alteration of autoantibodies in sera and aqueous humor, but less is known about the role of these autoantibodies. It is currently unknown whether IgG accumulates in the glaucomatous retina and which cellular components, e.g. B-cells and microglia, are involved or how the retinal homeostasis is affected in a pathogenetic way.

**Methods:** Globes of six human glaucomatous donor eyes and nine healthy samples were split and antibody microarrays were used to examine the patterns of pro-inflammatory proteins and microbial inflammation. However, the role of DC-produced TSLP remains to be elucidated. The present study was to explore an autocrine mechanism of DCs in amplifying local allergic inflammation by producing TSLP that links microbial pathogens to allergy.

**Methods:** Bone marrow-derived DCs from BALB/c and MyD88 knockout mice were treated with or without microbial pathogens, ligands to Toll-like receptors (TLRs) 1-9, or TSLP. Murine models of the ocular topical challenge and experimental allergic conjunctivitis were used for in vivo study. The mRNA expression was determined by reverse transcription and quantitative real-time PCR (RT-qPCR). The protein production was evaluated by ELISA, Western blotting, flow cytometry, and immunofluorescence staining.

**Results:** TSLP mRNA and protein were largely induced in DCs from BALB/c mice by lipopolysaccharide (LPS) and flagellin, ligands to TLR4 and TLR5 respectively. LPS and flagellin promoted DC maturation with enhanced expression of CD40, CD80, CD86 and MHC class II, as evaluated by flow cytometry. The expression of MyD88, NFKB1, NFkB2 and RelA, the nuclear translocation of NFKB p65, and the induction of TSLP mRNA and protein were significantly stimulated in DCs by flagellin, but blocked by TLR5 antibody or NFkB inhibitor, as analyzed by RT-qPCR, immunofluorescence staining, ELISA and Western blotting. These stimulatory effects of flagellin were also observed in DCs from MyD88+/- but not in MyD88-/- mice. TSLP was found to promote expression of CD40, CD80, OX40 ligand, IL-13 and CCL17 by DCs. TSLP-producing DCs were further identified in vivo in mouse ocular surface and draining cervical lymph nodes by topically challenged LPS or flagellin. TSLP/OX40L signaling by DCs was also observed in ocular surface and cervical lymph nodes of BALB/c mice with experimental allergic conjunctivitis induced by short ragweed pollen.

**Conclusions:** Our findings demonstrated that DCs not only respond to TSLP, but also produce TSLP via TLR4/MyD88/NFkB pathways in response to microbial pathogens, suggesting local allergic inflammation may be amplified by DC-produced TSLP through a potential autocrine mechanism.

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- **Commercial Relationships:** De-Quan Li, None; Zhitao Su, None; Lili Zhang, None; Jing Lin, None; Cintia S. De Paiva, Glaxo Smith Kline (C), Baylor College of Medicine (P); Stephen C. Pflugfelder, Allergan (C), Glaxo Smith Kline (C), Bausch and Lomb (C), Baylor College of Medicine (P)
- **Support:** NIH NEI grants EY11915 (SCP) and Core Grant for Vision Research EY002520, Alkek Foundation (DQL), an unrestricted grant from Research to Prevent Blindness, the Oshman Foundation and the William Stamps Farish Fund.

### Program Number: 3175
**Presentation Time:** 11:00 AM - 11:15 AM
**Dendritic Cell-produced TSLP Links Microbial Pathogens to Allergic Inflammation**

*De-Quan Li1, Zhitao Su2, Lili Zhang3, Jing Lin1, Cintia S. De Paiva1, Stephen C. Pflugfelder1.* Ophthalmology, Baylor College of Medicine, Houston, TX; 1School of Optometry and Ophthalmology, Wenzhou Medical College, Wenzhou, China; 2Ophthalmology, Qingdao University Medical College, Qingdao, China.

**Purpose:** Thymic stromal lymphopoietin (TSLP) has been identified to activate dendritic cells (DCs) to trigger Th2-dominant allergic inflammation. However, the role of DC-produced TSLP remains to be elucidated. The present study was to explore an autocrine mechanism of DCs in amplifying local allergic inflammation by producing TSLP that links microbial pathogens to allergy.

**Methods:** Bone marrow-derived DCs from BALB/c and MyD88 knockout mice were treated with or without microbial pathogens, ligands to Toll-like receptors (TLRs) 1-9, or TSLP. Murine models of the ocular topical challenge and experimental allergic conjunctivitis were used for in vivo study. The mRNA expression was determined by reverse transcription and quantitative real-time PCR (RT-qPCR). The protein production was evaluated by ELISA, Western blotting, flow cytometry, and immunofluorescence staining.

**Results:** TSLP mRNA and protein were largely induced in DCs from BALB/c mice by lipopolysaccharide (LPS) and flagellin, ligands to TLR4 and TLR5 respectively. LPS and flagellin promoted DC maturation with enhanced expression of CD40, CD80, CD86 and MHC class II, as evaluated by flow cytometry. The expression of MyD88, NFKB1, NFkB2 and RelA, the nuclear translocation of NFkB p65, and the induction of TSLP mRNA and protein were significantly stimulated in DCs by flagellin, but blocked by TLR5 antibody or NFkB inhibitor, as analyzed by RT-qPCR, immunofluorescence staining, ELISA and Western blotting. These stimulatory effects of flagellin were also observed in DCs from MyD88+/- but not in MyD88-/- mice. TSLP was found to promote expression of CD40, CD80, OX40 ligand, IL-13 and CCL17 by DCs. TSLP-producing DCs were further identified in vivo in mouse ocular surface and draining cervical lymph nodes by topically challenged LPS or flagellin. TSLP/OX40L signaling by DCs was also observed in ocular surface and cervical lymph nodes of BALB/c mice with experimental allergic conjunctivitis induced by short ragweed pollen.

**Conclusions:** Our findings demonstrated that DCs not only respond to TSLP, but also produce TSLP via TLR4/MyD88/NFkB pathways in response to microbial pathogens, suggesting local allergic inflammation may be amplified by DC-produced TSLP through a potential autocrine mechanism.
Purpose: Blau's syndrome (BS) is an inherited multisystem autoimmune granulomatous inflammation due to mutations in the CARD15/NOD2 gene located on chromosome 16q. 3 sites of clinical involvement include the eyes, skin and joints. While the eyes are the most important affected tissues, to date, no examples of the ocular pathology of this condition exist. We report the first ocular histopathologic and immunopathologic findings from an enucleated BS eye as well as from an iridectomy specimen.

Methods: A 14-year-old patient with BS had a blind painful eye that underwent enucleation. Patient 2 underwent incidental iridectomy at the time of cataract surgery at age 16. The clinical medical and ophthalmologic histories of both cases were reviewed. The eye underwent standard pathologic processing and the sections stained for H&E PAS. Granulomas were seen and special stains for microorganisms and polarization were performed. Immunopathologic analysis of inflammatory cells was done for CD3, CD20, CD27 and Iba1 were used for immunohistochemistry to identify possibly involved cellular players. Data underwent student’s t-test analysis.

Results: Antibody microarray analysis revealed an increased level of pro-inflammatory components in the glaucomatous group like TNF-α (+68%), IL 1β (+38%), IL 6 (+45%) and IL 8 (+34%). Ratios of single complement components were altered, while the total amount of all complement proteins did not differ. In glaucoma tissues, a significantly reduced amount of remaining cells in the retinal ganglion cell-layer was found (healthy: 104±7 nuclei/mm; glaucoma: 67±9 nuclei/mm; p=0.0007). Cell loss was accompanied by a twofold higher amount of retinal IgG accumulations (healthy: 5.0±0.5 IgG/100 cells; glaucoma: 9.4±1.9 IgG/100 cells; p=0.004), and likewise co-localized by microglia. Appropriately, iba1 levels were increased by +29% in glaucoma. Moreover, B-cells (1.8±1 100/cells), CD27+ cells and CD27+/IgG+ plasma cells were observed exclusively in glaucomatous retinas.

Conclusions: The study provides evidences for IgG deposition and occurrence of plasma cells in human glaucomatous retina. The result suggests that these IgG deposits occurred in a pro-inflammatory environment, which seems to be maintained locally by immune-competent cells like microglia. There are hints that ganglion cell death in glaucoma is partly mediated by antibody-dependent-cellular-cytotoxicity.

Commercial Relationships: Oliver W. Graumlich, None; Sabine Beck, None; Anika Ziegler, None; Norbert Pfeiffer, Sensimed AG (F), Sensimed AG (R), MSD (F), MSD (R), Alcon (F), Allergan (F), Novartis (F), Novartis (R), Bayer (F), Heidelberg Engineering (F), Bausch&Lomb (F), Boehringer-Ingelheim (F), Carl Zeiss Meditec (F), Chibret (F), Nidek (F), Pfizer (F), Santen (F), Santen (R), Topcon (F), Ivantis Inc (F), Ivantis Inc (R); Franz H. Grus, None

Program Number: 3177
Presentation Time: 11:30 AM - 11:45 AM
Ocular Pathology and Immunopathology of Blau’s Syndrome: First Cases
David S. Bardenstein1, Atif B. Collins3, Faruk Orge1, Deepak P. Edward2, Elizabeth B. Brooks3, Debra A. Goldstein2, Rachida Bouhenni2, Eric Pearlman1, Elias I. Traboulsi2

1UH Eye Institute, Case-University Hospitals Medical Center, Cleveland, OH; 2Medicine, Case University Hospitals Medical Center, Cleveland, OH; 3Ophthalmology, Summa Health System, Akron, OH; 1Ophthalmology, King Khaled Hospital and Wilmer Eye Institute, Baltimore, MD; 2Ophthalmology & Visual Sciences, University of Illinois, Chicago, IL; 3Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH.

Purpose: Blau’s syndrome (BS) is an inherited multisystem autoimmune granulomatous inflammation due to mutations in the CARD15/NOD2 gene located on chromosome 16q. 3 sites of clinical involvement include the eyes, skin and joints. While the eyes are the most important affected tissues, to date, no examples of the ocular pathology of this condition exist. We report the first ocular histopathologic and immunopathologic findings from an enucleated BS eye as well as from an iridectomy specimen.

Methods: A 14 year old patient with BS had a blind painful eye that underwent enucleation. Patient 2 underwent incidental iridectomy at the time of cataract surgery at age 16. The clinical medical and ophthalmologic histories of both cases were reviewed. The eye underwent...
thirds increasing expression and one-third decreasing expression. Some notable auto-antibodies which were increased dramatically included complement C3, CRP, PKM2, Calretiolin, Collagen V, Collagen VI, SOD1, aldolase, L-glutamine synthetase, annexin II, CNPase, RPE, and Factor X.

**Conclusions:** Laser injury, specifically intense rupture burns which induce choroidal neovascularization, cause a dramatic change in the serum autoantibody profile after 2 months. Age-related macular degeneration and cancer-associated retinopathy in humans show a similar elevation in serum complement, CEP, α-enolase, and hsp antibodies. Laser-induced choroidal neovascularization could serve as a novel animal model for studying the pathogenesis, immune response, and potential pharmaceutical targets of retinal degenerations.

**Commercial Relationships:** Yannis M. Paulus. None; Chuan-Hui Kuo. None; Kei Morohoshi. None; Alex Nugent. None; Luo Luo Zheng. None; Hiroyuki Nomoto. None; Mark S. Blumenkranz, avalanche biotechnologies (I), avalanche biotechnologies (P), optimedia (I); Daniel V. Palanker. None; Santa Ono. None

**Support:** Alcon Research Institute grant, the Hornsgen and Miller Family Foundations, OptiMedica Corp., the Angelos and Penelope Dellaporta Research Fund, and the L. Boltzmann Institute for Retinology and Biomicroscopic Laser Surgery in Vienna, Austria

**Program Number:** 3179

**Presentation Time:** 12:00 PM - 12:15 PM

**Promoting CD200R signalling inhibits laser-induced choroidal neovascularisation due to altered proangiogenic macrophage gene expression**

David A. Copland¹, Scott J. Robbie², Jian Liu¹, Wei-Kang Wd¹, Robin R. Ali³, James W. Bainbridge¹, Andrew D. Dick¹, ²Ophthalmology, School of Clinical Sciences, University of Bristol, Bristol, United Kingdom; ³Genetics, UCL Institute of Ophthalmology & NIHR Biomedical Research Centre London, London, United Kingdom.

**Purpose:** Manipulating macrophage activation to prevent choroidal neovascularisation (CNV) may offer a new strategic approach for therapeutic intervention in AMD. The recruitment and infiltration of macrophages to the choroid are essential for the development of CNV, and whilst the exact mechanisms that drive CNV are still poorly defined, experimental evidence suggests macrophages initiate angiogenesis. The purpose of this study was to interrogate whether suppressing angiogenic macrophage phenotype (Arg-1+VEGF+) via targeting inhibitory myeloid CD200R modulates the early infiltrating macrophage phenotype and function, and thereby influence the clinical outcome of disease.

**Methods:** CNV was induced in B10.RIII, C57BL/6J, CD200-/- and CD200R-/- mice by laser photoagulation. The agonist monoclonal rat anti-mouse CD200R antibody (DX109) or isotype control antibody was administered by intravitreal injection either at time of laser or on day 3 following induction of CNV (6 lesions per fundus). The effect of DX109 (a CD200R agonist) treatment on both size and permeability of induced CNV was assessed by digital image analysis of fundus fluorescein angiograms. Expression of macrophage activation-related mediators from choroid and retinal tissue was assessed by quantitative RT-PCR.

**Results:** In vivo assessment demonstrated that the local delivery of DX109 resulted in significant reduction in the mean CNV size at 2 weeks post-laser. Administration of DX109 led to reduced expression of the macrophage chemokine CCL2, as well as a proangiogenic phenotype including reduced Arginase-1 and IL-1β at day 3 post-laser. In further experiments examining the influence of CD200R ligation on modulating macrophage phenotype, DX109 was administered following CNV induction in CD200 and CD200R deficient mice. A corroborative reduction in gene expression was demonstrated in CD200-/- as in wild-type mice, whilst no effect was observed in CD200R-/- animals.

**Conclusions:** These results demonstrate that modulating macrophage activity via myeloid CD200R ligand can suppress the pro-angiogenic phenotype of recruited cells, and proffers mechanisms whereby laser-induced choroidal neovascularisation is inhibited. Approaches to target CD200R and promote such a change in function may prove beneficial for the treatment of AMD.

**Commercial Relationships:** David A. Copland. None; Scott J. Robbie. None; Jian Liu. None; Wei-Kang Wu. None; Robin R. Ali. None; James W. Bainbridge. Novartis (F), Alimera (C), Gene Signal (C), Advanced Cell Technology (F), Targeted Genetics (P), Oxford Biomedica (C), GSK (F); Andrew D. Dick. Novartis (C), Novartis (F), GSK (F), Abbott (F)

**Program Number:** 3180

**Presentation Time:** 12:15 PM - 12:30 PM

**Paradoxical Role of Caveolin-1 in Retinal Inflammation**

Michael H. Elliott¹, Xiaoman Li², Xiaowu Gu³, Alaina M. Reagan³, Timothy M. Boyce², Ilya Slech¹, Md Nawajes A. Mandal³, Michelle C. Callegan¹, Daniel J. Carr¹. "Ophthalmology, OUHSC, Oklahoma City, OK; ³Key Laboratory of Medical Cell Biology, China Medical University, Shengyan, China.

**Purpose:** Recent evidence indicates that caveolin-1 (Cav-1), the signature protein of caveola membrane microdomains, modulates inflammatory responses and innate immunity. In the uveitic retina, Cav-1 expression is dramatically upregulated, particularly in Müller glial cells (Hauck et al., 2010, Mol Cell Proteomics). However, Cav-1’s role in retinal inflammation has not been rigorously tested. In the current study, we examined the effect of Cav-1 ablation on the sensitivity of the retina to inflammation.

**Methods:** Cav-1 knockout (KO) mice were challenged by intravitreal injection of the Toll-like receptor-4 (TLR4) agonist, lipopolysaccharide (LPS), and inflammation was assessed by flow cytometry, immunohistochemistry, and spectral domain optical coherence tomography. Levels of chemoattractants were determined by multiplex immunoassays. Leukostasis was assessed in retinal flatmounts after perfusion with FITC-labeled Concanavalin A (FITC-ConA). Microarray analysis was performed on Cav-1 KO and control retinas/eyecups and the dataset was analyzed by Gene Set Enrichment Analysis (KEGG pathways, gene ontologies, and Ingenuity Pathway Analysis).

**Results:** Analysis of microarray data (n = 6) revealed a remarkable upregulation of mRNAs associated with inflammatory processes and innate immune responses. Intravitreal challenge with LPS induced a significant increase in the number of infiltrating bone marrow-derived (CD45hi) cells in Cav-1 KO retinas compared to controls as measured by flow cytometry. Increased leukostasis was visualized by FITC-ConA labeling of retinal flatmounts. Given the role of Cav-1 modulation of TLR signaling, we predicted that Cav-1 ablation would result in enhanced TLR4 signaling. Paradoxically, the protein levels of chemoattractant effectors downstream of TLR4 (monocyte chemotactic protein-1/CCL2, CXCL1/KC, interleukin-6, and interleukin-1b) were all significantly reduced in retinal extracts from Cav-1 KO compared to control mice.

**Conclusions:** This paradox suggests that Cav-1 modulates inflammatory signaling and leukocyte infiltration through distinct mechanisms. We hypothesize that Cav-1 expression may enhance inflammatory signaling while at the same time supporting the physical properties of the blood-retinal barrier.

**Commercial Relationships:** Michael H. Elliott. None; Xiaowu Li. None; Xiaowu Gu. None; Alaina M. Reagan. None; Timothy
Minocycline Prevents Inflammatory Leukocyte Infiltration Following Retinal Ischemia-Reperfusion Injury

**Purpose:** Retinal ischemia-reperfusion (IR) injury is an acute model of retinal neurodegeneration. The tetracycline derivative minocycline (Mino) exhibits anti-inflammatory and neuroprotective properties independent of its bacteriostatic function. We tested the ability of Mino to prevent the infiltration of leukocytes, neurodegeneration and gene expression changes following retinal IR injury.

**Methods:** Rats were treated with Mino by repeated intraperitoneal injection prior to and following IR. Retinas were made ischemic by elevation of intraocular pressure for 45 min and allowed to naturally reperfuse. Leukocyte infiltration was evaluated by flow cytometry of retinal cells after enzymatic-dissociation of pooled retinas, employing antibodies to the common leukocyte antigen CD45, the monocyte marker CD11b and major histocompatibility complex class II (MHC II). Microglia were identified as CD11b+/CD45low cells, invading monocytes and granulocytes as CD11b+/CD45high cells, and invading lymphocytes as CD11bneg/CD45high cells. Neuronal cell death and neurodegeneration was evaluated by caspase-3/7 activation, internucleosomal DNA cleavage and retinal layer thinning. Retinal mRNA expression was evaluated by qRT-PCR.

**Results:** IR caused a marked and significant increase in the numbers of invading leukocytes present after 48 h of reperfusion, including those with high MHC class II expression indicative of an inflammatory and antigen presenting phenotype. Mino treatment inhibited the IR-induced infiltration of CD11b-positive and-negative leukocytes: this effect was greatest and most significant for MHC II-positive cells within each population (p<0.01). Surprisingly, Mino treatment had no effect on neuronal death or neurodegeneration following IR. Mino significantly inhibited the upregulation of several IR-responsive genes, including monocyte chemoattractant protein 1 (MCP-1, CCL2, p<0.05) and intracellular adhesion molecule 1 (ICAM-1, p<0.001).

**Conclusions:** Mino inhibited the infiltration of inflammatory leukocytes following IR injury and this effect was associated with prevention of neurodegeneration, but did coincide with inhibition of chemoattractant and adhesion molecule expression. Tetracycline derivatives may thus represent effective means to treat neural inflammation associated with retinal ischemic diseases by preventing the infiltration of inflammatory cells.

**Commercial Relationships:** Steven F. Abcouwer, None; Sumathi Shanmugam, None; Cheng-mao Lin, None; Heather Lindner, None; Alistair J. Barber, None; Arivalagan Muthusamy, None; David A. Antonetti, None

**Support:** NIH Grant R01EY007739, Juvenile Diabetes Research Foundation Grant - JDRF Center for Mechanisms and Intervention of Diabetic Retinopathy

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Corneal infection induces migration of dendritic cells with CD1a expression
Mari Narumi1, Yoshiko Kashiwagi2, Hiroyuki Namba1, Ritaro Ohe2, Mitsunori Yamakawa2, Hitodoshi Yamashita1. 1Ophthalmology & Vis Sciences, Yamagata Univ Faculty of Med, Yamagata, Japan; 2Department of Diagnostic Pathology, Yamagata University Faculty of Medicine, Yamagata, Japan; 3Department of health and nutrition, Yamagata prefectural yonezawa women’s junior college, Yamagata, Japan.

Purpose: Dendritic cells (DCs) migrate to the central part of infected cornea, of which mechanisms have not been fully clarified. We analyzed the factors relevant to the migration including the patients’ clinical backgrounds, and also investigated the chemotactic factors of DCs in cornea with pathological states.

Methods: The present study was approved by the Ethical Committee of Yamagata University Faculty of Medicine. We obtained surgically 22 corneal tissues, and blood for migration assay from 2 healthy volunteers. We observed immunohistochemically the expression of specific DC markers (CD1a, DC-SIGN, CD83, Langerin, S-100 protein) and also the numbers of newly formed vessels and lymphatic vessels with CD31 and D2-40, and the densities of DCs. We investigated statistically the factors relevant to the distributions of each DCs. The factors were the states of corneal infection, perforation, history of previous penetrating keratoplasty (PKP), and newly formed lymphatic vessels and/or blood vessels. To investigate the migration mechanisms, immature monocyte derived DCs (immoDCs) were isolated from blood and CD1a was used as a marker. The migration was observed using Boyden’s chamber method with TNF-α, TGF-β1, IL-6 as chemotactic factor candidates.

Results: The lymphatic vessels were detected in 15, 10 with newly formed vessels, and the vessel formation was correlated with the infection and/or the perforation and/or the PKP. Immature DCs (CD1a+ DC) were mainly observed in the corneal epithelial and the sub-epithelial layers. The density of CD1a+ DCs increased significantly in the cases with corneal infection and/or perforation. DCs with S-100 protein were increased in the cases with infection and/or with newly formed vessels. Lymphatic vessels were observed...
only in the cases with infection, and were not correlated with the DC distribution. The migration assay showed that TNF-α and IL-6 (100ng/ml each) were chemotactic factors for immDCs, and TGF-β1 was less effective.

**Conclusions:** The states of infection, perforation, neovascularization may affect the distributions of the corneal DCs, especially the CD1a+ DCs in the central part. The inflammatory cytokines accelerated the migration of CD1a+ DCs. Taken together, the corneal pathological states above shown may accelerate the migration of immature DCs by inflammatory cytokines, which may be correlated with formation of blood vessels and lymphatic vessels.

**Commercial Relationships:** Mari Narumi, None; Yoshiko Kashiwagi, None; Hiroyuki Namba, None; Ritaro Ohe, None; Mitsunori Yamakawa, None; Hidetoshi Yamashita, Senju (C), Senju (P)

**Program Number:** 3669
**Presentation Time:** 3:45 PM - 4:00 PM

**Conneal Endothelial Cell Changes In Children with Uveitis and Ahmed Valves**


**Purpose:** To determine whether Ahmed valves (AV) are associated with corneal endothelial cell changes in children with non-infectious chronic anterior uveitis (CAU); and to investigate possible risk factors for such changes.

**Methods:** In a cross-sectional study, we evaluated patients with unilateral or bilateral CAU (onset of uveitis, age ≤16 years) who had undergone AV implantation in one or both eyes for control of uveitic glaucoma. Non-contact specular microscopy was used to measure the following variables associated with the corneal endothelium: central corneal thickness (CCT); percent hexagonality; and coefficient of variation (CV). Central corneal thickness was measured by ultrasound pachymetry. The following potential risk factors for endothelial changes were determined by slit lamp biomicroscopy: total intraocular length of tube; length of tube segment in contact with clear cornea at its entry site ("tube touch"); separation of tube tip from the endothelium (≤1 corneal thickness vs. >1 corneal thickness). Interval since AV implantation was collected from medical records as an additional potential risk factor. Relationships were evaluated with the Kruskal-Wallis test and Spearman correlation coefficients. Results were adjusted for correlation between eyes in patients with bilateral uveitis using mixed effect models.

**Results:** We evaluated a total of 46 eyes of 23 patients. After excluding 4 eyes of 3 patients that underwent prior cataract surgery, and 9 eyes of 9 patients without uveitis, 33 eyes of 20 patients (25 with uveitis and AV; 8 with uveitis and no AV) were included for analysis. Mean CCT was 2347.4±755.3 cells/mm² for eyes with uveitis and AV, and was 2997.3±140.4 cells/mm² for eyes with uveitis and no AV (p=0.05). There was no significant difference in percent hexagonality or CV between groups. Mean CCT was significantly lower in eyes with increased tube touch (r = -0.46, p=0.03), and increasing interval post AV implantation (r = -0.67, p=0.02). Lower CCT was associated with AV tubes closer to the endothelium (unadjusted p<0.01). There was no significant difference in central corneal thickness between groups.

**Conclusions:** Although causal relationships cannot be established in cross-sectional studies, our results suggest that corneal endothelial cell damage results from mechanical trauma and may progress over time. Despite endothelial changes, function, as indicated by corneal thickness, remains normal in most eyes.

**Commercial Relationships:** Mathew S. Margolis, None; Gary N. Holland, None; Joseph Caprioli, Allergan Inc. (F), Allergan Inc. (C), Allergan Inc. (R); Fei Yu, None; Simon K. Law, None; JoAnn A. Giaconie, Allergan (C); Anthony J. Aldave, Alcon (R), Allergan (R), NIH (F), Bausch + Lomb (C), Allergan (C)

**Support:** Research to Prevent Blindness, Inc.

**Program Number:** 3670
**Presentation Time:** 4:00 PM - 4:15 PM

**The Comparison of Podoplanin Expression Between Choroidal and Conjunctival Melanoma**

Kazuichi Maruyama1, Yoshihiko Usui2, Shunichiro Ueda4, Yuku Maruyama5, Hiroshi Goto1, Toru Nakazawa1.

1Ophthalmology, Tohoku University Graduate School of Medicine, Sendai, Japan; 2Ophthalmology, Tokyo Medical University, Tokyo, Japan; 3Ophthalmology, Kyoto Prefectural Univ of Med, Kyoto, Japan.

**Purpose:** Ocular melanoma is the most common primary eye tumor observed in adults. It has been reported that conjunctival or ciliary body melanoma are associated with lymphatic metastasis due to the induction of lymphangiogenesis. However, the formation of lymphatic vessels in choroidal melanoma is a subject that has not well investigated. The purpose of this present study was to investigate if the discriminative expression of podoplanin (D2-40) is confirmed in choroidal melanoma in comparison to conjunctival melanoma.

**Methods:** This study involved 22 patients (13 males and 9 females) with conjunctival melanoma (12 cases) or choroidal melanoma (10 cases). To visualize the podoplanin or macrophage, anti-podoplanin (D2-40) or anti-CD68 antibody was used. The area covered by a podoplanin-positive structure or CD68-positive cells was determined using National Institutes of Health (NIH) image software. Moreover, the number of podoplanin-positive vascular lumen was counted in both groups.

**Results:** The number of podoplanin-positive vascular lumen was found to be significantly less in the cases with choroidal melanoma than in those with conjunctival melanoma (p=0.044). However, no difference of podoplanin or CD68 expression was found between conjunctival melanoma and choroidal melanoma. Moreover, no discriminative podoplanin or CD68 expression was found between the metastatic and non-metastatic groups.

**Conclusions:** The findings of this study show that the number of podoplanin-positive lymphatic lumen, but not podoplanin expression, in choroidal melanoma was significantly less than in conjunctival melanoma. This data implicates that the formation of lymphatic lumen in melanoma might depend on the location of the tumor formation.

**Commercial Relationships:** Kazuichi Maruyama, None; Yoshihiko Usui, None; Shunichiro Ueda, None; Yuku Maruyama, None; Hiroshi Goto, None; Toru Nakazawa, Kowa Company Ltd. (F), Kowa Company Ltd. (C)

**Support:** KAKEN 23592613

**Program Number:** 3671
**Presentation Time:** 4:15 PM - 4:30 PM

**Therapeutic use of chimeric bacteriophage (phage) endolysins in staphylococcal endophthalmitis**

Pawan Kumar Singh1, David M. Donovan2, Ashok Kumar1, 2. 1Ophthalmology, Kresge Eye Institute, Wayne State University, Detroit, MI; 2Anatomy & Cell Biology, Wayne State University, Detroit, MI; 3Animal Biosciences and Biotechnology, Beltsville Agricultural. Res. Center, ARS, USDA, Beltsville, MD.

**Purpose:** Phage endolysins are peptidoglycan hydrolases that are produced at the end of phage lytic cycle to digest host bacterial cell wall. The Comparison of Podoplanin Expression Between Choroidal and Conjunctival Melanoma Kazuichi Maruyama1, Yoshihiko Usui2, Shunichiro Ueda3, Yuku Maruyama4, Hiroshi Goto5, Toru Nakazawa1.

1Ophthalmology, Tohoku University Graduate School of Medicine, Sendai, Japan; 2Ophthalmology, Tokyo Medical University, Tokyo, Japan; 3Ophthalmology, Kyoto Prefectural Univ of Med, Kyoto, Japan.

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**Conclusions:** The findings of this study show that the number of podoplanin-positive lymphatic lumen, but not podoplanin expression, in choroidal melanoma was significantly less than in conjunctival melanoma. This data implicates that the formation of lymphatic lumen in melanoma might depend on the location of the tumor formation.

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**Support:** KAKEN 23592613

**Program Number:** 3671
**Presentation Time:** 4:15 PM - 4:30 PM

**Therapeutic use of chimeric bacteriophage (phage) endolysins in staphylococcal endophthalmitis**

Pawan Kumar Singh1, David M. Donovan2, Ashok Kumar1, 2. 1Ophthalmology, Kresge Eye Institute, Wayne State University, Detroit, MI; 2Anatomy & Cell Biology, Wayne State University, Detroit, MI; 3Animal Biosciences and Biotechnology, Beltsville Agricultural. Res. Center, ARS, USDA, Beltsville, MD.

**Purpose:** Phage endolysins are peptidoglycan hydrolases that are produced at the end of phage lytic cycle to digest host bacterial cell wall.
wall, facilitating the release of mature phage progeny. The aim of this study is to determine the antimicrobial activity of chimeric phage lysins against clinical isolates of *Staphylococcus aureus* and to test their therapeutic efficacy in a mouse model of *S. aureus* endophthalmitis.

**Methods:** Chimeric fusion proteins containing multiple catalytic domains and cell wall binding domains were genetically engineered. Their activity against 19 clinical isolates of *S. aureus* from cases of endophthalmitis was tested using three functional assays (plate lysis, turbidity reduction and MIC determination). Their ability to disrupt biofilm formation was also tested. To determine the therapeutic potential in vivo, a chimeric Ply187 endolysin was injected intravitreally in C57BL/6 mouse eyes at 6 h and 12 h post *S. aureus* infection. Eyes were examined clinically and subjected to quantification of viable bacteria, retinal function, inflammatory cytokine levels as well as histological analysis.

**Results:** All the chimeric lysins showed strong antimicrobial activity against clinical isolates as well as USA 300 and RN6390, a strain used in the mouse model. A chimeric fusion protein of the Ply187 endopeptidase domain and LysK SH3b cell wall binding domain showed strongest anti-staphylococcal activity as evidenced by complete inhibition of bacterial growth, turbidity reduction and biofilm disruption. Intravitreal injection of Ply187 (both at 6 and 12h post infection) significantly improved the outcome of *S. aureus* endophthalmitis, preserved retinal structural integrity, and maintained visual function as assessed by ERG analysis. Furthermore, phage lysin treatment dramatically reduced bacterial burden and inflammatory cytokines in the eyes.

**Conclusions:** Considering an increased antibiotic resistance among ocular isolates of *S. aureus*, our study suggests that the phage lysins could be used as potential therapeutic agents for management of bacterial endophthalmitis. Moreover, genetic engineering approaches can be used to generate potent chimeric lysins containing multiple lytic domains to avoid resistance development.

**Commercial Relationships:** Pawan Kumar Singh, None; David M. Donovan, None; Ashok Kumar, None

Support: NH Grant EY19888, Research to prevent Blindness

**405 Host Responses to Ocular Herpesvirus Infection - What Have We Learned and What Is the Future Direction? - Minisymposium**

**Program Number:** 4032

**Presentation Time:** 9:20 AM - 9:40 AM

**Chronic Murine Cytomegalovirus Infection results in more severe Experimental Choroidal Neovascularization**

**Richard D. Dix.** Department of Biology, Georgia State University, Atlanta, GA.

**Commercial Relationships:** Richard D. Dix, None

**Program Number:** 4033

**Presentation Time:** 9:40 AM - 10:00 AM

**Pathogenesis of Herpes Stromal Keratitis - A Focus on Corneal Neovascularization**

**Barry T. Rouse.** Pathobiology, Univ of Tennessee Coll of Vet Med, Knoxville, TN.

**Commercial Relationships:** Barry T. Rouse, None

**Program Number:** 4034

**Presentation Time:** 10:00 AM - 10:15 AM

**Discussion**

**Todd P. Margolis.** UCSF Proctor Foundation, Univ of California-San Francisco, San Francisco, CA.

**Commercial Relationships:** Todd P. Margolis, Peregrine (C), UCSF (P)

**427 Ocular Immune Responses**

Wednesday, May 08, 2013 11:00 AM-12:45 PM
606/607 Paper Session

**Program Number:** 4517

**Presentation Time:** 11:00 AM - 11:15 AM

**Loss of MicroRNA-124 and MicroRNA-126 expression regulates inflammatory microglial activation in inherited retinal degeneration**

**Thomas Langmann1,2, Christoph Moehle3, Marcus Karlstetter3, Bernhard H. Weber2.** 1Department of Ophthalmology, University of Cologne, Cologne, Germany; 2Institute of Human Genetics, University of Regensburg, Regensburg, Germany; 3Center of Excellence for Fluorescent Bioanalytics, University of Regensburg, Regensburg, Germany.

**Purpose:** Activation of microglia is a common hallmark of inherited and induced retinal degenerations. It is currently unknown whether MicroRNAs (miRNAs) play a role in gene regulation associated with this immune process. The purpose of this study was to identify key miRNAs and their target genes using a complementary genome-wide analysis of miRNAs and mRNAs in purified retinal microglia from retinoschisin-deficient mice, a prototypic model for inherited retinal degeneration.

**Methods:** Transgenic MacGreen reporter mice were crossed with retinoschisin-deficient animals to label alerted, amoeboid retinal microglia with GFP. Microglial cells from MacGreen mice served as ramified control cells. Small RNA fractions containing miRNAs as well as total RNA were isolated from both GFP-positive retinal microglia populations at postnatal day 21, an early phase of immune activation. Affymetrix miRNA 2.0 and Gene 1.0 microarrays were performed and selected transcripts were confirmed by qRT-PCR. Candidate miRNAs and their targets were deduced from microarray expression profiles and bioinformatic analyses using a compendium of 11 established miRNA target prediction programs. 3’ UTR regions containing miRNA seed matches were verified using the luciferase
Mice lacking TNFR1 develop less angiogenesis during late stage experimental autoimmune uveoretinitis (EAU)
Madeleine Stimpson1, Jian Liu1, David A. Copland1, Tarnjit K. Khera1, Andrew D. Dick1
1Academic Unit of Ophthalmology, University of Bristol, Bristol, United Kingdom; 2Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom.

Purpose: TNF is a pivotal cytokine for the progression and clinico-pathological expression of EAU. Firstly, TNF regulates macrophage infiltration into the eye and, within the tissue, macrophage activation is responsible, in part, to the TNFR1-dependent tissue damage observed. After peak disease and damage, a persistent stage of EAU ensues (lasting up to 120 days). During this period a continual inflammatory infiltrate is observed alongside formation of retinal neovascular membranes (RNMs) and loss of remaining photoreceptors. Although TNF can induce VEGF secretion from macrophages and endothelial cells, the role of TNF during the later stages of EAU has not been defined. Our aim was to examine the impact of TNFR1-dependent TNF signalling on retinal angiogenesis in mice during late stage EAU.

Methods: Disease progression was monitored in TNFR1-/- and wild type (WT) C57BL/6J mice through topical endoscopic fundus imaging (TEFI). At day 90 post immunisation (p.i.), collagen IV-immunofluorescence on retinal whole mounts was used to quantify morphologically determined angiogenesis (RNMs). Cellular infiltration was quantified by flow cytometry and gene expression of cytokines was analysed using qPCR.

Results: Clinical disease in TNFR1-/- mice was previously described at a consistently lower level compared to WT. Here, we show there was no significant difference in cell numbers of CD4+ and CD11b+ cells in the eye at day 90. TNFR1-/- mice were, however, protected from developing angiogenesis reflected by not only a dramatic reduction in RNMs but also, when occurring, smaller angiogenic lesions. Despite equal infiltrating cell numbers, TNFR1-/- displayed a reduction in gene expression of pro-inflammatory and pro-angiogenic cytokines, in particular levels of IL-17A and TNF.

Conclusions: Lack of active TNFR1 signalling suppresses inflammatory-mediated angiogenesis in the late stages of EAU, during which a reduction in cytokines that normally lead to macrophage activation and VEGF secretion is observed. Understanding the role of TNF in angiogenesis during uveitis may benefit the development of therapies targeting retinal angiogenesis, a major cause of vision loss.

Commercial Relationships: Madeleine Stimpson, None; Jian Liu, None; David A. Copland, None; Tarnjit K. Khera, None; Andrew D. Dick, Novartis (C), Novartis (F), GSK (F), Abbott (F).

Support: National Eye Research Centre, Bristol, UK.
Monophasic PDSAg

Presentation Time: 11:45 AM - 12:00 PM

Demonstration of Donor T cell Recruitment and Correlation with Inflammatory Cytokine Presence in Experimental Ocular Graft-versus-Host Disease

Samantha Herretes1, Juan C. Murillo1, Duncan Ross2, Henry Barreras2, Yaohong Tan1, Ali M. Saeed1, Astrid Gonzalez1, Carolina Betancurt1, Robert B. Levy2, Victor L. Perez2, *1

1Ophthalmology, Bascom Palmer Eye Inst, Univ of Miami, Miami, FL; 2Immunology and Microbiology, University of Miami, Miami, FL.

Purpose: Graft-versus-host disease is a potentially blinding condition that may affect allogeneic hematopoietic stem cell transplant (HSCT) survivors. Alloreactive donor T cells are essential for the development of GVHD, but their direct role in local ocular GVHD has not been demonstrated. In this study, we examined immunological and ocular changes using a clinically relevant MHC-mismatched, minor antigen-mismatched HSCT transplantation model of systemic and ocular GVHD.

Methods: After high-dose TBI, C3H.SW (H2b) mice were transplanted with TCD-BM alone or together with T cells from either wild type B6 mice or CXC6-6/- GFP knockin mice, which express GFP regulated by the CXC6 promoter (B6-CXC6-6/-; GFP). GVHD was monitored weekly by clinical score composite and ocular surface fluorescein staining. In vivo fluorescent microscopy was used to measure the recruitment of B6-CXC6-6/-; GFP donor T cells. At 6-7 weeks goblet cells were quantified using PAS staining and T cell phenotype was characterized by flow cytometry analysis of corneal cells suspension. Total RNA was prepared and the production of mediators of inflammation analyzed by qPCR.

Results: Mice that received transplanted BM and T-cells from either wild type B6 or B6-CXC6-6/-; GFP developed evidence of systemic GVHD by day 21 as opposed to control animals. Beginning at day 28, the recruitment of donor B6-CXC6-6/-; GFP T cells correlated with the development of progressively increasing ocular surface disease in mice with GVHD. Interesting, relative expression of RNA of the T cell chemo-attractant CXCL10 and its receptor CXC6, also increased in the corneas. Similarly, the relative expression of the inflammatory cytokines, IFN-γ, IL-1 and IL-6 were significantly increased, correlating with a significantly decreased number of goblet cells in the conjunctiva of mice with GVHD but not controls.

Conclusions: C3H.SW mice that receive BM with T cells develop ocular GVHD characterized by donor T cell recruitment, increased expression of CXCL10/CXC6, inflammatory cytokines and the development of corneal staining, ulceration and loss of goblet cells. These findings support a critical role for donor T cells in the immune responses occurring at the ocular surface. Understanding kinetics and pathways will provide targets for the prevention and treatment of ocular GVHD.

Commercial Relationships: Samantha Herretes, None; Juan C. Murillo, None; Duncan Ross, None; Henry Barreras, None; Yaohong Tan, None; Ali M. Saeed, None; Astrid Gonzalez, None; Carolina Betancurt, None; Robert B. Levy, None; Victor L. Perez, Alcon (C), Bausch & Lomb (C), Genentech (C), Cleveland Clinic Foundation (P), Alcon (F), Alcon (R)

Support: Research to Prevent Blindness Physician-Scientist Award (VLP), unrestricted grant Research to Prevent Blindness, NEI P30 EY014801

Ulrike Kaufmann, Maria Diedrichs-Möhring, Gerhild Wildner.

Ophthalmology, Clinic of the Ludwig-Maximilians-University, Munich, Germany.

Purpose: Rat EAU can be induced with S-Ag peptide PDSAg, inducing monophasic, or IRBP peptide R14, inducing relapsing uveitis. We have previously shown different dynamics of intraocular T cell populations during the two types of EAU. but the exact mechanisms behind the disease courses remain elusive. Here we used different combinations of the two antigens for immunization to investigate the mutual influence on the disease course and the immune response.

Methods: EAU was induced with PDSAg or R14 in CFA and with combinations of both, either administered separately at contralateral sides or as a mixture of both. Disease course was analyzed daily and cytokine pattern (IFN-γ, IL-17, IL-10) and Foxp3 expression of intraocular cells was determined at onset, peak, resolution and late remission of disease by flow cytometry. Data of the combined immunizations were compared with that from the conventional PDSAg and R14 immunizations.

Results: While in R14-induced EAU 75% of the eyes developed relapses, none of the PDSAg- or PDSAg/R14-mixture immunized rats had recurrences. However, contralateral administration of both antigens allowed recurrences in 12.5% of eyes. The cytokine pattern of intraocular cells looked similar in those animals immunized with both antigens, irrespective of the application, but differed from the pattern of the rats which were immunized with PDSAg or R14 only. Rats immunized with both antigens showed an R14-like cytokine pattern at onset of EAU and a PDSAg-like cytokine expression at resolution of EAU. Cultivated lymph node cells of mixture-immunized rats had increased numbers of Foxp3+, but decreased IFN-γ+ cells compared to rats immunized with PDSAg or R14 only, respectively.

Conclusions: Disease course and cytokine pattern of intraocular cells confirmed a dominant role of the monophasic, PDSAg-specific immune response. Increased regulatory T cells expressing IL-10 or Foxp3 might be responsible for the prevention of relapses.

Commercial Relationships: Ulrike Kaufmann, None; Maria Diedrichs-Möhring, None; Gerhild Wildner, None

Support: Deutsche Forschungsgemeinschaft SFB571, Friedrich-Baur-Foundation, Münchener Medizinische Wochenschrift

Program Number: 4522

Presentation Time: 12:15 PM - 12:30 PM

Inhibition of Vascular Endothelial Growth Factor Ameliorates Ocular Allergy in the Murine Model

Daniel R. Saban1, Reza Dana2, Hyun Soo Lee3, Tomas Blanco4

1Ophthalmology, Duke University School of Medicine, Durham, NC; 2Ophthalmology, Scheepens Eye Res Inst, Mass. Eye & Ear, Harvard Medical School, Boston, MA.

Purpose: Vascular endothelial growth factor (VEGF) is one of the most potent proangiogenic cytokines, which also promotes vascular permeability. However, little is known about the possible pathophysiological function of VEGF in ocular allergy. In the present study, we investigated the possible involvement of VEGF in murine ocular allergy pathogenesis.

Methods: C57BL/6 mice were sensitized once with 100 ug ovalbumin (OVA) + pertussis toxin (300 ng) + aluminum hydroxide (1 mg). After 2 weeks, mice were challenged once/daily with OVA (250 ug) eye-drops and examined 20 min later for 10 d. Some mice received systemic VEGF receptor inhibitor (axitinib) to block VEGF function in this model. Corneal neovascularization was evaluated by FITC-CD31 antibody staining. Expressions of VEGFs at the mRNA level in the cornea and conjunctiva were evaluated by real-time PCR. Conjunctivae were also collected to enumerate eosinophil
recruitment by flow cytometry.

**Results:** Significant presence of corneal heme- and lymph-angiogenesis developed in this model, with increased mRNA expression of VEGF-A/C/D/R3 in the cornea and VEGF-A/D in the conjunctiva. Administration of axinitin led to reduced clinical signs of ocular allergy, as well decreased eosinophil infiltration in the conjunctiva.

**Conclusions:** These results suggest that VEGF is one of the major determinants of AC and that the inhibition of VEGF may be a good therapeutic strategy.

**Commercial Relationships:** Daniel R. Saban, Sclerenges Eye Res Inst, Mass Eye and Ear, (P), Eleven Biotherapeutics (R); Reza Dana, Allergan, (C), Alcon (C), Bausch & Lomb (C), Eleven Bio (I), GSK (F), Novabay (C), Revision Optics (C), Novaliq (C), Rigel (F); Hyun Soo Lee, None; Tomas Blanco, None

**Support:** R01EY021798

**Program Number:** 4523

**Presentation Time:** 12:30 PM - 12:45 PM

**Translational Modeling of Calpain-5 Vitreoretinopathy**

**Mechanisms in Mice**

Vinith B. Mahajan1,2, Katherine J. Wert1, Jessica M. Skeie1,2, Stephen H. Tsang1, 1Ophthalmology, University of Iowa, Iowa City, IA; 2Omics Laboratory, University of Iowa, Iowa City, IA; 3Institute of Human Nutrition, Columbia University, New York, NY; 4Ophthalmology, Columbia University, New York, NY.

**Purpose:** We identified CAPN5 (calpain-5) as the causative gene for autoimmune uveitis in patients with Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy (ADNIV). To understand the molecular genetic mechanisms of ADNIV and develop a preclinical model, retinal Capn5 was studied in mice.

**Methods:** Primary, secondary, and tertiary protein alignments were created with Accessory Protein Yasara Structure software. DNA sequencing, immunohistochemistry, and histology was performed using standard methods. A lentiviral vector expressing green fluorescent proteins (GFP) under a rhodopsin promoter was injected into the subretinal space of perinatal mice. Lentiviral expression was analyzed through six months of age using electroretinography and histological assessment.

**Results:** Mouse calpain-5 protein showed high homology to its human ortholog with over 80% sequence identity that included the ADNIV mutant residues. Three-dimensional protein modeling of calpain-5 to other calpains revealed a high structural homology. Expression of Capn5 was detected in a mouse photoreceptor-specific cDNA library. In mouse photoreceptor cells there was strong calpain-5 protein expression in the inner and outer segments of both rods and cones, which correlated with human eye expression. Phenotyping of Capn5 knockout mice did not show eye abnormalities. To study a gain of Capn5 function effect, a photoreceptor-specific expression lentiviral vector was designed and injected into the subretinal space of perinatal mice. Live imaging of a GFP reporter showed good uptake and distribution throughout the retina. Electroretinography and histological examination supported the use of this vector with CAPN5 mutants.

**Conclusions:** Taken together, our studies suggest that genetic models of ADNIV can be developed in the mouse. There are a very limited number of animal models for uveitis, ocular neovascularization, and intraocular fibrosis, and a Capn5-ADNIV mouse model may help address this gap.

**Commercial Relationships:** Vinith B. Mahajan, None; Katherine J. Wert, None; Jessica M. Skeie, None; Stephen H. Tsang, None

**Support:** The authors are supported by NIH Grants K08EY020530 (VBM), 5T32EY013933 (KJW), 1F32EY022280 (JMS), EY018213 (SHT), Research to Prevent Blindness, and the Foundation Fighting Blindness.

**473 Non-infectious Inflammation**

**Purpose:** Investigated the etiology of new-onset non-infectious uveitis in patients that presented after cataract extraction and intraocular lens (IOL) implantation. While it is critical to rule out infectious etiologies, there are multiple other common causes for uveitis in this setting. These include: lens-induced uveitis, IOL-related inflammation and non-surgery related causes of inflammation.

**Methods:** Studied a cohort of patients that presented over a one-year period to two uveitis specialists at New York Eye and Ear Infirmary. Most patients received a work-up for complete blood count (CBC), Sedimentation rate, anti-nuclear antibody (ANA), Rheumatoid Factor (RF), Anti-neutrophil cytoplasmatic antibody (ANCA), HLA-B27, syphilis serology and ultrasound biomicroscopy (UBM). Forty-three patients seen, twelve were excluded due to other known causes of uveitis or evidence of other unrelated causes of uveitis. The remaining patients (31) were examined for age, sex, time from cataract surgery to uveitis onset, unilateral vs bilateral, location of uveitis and positive labwork or UBM.

**Results:** There were a total of 31 patients that met the non-infectious uveitis following surgery criteria. 9/31 (29%) were male, 22/31 (71%) were female. Mean age at presentation was 68.6 years (range 47 - 92 years old). Location of uveitis was anterior in 30/31 (96.8%) and posterior in 1/31 (3.2%). In 21/31 (68%) the uveitis involved just the surgical eye and 10/31 (32%) were bilateral. 15/31 (48%) were idiopathic, of the remaining patients: 5/31 (16%) were due to lens position, 3/31 (9.6%) were ANA positive, 3/31 (9.6%) were RF positive, 3/31 (9.6%) were HLA-B27 positive, 1/31 (3.2%) was ANCA positive, 1/31 (3.2%) was biopsy proven sarcoid, 1/31 (3.2%) was due to retained cortex.

**Conclusions:** This study examined common causes for new-onset non-infectious uveitis following cataract surgery. While approximately half of the cases were idiopathic, the other half were found to have a diagnosed etiology. These are important to consider as the treatment approach is very different from one cause to another. These results will help ophthalmologists be aware of some of the common non-infectious causes of uveitis following cataract surgery.

**Commercial Relationships:** Zvi A. Kresch, None; Cynthia Yang, None; Vicente Diaz, None; Sanjay Kedhar, None; John V. Mauro, None; C. Michael Samson, CLS Pharmaceuticals (I), PCAsso (I)

**Program Number:** 5187 Poster Board Number: C0002

**Presentation Time:** 2:45 PM - 4:30 PM

**Trends in Patterns of Intermediate Uveitis in a Tertiary Institution in Singapore**

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Helen Mi1, Su Ling Ho2, Wee Kiak Lim3, Elizabeth Poh Ying Wong2, Stephen C. Teoh2, 1Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore; 2Ophthalmology, National Healthcare Group Eye Institute, Tan Tock Seng Hospital, Singapore, Singapore.

Purpose: The study aims to describe and analyze the clinical characteristics and etiologic causes of intermediate uveitis (IU) patients seen by a tertiary eye care center in Singapore over 8 years.

Methods: This was a retrospective analysis of all 66 consecutive cases of IU that presented to the Uveitis clinic, out of a total of 1168 uveitis patients, between 2004-2011. Data collected included demographics, clinical and laboratory findings, and complications. Diagnoses for specific ocular entities or systemic disease associations were based on standardized clinical history, systemic review, complete ophthalmological examination and laboratory investigations. Further ancillary investigations were performed where necessary. Cases were classified as idiopathic when a specific etiology was not identified.

Results: There was a male:female ratio of 1:1.2, and the disease was bilateral in 37 patients (56.1%). The median age of diagnosis was 40 years, with majority of the patients in the age group of 41-60 years (n=24, 36.4%). The majority was Chinese (n=38, 57.6%), followed by Indians (n=12, 18.2%) and Malays (n=11, 16.7%). The ethnicity distribution was dissimilar to our ethnic distribution in Singapore (p<0.001). Most were idiopathic (n=39, 59.1%) in etiology, followed by tuberculosis (TB) (n=10, 15.2%). Ocular complications developed in 21 patients (31.8%), with cystoid macular edema (CME) being the commonest (n=19, 28.8%), followed by severe vitritis (n=6, 9.1%). TB-associated IU was associated with complication of severe vitritis (p<0.001). There was a downward trend for the proportion of IU patients over the total uveitis patients (p=0.021), with Spearman’s rho of -0.786.

Conclusions: Our results suggest a downward trend for the proportion of IU over the total uveitis patients. However, TB-associated IU was of higher prevalence compared to less endemic areas, emphasizing the need for increased TB surveillance. A high index of suspicion for TB-associated IU is required in patients with severe vitritis. Comparisons with other countries revealed disparities in the IU etiologies, indicating possible genetic and geographical differences. Prevalence of autoimmune etiologies of IU is less compared to the western population. Our study showed an increased incidence of IU in the Indian population, suggesting a probable predisposition of the Singapore local Indian population for IU.

Commercial Relationships: Helen Mi, None; Su Ling Ho, None; Wee Kiak Lim, None; Elizabeth Poh Ying Wong, None; Stephen C. Teoh, None

Program Number: 5188 Poster Board Number: C0003
Presentation Time: 2:45 PM - 4:30 PM
Epidemiology of Infectious Uveitis in Alabama
Christopher J. Compton, Carrie Huisingsh, Gerald McGwin, Russell W. Read, Kinley Beck. Ophthalmology, Univ of AL - Birmingham, Birmingham, AL.

Purpose: To report the epidemiology of infectious uveitis among a referral population in the Southeast United States. In addition, to analyze the epidemiology of infectious uveitis in a United States population with a high percentage of African Americans.

Methods: We evaluated demographic and clinical data from 780 consecutive patients referred to the University of Alabama - Birmingham (UAB) Uveitis Clinic.

Results: The average age at initial visit for the total population was 46.5 years of age, 48.9 and 46.2 for infectious and non-infectious respectively (p=0.2717). The total population (n=780) included 259 (33.2%) males and 522 (66.8%) females. Among infectious cases (n=80): 42 patients (52.5%) were males and 38 (47.5%) were females; 22 (27.5%) were African American, 55 (68.8%) were Caucasian and 3 (3.8%) identified themselves as “other”. There were 325 (41.6%) African American patients, 433 (55.4%) Caucasians, and 23 (2.9%) “other”. There were a total of 700 non-infectious cases. Males comprised 217 (31.0%) of non-infectious cases while there were 484 (69%) females (p=0.0001). Among non-infectious cases, 303 (43.2%) were African American patients, 378 (53.9%) were Caucasian patients, and 20 (2.9%) “other” (p=0.0259).

The three most common non-infectious etiologies were: 388 idiopathic (55.3%), 79 HLA-B27 associated (11.3%), and Sarcoid 44 (6.3%). The most common infectious etiologies were: Herpes Zoster 19 (23.8%), Toxoplasma retinitis (17.5%), and Herpes Simplex (11.3%). Among the cases of Herpes Zoster related uveitis, 17 (90.9%) were Caucasian (p=0.0135). All 3 cases of HTLV related uveitis were African Americans (p=0.0211). Of cases of toxoplasma retinitis, 11 (78.6%) were males and 3 (21.4%) were females (p=0.0315).

Conclusions: Cases of uveitis with infectious etiologies were more likely to be posterior and non-infectious cases were more likely anterior (p=0.025). Among infectious etiologies, the most common diagnosis was Herpes zoster at 23.8%, followed by toxoplasmosis at 17.5% and Herpes simplex at 11.3%. Our study had a higher incidence of syphilis (8.8%) than previous epidemiologic studies (1-3%). Herpes zoster occurred significantly more often in Caucasians than in African Americans (p=0.013). In African Americans, there was a relatively higher incidence of Syphilis and Lyme disease although not statistically significant (p=0.097 and p=0.068). Males were statistically more likely to have a diagnosis of toxoplasmosis (p=0.032).

Commercial Relationships: Christopher J. Compton, None; Carrie Huisingsh, None; Gerald McGwin, None; Russell W. Read, None; Kinley Beck, None
Support: Research to Prevent Blindness; EyeSight Foundation of Alabama

Program Number: 5189 Poster Board Number: C0004
Presentation Time: 2:45 PM - 4:30 PM
Characteristics of HLA-B27/Ankylosing Spondylitis Associated Uveitis in Different Ethnicities
Russell W. Read1, 2, Kinley Beck1, 1Ophthalmology, University of Alabama at Birmingham, Birmingham, AL; 2Pathology, University of Alabama at Birmingham, Birmingham, AL.

Purpose: To determine if variations exist in the disease characteristics of HLA-B27/Ankylosing Spondylitis associated uveitis between varying ethnicities (primarily Caucasians and African Americans).

Methods: Retrospective chart review of all patients seen between 2007 and 2010 (inclusive) at a single tertiary care academic uveitis center in Birmingham, Alabama, USA. Characteristics were compared between racial subgroups using t-test and chi-square test for continuous and categorical variables, respectively.

Results: Seven hundred thirty two (732) patients were identified as having uveitis from the chart review.42% of which were African-American, 55% Caucasians, and 3% categorized as “other.” A diagnosis of HLA-B27/AS was established in 97 patients (13% of the total uveitis cases) and was the diagnosis in 18% of Caucasians, 7% of African Americans, and 9% of other ethnicities (2 patients, which due to the small number were excluded from further analysis (p=0.000007). Of patients with HLA-B27/AS uveitis, the mean age at presentation was 38 years in Caucasians, 40 in African Americans (p=0.57). Females made up 46% of Caucasians, 60% of African Americans.
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Americans (p=0.26). Caucasians had acute disease 95% of the time, African Americans 86% (p=0.17); unilateral disease was present in 96% of Caucasians, 95% of African Americans (p=0.89). At presentation, 81% of Caucasians had VA in the affected eye of 20/40 or better while 62% of African Americans did. Vision of 20/50 to 20/150 was present in 12% of Caucasians and 29% of African Americans. Vision of 20/200 or worse was present in 7% of Caucasians and 10% of African Americans (p=0.1539). Severity of anterior chamber reaction was skewed to the extreme ends of the scale in African Americans while Caucasians had generally lower levels of inflammation at presentation (p=0.11).

Conclusions: HLA-B27/AS uveitis is significantly more common among Caucasians than African Americans. While African Americans were overrepresented in the lower visual acuity categories and had a higher percentage of patients with the most significant level of inflammation, no statistically significant differences were found between the two groups.

Commercial Relationships: Russell W. Read, None; Kinley Beck, None

Support: Research to Prevent Blindness; EyeSight Foundation of Alabama

Program Number: 5190 Poster Board Number: C0005
Presentation Time: 2:45 PM - 4:30 PM
A Phase I/II Dose-Ranging, Randomized Clinical Trial of Abatacept (Orencia) in the Treatment of Refractory Non-Infectious Uveitis: Preliminary Results
Eric B. Suhler1, Tracy R. Giles2, Shelley A. Hanel2, Teresa L. Liesegang2, Robert M. Beardsley3, Kelly L. Larkin2, Phoebe Lin2, James T. Rosenbaum3, 1Ophthalmology, Portland VA Medical Center, Portland, OR; 2Ophthalmology, Casey Eye Institute-OHSU, Portland, OR; 3Ophthalmology, Devers Eye Institute, Portland, OR.

Purpose: To ascertain the safety and effectiveness of abatacept (Orencia, Bristol-Myers-Squibb), a recombinant fusion protein which inhibits T-cell costimulation by binding CTLA-4 antigen, for the treatment of refractory non-infectious uveitis.

Methods: We received IRB and FDA approval to recruit 20 patients into a 24 week open-label study of the effectiveness of abatacept 10 mg/kg infusions for the treatment of refractory uveitis, followed by randomization to either 5 or 10 mg doses for another 80 weeks. Patients characterized as refractory had failed treatment with corticosteroids and at least one standard immunosuppressive. Initial treatment outcome was ascertained at 24 weeks after study initiation by a composite clinical endpoint comprised of improvement in visual acuity, intraocular inflammation, ability to taper corticosteroids or immunosuppressives, and fluorescein angiography or ocular coherence tomography. Patients who meet criteria for clinical success at 24 weeks will be allowed to complete up to two years of therapy at the randomized 5 or 10 mg/kg dose, with repeat outcome assessment at weeks 52, 76, and 104.

Results: Study recruitment began in April 2012 and three subjects have enrolled thus far, two of whom have reached the 24 week endpoint at this writing. Both of these patients met the composite endpoint for clinical success at week 24. One patient had improvement in vision and was able to taper off corticosteroids at week 24, but subsequently flared symptomatically at week 28 and left the study. One patient achieved control of active inflammation and was able to taper off corticosteroids at week 24. The third patient has demonstrated preliminary benefit at week 8, having reduced prednisone from 30 to 15 mg daily and with improvement in inflammation as well. Significant adverse effects have included upper respiratory infection and fungal skin rash in the second patient, both which were treated with antimicrobials and neither of which required study discontinuation.

Conclusions: Our preliminary results suggest that abatacept may be effective for the treatment of refractory uveitis. No treatment-limiting toxicity was noted. Enrollment is ongoing and further study is required to define patient populations who may derive the most benefit from abatacept therapy.

Commercial Relationships: Eric B. Suhler, Abbott (F), Abbott (C), Bristol-Myers-Squibb (F), EyeGate (F), Genentech (F), LuxBio (F), LuxBio (C), Eleven Biotherapeutics (C), XOMA (F), Tracy R. Giles, None; Shelley A. Hanel, None; Teresa L. Liesegang, None; Robert M. Beardsley, None; Kelly L. Larkin, None; Phoebe Lin, None; James T. Rosenbaum, Genentech (C), Abbott (F), Xoma (F), EyeGate (F), Bristol Myers (F), Lux (C), Novartis (C), Regeneron (C), Teva (C), Therakine (F), Mitotech (F), Aquinox (F), Allergan (C), Santen (C)

Support: Bristol-Myers-Squibb; providing study expenses and gratis drug; Research to Prevent Blindness (institutional support); Portland VA Medical Center; NIH via support of Oregon Clinical and Translational Research Institute (OCTRI)

Clinical Trial: NCT01279954

Program Number: 5191 Poster Board Number: C0006
Presentation Time: 2:45 PM - 4:30 PM
Effectiveness of anti-Tumor Necrosis Factor agents in Pediatric Population with Uveitis
Rula Hajj-al1, Maria M. Choudhary2, Andrew Zeft3, Steven Spalding1, Sunil K. Srivastava1, Careen Y. Lowder1, 1Orthopedics and Rheumatologic Institute, Cleveland Clinic Foundation, Cleveland, OH; 2Internal Medicine, Cleveland Clinic Foundation, Cleveland, OH; 3Ophthalmology, Cleveland Clinic Foundation, Cleveland, OH; 4Pediatric Rheumatology, Cleveland Clinic Foundation, Cleveland, OH.

Purpose: Anti-tumor necrosis factor (anti-TNF) agents play an important role in controlling inflammation associated with non-infectious uveitis. Most commonly used agents include infliximab, adalimumab and etanercept. We compared these three agents in the pediatric population with non-infectious uveitis that is resistant to conventional immunosuppressive therapy.

Methods: A retrospective chart review of pediatric patients < 18 years old, with non-infectious uveitis followed at Cole Eye Institute between the year 2003 to 2011. All patients with non-infectious uveitis on anti-TNF agents were included in the study. Primary outcomes included achieving sustained remission (SR, defined as absence of inflammation for at least 3 consecutive months on a prednisone (or its equivalent) dose of 10 mg or less), time to SR and duration of SR. Proportional hazard regression model was used for sustained remission analysis where as categorical data was analyzed using the Chi-square tests.

Results: The study cohort consisted of thirty eight patients consisting of 29 (76.3%) females and 9 (23.7%) males with a mean age of 11.2 (range: 3 - 17) years at the time of start of anti-TNF. Out of these, 23 (60.5%) had anterior uveitis, 4 (10.5%) had intermediate, 3 had posterior (7.9%) and 8 (21.1%) had panuveitis. 5 patients were on etanercept, 21 on infliximab and 12 on adalimumab. The mean duration to achieve SR was 8.46 (SD: 10.3) months for patients on etanercept, 11.42 (SD: 17.24) months on infliximab, and 12.86 (SD: 17.65) months on adalimumab (p = 0.96) (Figure 1). 80% patients on etanercept, 73% on infliximab and 81.3% on adalimumab were able to achieve SR (p = 0.88). SR was maintained for a mean duration of 28.7 (SD: 20.1) months on infliximab, 11.3 (SD: 10.2) months on adalimumab and 12.8 (SD: 6.5) months on etanercept (p = 0.043).

The mean dose of infliximab at which SR was achieved was 6.7 mg/kg (SD: 3.1) at mean dosing interval of 3.6 (SD: 2.4) weeks.

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Conclusions: Anti-TNF agents are effective in controlling inflammation in pediatric patients with non-infectious uveitis. Patients on infliximab tend to do better than those on etanercept and adalimumab by maintaining disease control for a longer period.

Table 1: Kaplan Meier curve showing comparison of the three agents for time to achieve steroid sparing sustained remission.

Commercial Relationships: Rula Haji-ali, None; Maria M. Choudhary, None; Andrew Zelt, Pfizer (I); Merck (I); Steven Spalding, None; Sunil K. Srivastava, Bausch and Lomb (F), Bausch and Lomb (C), Novartis (F), Allergan (F); Careen Y. Lowder, None

Program Number: 5192 Poster Board Number: C0007
Presentation Time: 2:45 PM - 4:30 PM
Multicenter study of TNF-a antagonists for refractory Behçet’s uveitis in Spain
Marina Mesquida1, David Diaz-Valle2, Miguel Cordero Coma3, Alejandro Fonollosa4, Victor Llorens5, Laura Pelegrin6, Maria Victoria Hernandez7, Gerard Espinosa8, Blanca Molins1, Alfredo Adan Civera1. 1Ophthalmology, Hospital Clinic de Barcelona, Barcelona, Spain; 2Ophthalmology, Hospital Clinic San Carlos, Madrid, Spain; 3Ophthalmology, Hospital de Leon, Leon, Spain; 4Ophthalmology, Hospital Universitario Cruces, Bilbao, Spain; 5Rheumatology, Hospital Clinico de Barcelona, Barcelona, Spain; 6Autoimmune Disease, Hospital Clinic de Barcelona, Barcelona, Spain.
Purpose: To assess the long-term efficacy and safety of tumour necrosis factor alpha (TNF-a) antagonists (infliximab, IFX); adalimumab, [ADA]; and golimumab [GLM]) for the treatment of patients with Behçet’s disease (BD) and uveitis who failed to respond or did not tolerate conventional immunosuppressive (IS) treatment.
Methods: Retrospective study of patients with Behçet’s uveitis treated with anti-TNF therapy in four tertiary referral hospitals of Spain. Data analyzed were the following: demographic characteristics, disease duration and type of uveitis; best-corrected visual acuity (BCVA); previous treatments; type, regime and duration of each biological agent used; outcomes (remission, loss of efficacy, number of uveitis attacks).
Results: We included 59 eyes of 32 patients (16 males; mean age, 39±11.9 years old) with BD and refractory uveitis. Mean disease duration was 6.4±4.8 years. 18 patients (56.3%) were HLA-B51 positive. 66% of patients had panuveitis, 25% posterior, 6% anterior, and 3% intermediate uveitis. 84% of patients had bilateral ocular involvement. All patients received oral corticosteroids (CS), and 63% had received ≥2 IS drugs at baseline. 25 patients (78%) were treated with IFX and 7 (22%) with ADA as initial therapy, 11/25 patients treated initially with IFX were switched to another anti-TNF agent due to adverse events (3 patients), loss of efficacy (3), patient’s choice (1), or uveitis relapse after IFX withdrawal (4); 9/11 were switched to ADA and 3/11 to GLM. Globally, 16/32 patients were treated with ADA and 3 with GLM. IFX was infused for a mean of 16.6 months (range, 2-48), ADA was administered for a mean of 30.2 months (range, 3-52), and GLM was given for a mean of 4 months (range, 3-6). Mean follow-up was 77.3 months (range, 8-276). 28 (87%) achieved uveitis remission. 12/32 patients were able to discontinue all systemic IS and CS. BCVA remained stable or improved in 53/59 eyes. Mean BCVA improved from 0.5±0.2 to 0.5±0.2 (p<0.05), and ocular attacks per year dropped from 37 in the year before therapy to 5 at final follow-up visit (p<0.05). 3 serious adverse events requiring IFX withdrawal were reported: 1 severe infusion reaction, 1 pulmonary tuberculosis, and 1 prostatitis.

Conclusions: Anti-TNF agents are effective biological drugs for the treatment of Behçet’s uveitis. Treatments were generally well tolerated and only 3 patients required withdrawal.

Commercial Relationships: Marina Mesquida, None; David Diaz-Valle, None; Miguel Cordero Coma, Abbott laboratories (R), Merck (R); Alejandro Fonollosa, None; Victor Llorens, None; Laura Pelegrin, None; Maria Victoria Hernandez, None; Gerard Espinosa, None; Blanca Molins, None; Alfredo Adan Civera, None
Support: Catalan Society of Ophthamology Grant

Program Number: 5193 Poster Board Number: C0008
Presentation Time: 2:45 PM - 4:30 PM
Pharmacological blockade of interleukin 6 receptor (IL-6R) inhibits the development of ocular inflammation in the murine model of experimental autoimmune uveitis (EAU)
Purpose: To evaluate the effects of systemic administration of a mouse monoclonal antibody against murine IL-6R on ocular inflammation in a murine model of EAU.
Methods: EAU was induced by immunization with interphotoreceptor retinoid binding protein (IRBP) in male C57BL/6 mice. In Study 1, a monoclonal murine IL-6R antibody was administered IP at 25 mg/kg or 100 mg/kg on days 5, 7, 9, 11, 14, and 17 after EAU induction. Control animals in which EAU was induced were either untreated, or injected with a control protein (the Fc domain of murine IgG2, 33 mg/kg) following the same treatment schedule. The extent of vitreal and retinal inflammation was assessed in-life by optical coherence tomography (OCT) before IRBP injection (on day-1) and on days 7, 14 and 20 after immunization, and eyes were collected for histological analysis on day 21. A second study was conducted to confirm and extend the results of the first experiment. In Study 2, groups of mice were given one of 3 doses of anti-IL-6R (10 mg/kg, 35 mg/kg or 100 mg/kg IP), or mFc (33 mg/kg IP), every third day from day 5 post EAU induction through day 17.
Results: In Study 1, administration of anti-IL-6R resulted in a dose-related inhibition of uveitis as evidenced by reductions in retinal thickness, morphological abnormalities and inflammatory cell infiltration, as determined by both OCT measures and histological scores (p <0.0003, Kruskal-Wallis Test). Study 2 confirmed that systemic administration of anti-IL-6R resulted in a dose-dependent reduction in vitreoretinal inflammation as determined by OCT, compared to untreated mice, or mice treated with the control protein.

Conclusions: Treatment with an anti-IL-6R mAb starting 5 days post immunization produced a dose-related reduction in inflammation and retinal damage. These results indicate that pharmacological inhibition of IL-6 signaling may have utility in the treatment of inflammatory diseases affecting the retina and uvea track, particularly autoimmune forms of uveitis.

Commercial Relationships: Jingtai Cao, Regeneron Pharmaceuticals, Inc. (E); Thomas C. MacPherson, Regeneron

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Characteristics of patients who attain remission of inflammatory eye disease following treatment and discontinuation of mycophenolate mofetil


Purpose: This study aimed to characterize the long-term outcomes of patients with uveitis following successful disease suppression with mycophenolate mofetil (MM), a form of steroid-sparing immunomodulatory therapy (IMT). Experience suggests that once patients’ inflammation can be successfully controlled with IMT, there may be long-term benefit to disease control even following discontinuation of the medication.

Methods: A retrospective chart review was conducted to identify patients treated by a single oculocutaneous immunologist at a tertiary referral center who were started on mycophenolate mofetil (MM) for uveitis. Inclusion criteria were successful disease suppression with MM, discontinuation of the medication and at least three years of subsequent follow-up. 36 patients met these criteria. Additional factors examined included duration of MM use, concurrent use of other IMT, age and gender.

Results: 28/36 (78%) consecutive patients meeting the inclusion criteria achieved successful disease remission following treatment with mycophenolate mofetil (MM). These patients were treated for a variety of anterior, intermediate as well as posterior uveitic diseases. 72% of patients who attained remission were female and their average age at beginning of MM treatment was 43 years old (range 30 to 61). 75% of patients experiencing relapse of disease were female and their average age was 31 years old (range 17 to 38). Average duration of MM treatment in patients who attained remission was 29 months (range 16 to 40) and 32 months (24 to 40) for those who developed recurrent disease. The average time to relapse was 25 months (range 20 to 28). 14% of patients who achieved remission, versus 38% of those who did not, required concurrent treatment with other IMT during the time of administration of MM.

Conclusions: 78% of patients treated with mycophenolate mofetil (MM) achieved long-term remission after control and discontinuation of the medication. The need for concurrent IMT was greater in the group who relapsed within three years following treatment with MM. This is thought to be related to cases that were more difficult to control. Duration of treatment with MM, age and gender were not correlated with post-treatment relapse. These results will aid ophthalmologists when counseling patients with uveitis and choosing immunomodulatory therapy.

Commercial Relationships: Travis Jenkins, None; Tiffany Truong, None; Kevin Lai, None; Zvi A. Kreshch, None; Vicente Diaz, None; John V. Mauro, None; Sanjay Kedhar, None; C. Michael Samson, CLS Pharmaceuticals (I), PCAsso (I)

Program Number: 5196 Poster Board Number: C0011
Presentation Time: 2:45 PM - 4:30 PM

Risk Factors Associated with Intraocular Pressure Rise in Patients with Uveitis Treated with the Fluocinolone Acetonide Implant

Anjali Parekh¹, Sunil K. Srivastava², Thomas A. Albini³, Quan Dong Nguyen⁴, Debra A. Goldstein⁵. ¹Ophthalmology, Northwestern Medical Faculty Foundation, Chicago, IL; ²Ophthalmology, Cole Eye Institute, Cleveland, OH; ³Ophthalmology, Bascom Palmer Eye Institute, Miami, FL; ⁴Ophthalmology, Wilmer Eye Institute, Baltimore, MD.

Purpose: The fluocinolone acetonide (FA) intravitreal implant (Retisert; Bausch & Lomb Inc., Rochester, New York, USA) is an FDA approved therapy for the treatment of chronic non-infectious intermediate, posterior, and pan-uveitis. The efficacy of this implant has been demonstrated by three 3 year multicenter, prospective, randomized clinical trials. Elevated intraocular pressure is a well-
known adverse event secondary to the use of the FA intravitreal implant. In a study evaluating the incidence and management of elevated IOP in patients with uveitis treated with the FA implant, topical IOP lowering medications were administered in 74.8% of implanted eyes, and IOP lowering surgeries were performed in 36.6% of implanted eyes by 3 years. The purpose of this investigation is to report associated patient risk factors that may predispose to elevated intraocular pressure following treatment with the intravitreal FA implant.

Methods: Pooled data from the previously published 3 multicenter, double-masked, randomized, controlled phase 2b/3 clinical trials evaluating the safety and efficacy of the 0.59 mg or 2.1 mg FA intravitreal implant were analyzed to characterize risk factors associated with elevated IOP. Risk factors studied included age, gender, race, and location of uveitis. All eyes implanted with either the 0.59 mg FA implant or the 2.1 mg FA implant were included in the analysis. Frequencies of patients with glaucoma surgery were compared using Pearson chi-square or Fisher exact tests for categorical factors and t-tests for continuous measures. Analyses were performed in SAS software and used a 0.05 significance level.

Results: 290 eyes received an FA implant. Male gender was associated with an increased risk of requiring glaucoma surgery (p = 0.037), as was younger age (p = 0.006). No statistically significant difference was found between Caucasians and non-Caucasian patients. (p = 0.38) Patients with posterior uveitis were more likely than those with anterior, intermediate, or panuveitis to require surgery (p = 0.006).

Conclusions: The risk of requiring glaucoma surgery in this series was highest in males, younger patients and those with posterior uveitis. This information can be used to counsel patients considering treatment with the FA implant.

Commercial Relationships: Anjali Parekh, None; Sunil K. Srivastava, Bausch and Lomb (F), Bausch and Lomb (C), Novartis (F), Allergan (F); Thomas A. Albini, Bausch and Lomb (C), Allergan (C), Genentech (F), Eleven Biotherapeutics (C); Quan Dong Nguyen, Genentech (F), Regeneron (F), Lux Biosciences (F), Abbott (F), GSK (F), Santen (F), Santen (C), Bausch and Lomb (C), Optos (F), Heidelberg Engineering (F); Debra A. Goldstein, Bausch and Lomb (C), Bausch and Lomb (R)

Support: University of Illinois at Chicago and Northwestern Memorial Department of Ophthalmology is/are supported by an unrestricted grant from the Research to Prevent Blindness Foundation (NY)

Clinical Trial: NCT00456482

Program Number: 5198 Poster Board Number: C0013

Presentation Time: 2:45 PM - 4:30 PM

Combination chemotherapy with Mycophenolate mofetil and Cyclosporin in recalcitrant uveitis

Reena A. Rasheed1,*, Guergour T. Markov1,2, C. Stephen Foster1.

1Ocular Immunology, Massachusetts Eye Research and Surgery Institution, Massachusetts, MA; 2Regional Institute of Ophthalmology, Trivandrum, India; 3University Eye Hospital, Pashev, Bulgaria.

Purpose: To evaluate the effectiveness of combination chemotherapy (CC) with Mycophenolate mofetil (MMF) and cyclosporin A (CsA) in refractory uveitis.

Methods: Retrospective, non-comparative, cohort study. Ninety-three patients (177 eyes) who had CC with CsA and MMF during their course of treatment for uveitis and had a minimum follow up of 12 months with combination therapy. We analysed the efficacy, complications, and outcomes of CC. Ability to control ocular inflammation with CC at 6 and 12 months and corticosteroid sparing effect were main outcome measures. Discontinuation of therapy due to intolerance to medications or other reasons were also analysed. Efficacy of control of inflammation by CC was evaluated from the start of CC to sustained improvement of global disease activity by CC. Corticosteroid sparing effect was defined as the ability of CC to control ocular inflammation with total absence (0 mg) of oral, periocular or topical corticosteroids.

Results: In the study group, 75.3% patients by 6 months and 80.8% by 12 months achieved complete control of inflammation. Twenty-eight percent of patients required additional therapy with biologics. Additional surgeries were done for complete control of inflammation in 4.3% patients. Control of inflammation was 80.8% at 6 months and 84.2% at 12 months follow up in birdshot retinochoroidopathy (BSRC) patients and 77.3% at 6 months and 81% at 12 months for panuveitis patients. For patients with associated vasculitis, control of inflammation was 52.9% at 6 months and 62.5% at 12 months. Pars planitis patients had a very poor outcome (25% at 6 months and 0% at 12 months) with CC. Complete corticosteroid sparing success was achieved in 69.9% patients. Oral steroids were used in 10.8% patients and episodic periocular or topical steroids were given in 17.2% patients. Sixteen
percent of patients stopped CC due to intolerance; 19.4% and 7.5% developed toxicity to CsA and MMF, respectively. In 4.3% patients, CC was stopped due to insurance/non-compliance.

**Conclusions:** Combination chemotherapy with CsA and MMF is highly effective in controlling ocular inflammation in patients with BSRC and with panuveitis, but not with retinal vasculitis or with pars planitis. Efficacy and tolerance were better with these medications when they were given as combination than as monotherapy.

**Commercial Relationships:** Reena A. Rasheed, None; Gueorgui T. Markov, None; C. Stephen Foster, Abbott Medical Optics (C), Abbott Medical Optics (F), Alcon Laboratories, Inc. (C), Alcon Laboratories, Inc. (F), Allergan, Inc. (C), Allergan, Inc. (F), Eyegate Pharmaceuticals, Inc. (I), Eyegate Pharmaceuticals, Inc. (F), IOP Ophthalmics (C), Ista Pharmaceuticals (C), Lux Biosciences, Inc. (C), Lux Biosciences, Inc. (F), Novartis Pharmaceuticals Corporation (C), Novartis Pharmaceuticals Corporation (F), XOMA Ltd (C)

**Program Number:** 5199 **Poster Board Number:** C0014  
**Presentation Time:** 2:45 PM - 4:30 PM  
**Macular thickness measurements with spectral domain optical coherence tomography during active episode of Unilateral Acute Anterior Uveitis**  
David Diaz-Valle, Sara Elena Garcia-Vidal, Pedro Arriola-Villalobos, Ricardo Cuina, Jose A. Gegundez-Fernandez, Jose M. Benitez-del-Castillo. OPHTHALMOLOGY, HOSPITAL CLINICO SAN CARLOS, Madrid, Spain.  
**Purpose:** To assess the macular thickness using high-resolution spectral domain optical coherence tomography (OCT) in patients with active unilateral acute anterior uveitis (UAAU) and to compare with their contralateral unaffected eye.  
**Methods:** A descriptive comparative study of patients with an active flare of UAAU has been performed using spectral domain OCT (Spectralis HRA + OCT, Heidelberg, Germany). Forty eyes of twenty patients with idiopathic non-infectious UAAU were included. Data analysed were: best-corrected visual acuity (BCVA); anterior chamber cells; macular volume and macular thickness in nine sectors of the macular area -central region and in eight sectors surrounding the center (inner-superior, inner-nasal, inner-temporal and inner-inferior and outer superior, nasal, temporal and inferior-). Statistical analysis was performed using paired t-test.  
**Results:** We included 20 patients with idiopathic UAAU during an active episode (14 males; mean age, 32±7.9 years old). BCVA was 0.82 ± 0.33 in the affected eye and 0.93 ± 0.27 in the healthy eye. Mean anterior chamber cells according to SUN classification were 2.40 ± 0.82 in the affected eye and 0.15± 0.67 in the healthy eye. Mean central macular thickness was 274.55 ± 31.87 μ in the affected eye and 267.90 ± 28.20 μ in the healthy eye (p=0.337). There were no statistical differences in macular thickness in any macular region between both eyes of UAAU patients. Macular volume was 10.395 ± 0.7265 mm³ in the involved eye and 10.00 ± 0.8838 mm³ in the healthy eye (p=0.023).  
**Conclusions:** There are no significant differences in macular central thickness between the involved eyes versus the healthy eyes during an acute episode of UAAU. The only parameter that is significantly altered is the macular volume, which could be a useful clinical indicator to monitor uveitis activity. Further studies are needed to clarify these findings.

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treatment. These data could be used as reference in future controlled trials assessing new treatment methods in acute anterior uveitis.

**Commercial Relationships:** Margaux Guillard, None; Emilie Perrin, None; Bridget-Anne Kirwan, None; Dominique Monnet, None; Antoine P. Brezin, None

**Program Number:** 5201 Poster Board Number: C0016

**Presentation Time:** 2:45 PM - 4:30 PM

**Use of Immunosuppressive Medications for Treatment of Pediatric Intermediate Uveitis**

Spencer R. Cope, Aimee O. Hersh, Akbar Shakoor, John F. Bohnsack, Albert T. Vitale.

**Division of Rheumatology, University of Utah, Salt Lake City, UT; 2:Ophthalmology and Visual Sciences, John A. Moran Eye Center, Salt Lake City, UT.**

**Purpose:** The optimal treatment strategy for pediatric patients with intermediate uveitis is not well known. The purpose of this study is to describe the demographics, clinical presentation, treatment, and outcomes of a cohort of pediatric patients with intermediate uveitis, with a particular focus on the use of immunosuppressive medications.

**Methods:** This retrospective cohort study included all pediatric intermediate uveitis patients treated in the Uveitis Clinic at the University of Utah Moran Eye Center from 1999-2012. Medical records were reviewed and data abstracted at specific time points including initial presentation, 6 months, 1 year, and then annually. Data abstracted included examination findings, disease related complications, and treatment including surgical interventions and immunosuppressive medications. Responsiveness was determined based on Standardization of Uveitis Nomenclature (SUN) definitions for grading inflammation, inactive disease, and remission. Summary statistics were used to describe this cohort.

**Results:** The mean age at presentation was 7.7 years (SD 3.1). 56% of subjects were male, 95% had bilateral involvement. 36 subjects had idiopathic disease; 3 subjects had an underlying condition (juvenile idiopathic arthritis (n=2), multiple sclerosis (n=1)). Average length of follow-up was 37 months (range (6-96) months). Out of 76 total eyes involved, findings at presentation included: snowbanks (n=48, 63%), snowbanks (n=38, 50%), vitreous debris (n=32, 42%), and visual acuity < 20/50 (n=43, 57%). The most frequent disease complications were hyperton (n=45, 59%), cataracts (n=38, 50%), cystoid macular edema (n=30, 39%), visual acuity < 20/50 (n=46, 61%) and visual acuity < 20/200 (n=20, 26%). Common surgical treatments included periocular steroid injection (n=25 subjects, 64%) and pars plana vitrectomy (n=10, 26%). Oral steroids were received by 17 subjects (44%), and Table 1 describes immunosuppressive medication and responses. Overall, 49 subjects (64%) had inactive disease at their final follow-up visit, and 9 subjects (23%) achieved remission after at least 3 months off all medications.

**Conclusions:** In this cohort, patients treated with immunosuppressive therapy had interval improvement in their degree of inflammation. Prospective studies are needed to determine the best therapy for this high risk population.

<table>
<thead>
<tr>
<th>Immunosuppressive Medication</th>
<th>No. of eyes</th>
<th>No. of eyes with vitreous base inflammation improvement (%)</th>
<th>No. of eyes with anterior chamber involvement</th>
<th>No. of eyes with anterior chamber improvement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>36</td>
<td>26 (72)</td>
<td>33</td>
<td>24 (73)</td>
</tr>
<tr>
<td>Mycophenolate</td>
<td>12</td>
<td>6 (50)</td>
<td>10</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Mofetil</td>
<td>8</td>
<td>6 (75)</td>
<td>7</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>4</td>
<td>4 (100)</td>
<td>4</td>
<td>4 (100)</td>
</tr>
</tbody>
</table>

Table 1. Use of immunosuppressive treatment for pediatric intermediate uveitis.

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Purpose: This purpose of this study was to evaluate the association between inflammatory activity in uveitis patients and the amount of optic nerve head elevation measured by Spectralis spectral domain optical coherence tomography (SD-OCT).

Methods: Consecutive patients who had papillitis secondary to uveitis and who were examined on the Uveitis and Ocular Immunology Service at the Massachusetts Eye and Ear Infirmary between January 2010 and December 2012 were identified. Patients who had optic nerve imaging by Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) during and after an episode of active inflammation were included. The SD-OCT optic nerve imaging protocol included a manual measurement of the highest point of optic nerve head elevation in each eye. SD-OCT optic nerve head height and clinical variables including grade of inflammation and anatomic location of uveitis were recorded. A student’s paired t-test was used to assess the association between optic nerve head thickness and intraocular inflammation grade.

Results: Eight eyes from six patients were included in this series. The location of uveitis was anterior in 3 patients, retinal vasculitis in 1 patient, multifocal choroiditis in 1 patient and idiopathic papillitis in 1 patient. Mean maximum optic nerve head thickness in eyes without inflammation was 651 microns (n=8). The mean maximum never fiber layer elevation in optic nerves with inflammation was 916 microns (n=8). There is a statistically significant difference between optic nerve head elevation in inflamed vs. not inflamed eyes (p = 0.016).

Conclusions: Optic nerve elevation in papillitis secondary to uveitis can be quantified using SD-OCT. The amount of optic nerve elevation by SD-OCT in uveitis patients with secondary papillitis correlates with the presence of active intraocular inflammation. Further study is required to determine if optic nerve SD-OCT could be a clinically useful, non-invasive adjunct for monitoring inflammation in uveitis patients.

Commercial Relationships: Parvathy Pillai, None; Lucia Sobrin, None

Program Number: 5204 Poster Board Number: C0019
Presentation Time: 2:45 PM - 4:30 PM

Correlation between Disease Activity and Choroidal Patterns by OCT-EDI in Birdshot Chorioretinopathy

Andrea D. Birnbaum, Amani A. Fawzi, Lana M. Rifkin, Debra A. Goldstein, Ophthalmology, Northwestern Univ Feinberg Sch of Med, Chicago, IL.

Purpose: Birdshot chorioretinopathy (BCR) is characterized clinically by chorioretinal lesions, vasculitis, and macular edema. Visual acuity is often preserved, despite significant loss of functional vision. Patients are followed with clinical examination, OCT, fluorescein angiography (FA). Serial electroretinography and visual field testing are often performed to detect functional changes missed on other examination, although these tests can show fluctuations and are often cumbersome to perform. OCT enhanced depth imaging (EDI) allows visualization of structures posterior to the retinal pigment epithelium, and is easy to perform. The aim of this study is to correlate clinical signs of disease activity with choroidal patterns apparent on OCT-EDI.

Methods: Medical records of patients evaluated by a single investigator from July - December 2012 with a diagnosis of BCR were reviewed and disease activity was graded based on subjective complaints; clinical examination findings; FA including assessment for late leakage and cystoid macular edema (CME); and retinal OCT revealing CME. Only patients who were HLA-A29 positive were included in the study. Choroidal assessment was not included in these criteria. The OCT-EDI of each patient was then evaluated in a masked fashion based on the presence of following choroidal changes: visibility and ability to discern individual vascular structures (capillary, middle and deep vessels), interruptions of regular vascular pattern, intensity and size of inter-vascular stromal regions, presence of diffuse pockets of suprachoroidal hyporeflectivity.

Results: 13 patients were evaluated clinically and by OCT-EDI at several time points. Several clear clinical patterns of EDI changes were observed, and disease activity judged clinically could be correlated with EDI findings. As well, specific EDI findings including the vascular pattern and pockets of suprachoroidal hyporeflectivity correlated well with subjective complaints of photopsias.

Conclusions: Disease activity assessed clinically can be correlated with specific choroidal patterns on OCT-EDI. As well, subjective symptoms of photopsias, which do not always have easily recognized objective clinical, OCT or FA findings, were able to be clearly correlated with EDI features. OCT-EDI offers clinicians another tool to monitor disease activity and response to treatment in patients with BCR.

Commercial Relationships: Andrea D. Birnbaum, None; Amani A. Fawzi, None; Lana M. Rifkin, None; Debra A. Goldstein, None; Bausch and Lomb (C), Bausch and Lomb (R)
Support: NA

Program Number: 5205 Poster Board Number: C0020
Presentation Time: 2:45 PM - 4:30 PM

Segmentation and Analysis of Retinal Layers in Eyes with Uveitis and Comparison with Normal

Syed Mahmood A. Shah, Yasir J. Sepah, Mohammad A. Sadiq, Saleema Kherani, Mohamed A. Ibrahim, Zubir S. Rentiya, Mehreen Ansari, Diana V. Do, Quan Dong Nguyen, Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD.

Purpose: To examine the change in thickness of different retinal layers in eyes with intermediate (I), posterior (P) and panuveitis (Pan) Uveitis (U) in comparison to eyes with no known retinal disease.

Methods: This was a cross-sectional study. High-resolution 5 mm horizontal line scan passing through the fovea was acquired from 26 eyes with uveitis using Spectral Domain Optical Coherence Tomography. Eyes with macular edema and anterior uveitis were excluded from the analysis. Retinal layers were segmented to allow calculation of average thickness of retinal pigment epithelium (RPE), photoreceptor layer (PRL), bipolar cell layer (BPL), and combined ganglion cell and nerve fiber layers (GCL-NFL). Spatial changes in layers’ thicknesses and contribution of each layer to full retinal thickness (FRT) were assessed in 0.5mm increments. The measured values were compared to those from 50 normal eyes with no known retinal disease (Ibrahim et al., ARVO 2012).

Results: Four patients contributed both eyes and 18 had one eye included in the analysis. In 44% of the PU eyes decrease in FRT was observed (p<0.05) primarily in RPE (44%), PRL (31%) and GCL-NFL (37%). Sixty percent of the IU eyes showed increase in FRT (p<0.05), primarily in the BPL (50%) and GCL-NFL (42%). Fewer PU eyes (6.2%) showed an increase in FRT, primarily localized to the BPL. See Figure 1/Table 1 for percentages (%) of eyes with significant thinning/thickening in the individual retinal layers in patients with uveitis.

Conclusions: Compared to normal eyes, eyes with IU had thickening mainly in the BPL and GCL-NFL, while eyes with panuveitis had thickening across PRL, BPL, and GCL-NFL. Eyes with posterior uveitis had overall decrease in retinal thickness, mainly due to attenuation in RPE, PRL, and GCL-NFL. Segmenting individual
retinal layers may allow better understanding of the inflammatory process in different layers of the retina.

### Table 1: Percentages (%) of eyes with significant thinning/thickening in the individual retinal layers in patients with uveitis (n=25, p<0.05)

<table>
<thead>
<tr>
<th>Type of Uveitis (N)</th>
<th>Thinning</th>
<th>Thickening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FRT</td>
<td>RPE</td>
</tr>
<tr>
<td>Intermediate (5)</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Panuveitis (5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Posterior (16)</td>
<td>43.7</td>
<td>63.7</td>
</tr>
</tbody>
</table>

### Conclusions:
As a single marker, we propose a combination of ACE and sIL-2R as the standard serology to screen for sarcoidosis in uveitis patients. In patients that take ACE-inhibitors, ACE should be replaced by lysozyme. The combination of ACE and sIL-2R is the most reliable predictor of pulmonary involvement in ocular sarcoidosis.

### Commercial Relationships:
- Rafael Grajewski: None; Werner Adler: None; Konrad Frank: None; Mohamed Arfaoui: None; Simona L. Schlereth: None; Bernd Kirchhof: None; Claus Cursiefen: None; Mohamed A. Ibrahim: None; Yasar J. Sepah: None; Mohammad A. Sadiq: None; Saleema Kherani: None; Zubir S. Rentiya: None; Mehrseen Ansari: None; Diana V. Do: Genentech (F), Regeneron (F); Quan Dong Nguyen: Genentech (F), Regeneron (F), Lux Biosciences (F), AbbVott (F), GS K (F), Santen (F), Santen (C), Bausch and Lomb (C), Optos (F), Heidelberg Engineering (F)

### Program Number: 5207 Poster Board Number: C0126

### Presentation Time: 2:45 PM - 4:30 PM

### Program: Predictive Value of Angiotensin Converting Enzyme, Soluble Interleukin-2 Receptor and Lysozyme for Pulmonary Involvement in Human Ocular Sarcoidosis

### Purpose:
Sarcoidosis is an important cause of intraocular inflammation (uveitis) with very variable clinical presentation. Therefore, it is a clinical diagnosis that has to be supported by laboratory testing (pathology and serology). The most commonly used serological marker is angiotensin converting enzyme (ACE). In this study we analyzed additional markers (lysozyme and soluble Interleukin-2 Receptor: sIL-2R) that are associated with sarcoidosis and compared their single and combined values to predict pulmonary involvement that can confirm the diagnosis of ocular sarcoidosis.

### Methods:
ACE, lysozyme and sIL-2R levels have been determined in the serum of patients with noninfectious uveitis that was compatible with sarcoidosis. Patients (n=36) with elevation of at least one parameter have been classified according to the diagnostic criteria from the International Workshop on Ocular Sarcoidosis, Tokyo, 2006. Patients were further subdivided into groups with pulmonary involvement (biopsy proven: n=10, hilar lymphadenopathy: n=2) or without (n=24). Sensitivity, specificity and Youden index have been calculated to predict pulmonary sarcoidosis of single and combined serological values. None of the patients had been treated with ACE-inhibitors or systemic steroids.

### Results:
ACE as a single parameter revealed a lower sensitivity (0.50) than sIL-2R (0.83) or lysozyme (0.83), whereas the specificity was higher in ACE alone (0.88) than in sIL-2R (0.54) or lysozyme (0.50). The combination of ACE and sIL-2R demonstrated the highest Youden index (0.46) with a sensitivity of 0.50 and a specificity of 0.96, followed by the combination of ACE, sIL-2R and lysozyme (Youden index 0.42, sensitivity 0.42, specificity 1.00). Lysozyme combined with sIL-2R showed a Youden index of 0.38, a sensitivity of 0.67, and a specificity of 0.71, better than both values alone.

### Conclusions:
In contrast to the current practice with testing for ACE and sIL-2R as a single marker, we propose a combination of ACE and sIL-2R as the standard serology to screen for sarcoidosis in uveitis patients. In patients that take ACE-inhibitors, ACE should be replaced by lysozyme. The combination of ACE and sIL-2R is the most reliable predictor of pulmonary involvement in ocular sarcoidosis.
**Program Number:** 5208 **Poster Board Number:** C0127  
**Presentation Time:** 2:45 PM - 4:30 PM  
**An Experimental Model of Biofilm Formation in the Mouse Cornea**  
**Purpose:** Microbial keratitis is a severe and visually challenging outcome of corneal infection with antibiotic resistance being reported more frequently. The existence of bacteria in a protected state, the “Biofilm” a heterogeneous bacterial population within a biopolymeric extracellular matrix, shields bacteria from innate immune defence and antibiotics. Direct evidence of bacterial biofilm associated with ocular tissues is lacking. This study is an attempt to develop evidence for biofilm formation in a mouse model  
**Methods:** A corneal infection was created in a C57BL/6 wild type black mouse by the topical application of a bacterial suspension of 10^9 CFU/ml (Pseudomonas aeruginosa -ATCC 9027 and Staphylococcus aureus ATCC-29213). The progress of the infection was measured by monitoring corneal opacity through slit lamp examination. The infected eyes were enucleated in different time points of post infection, the cornea was excised and used for imaging biofilm formation. Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (CLSM) were used for this study  
**Results:** SEM demonstrated adherence of Pseudomonas on the corneal epithelial layer at early post infection times. Protruding clusters of Pseudomonas fixed within a matrix, as seen in a static culture, indicated the presence of extra polymeric substances (EPS) at day three post infection. Similarly, groups of Staphylococci were seen on the corneal epithelium in the early time of post infection. Assembly of cells connected with each other by fibre like structures and aggregates of bacteria enclosed in thick coatings of matrix substances observed by SEM suggested biofilm formation. CLSM images further confirmed the presence of bacteria within the biofilm  
**Conclusions:** These preliminary findings demonstrated that biofilm formation could develop on the corneal surface in an experimental mouse model of corneal infection. Use of this corneal biofilm model can provide new insights into the interaction of microbial communities on ocular pathophysiology and antibiotic resistance  
**Commercial Relationships:** Padmanabhan Saraswathi, None; Thet T. Aung, None; Shuhaida Salleh, None; Roger W. Beuerman, Allergan (F), SERI (P), Santen (R)  
**Support:** SERI Pilot Grant R959/68/2012, NMRC R-738 and TCR-618

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**Program Number:** 5209 **Poster Board Number:** C0128  
**Presentation Time:** 2:45 PM - 4:30 PM  
**The role of autophagy in Pseudomonas aeruginosa keratitis**  
Xiaoyu Jiang, Sharon A. McClellan, Ronald P. Barrett, Yunfan Zhang, Megan E. Foldenauer, Kerry Vistisen, Linda D. Hazlett. Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI.  
**Purpose:** Autophagy, an essential homeostatic process, is an important pro-survival mechanism involving the lysosomal degradation pathway in response to various signals, including dangerous stimuli such as infection. The role of autophagy and its potential regulation by TLR4, which is higher in C57BL/6 (B6) over BALB/c mice during Pseudomonas aeruginosa induced keratitis, is unknown and the purpose of this study.  
**Methods:** Wild type (WT) B6, BALB/c (with or without rapamycin treatment to induce autophagy) and TLR4 functionally deficient, C3-Tlr4<sup>−/−</sup> (Thr4<sup>−/−</sup>) mice were infected and tested using various approaches including: a Mouse Autophagy RT<sup>™</sup> Profiler<sup>™</sup> PCR array (containing 84 autophagy related genes), real time RT-PCR, and immunostaining (elastase and MPO) for neutrophil extracellular traps (NETs). In vitro, primary cultured corneal epithelium from WT B6 and BALB/c mice (confluent, 2nd passage cells) also was treated with ultrapure LPS (only activates TLR4 pathway; 1μg/ml for 18h) and tested by a PCR array, as above. Other, separate in vitro experiments were similarly done with or without the addition of rapamycin (1μM for 18h). For both of the above in vitro experiments, real time RT-PCR was used to further evaluate autophagy markers.  
**Results:** PCR array (3 days p.i.) and real-time RT-PCR (1-5 days p.i.) demonstrated that several pro-autophagy genes (beclin-1, LC3A, LC3B, and IRG1) were up-regulated, while autophagy degradation molecules (p62 and NBR1) were down-regulated in the cornea of B6 vs. BALB/c mice. Pro-inflammatory NETs, associated with autophagy-related signaling, also were more prominent and seen earlier in B6 vs. BALB/c mice. In vivo rapamycin treated BALB/c mice showed enhanced levels of TLR4 (and IFN-Υ) and a similar pattern for the above autophagy and degradation molecule related genes over controls. Conversely, RT-PCR showed that Thr<sup>−/−</sup> vs. WT BALB/c mice had decreased mRNA levels of beclin-1, LC3A, LC3B, and IRG1, while p62 and NBR1 were increased. In vitro PCR array studies revealed numerous (23) autophagy genes in the epithelium of B6 vs. BALB/c mice that were changed (two-fold or greater) and these were selectively confirmed by RT-PCR.  
**Conclusions:** These data provide evidence that autophagy is important in keratitis, is associated with NETs and that TLR4 appears to contribute to its regulation.  
**Commercial Relationships:** Xiaoyu Jiang, None; Sharon A. McClellan, None; Ronald P. Barrett, None; Yunfan Zhang, None; Megan E. Foldenauer, None; Kerry Vistisen, None; Linda D. Hazlett, None  
**Support:** NIH R01 EY016058, EY002986 and P30 EY004068 from the NEI

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Results: PCR array comparing LPS stimulated corneal epithelial cells from B6 vs BALB/c mice identified 19 apoptosis-related genes with 2 fold or greater change. RT-PCR comparing LPS stimulated with control B6 derived corneal epithelial cells showed increased expression of Bcl2, caspase 12, and FADD. In BALB/c derived epithelium, caspases 1, 3, 8, 12, Fas, and FADD were increased in LPS stimulated over controls, while caspase 9 levels were decreased. PCR array comparing infected corneas of BALB/c with Tlr4KO mice identified 8 genes with a 2 fold or greater difference between groups. In Tlr4KO vs wildtype mice, RT-PCR showed that Fas and FasL levels were decreased early, but unchanged later in disease; caspase 3 and FADD were initially down-regulated and then up-regulated, while caspase 8, Bcl2 and Bok were up-regulated at all times after infection.

Conclusions: The data provide evidence that TLR4 contributes to regulation/modulation of apoptosis in the cornea following P. aeruginosa infection.

Commercial Relationships: Sharon A. McClellan, None; Xiaoyu Jiang, None; Yunfan Zhang, None; Kerry Vistisen, None; Ronald P. Barrett, None; Linda D. Hazlett, None
Support: NIH/NEI R01EY002986, R01EY016058, and P30EY004068

Program Number: 5211 Poster Board Number: C0130
Presentation Time: 2:45 PM - 4:30 PM

Galectin-1-Mediated Suppression of Th17 Cell-Induced Corneal Immunopathology post Pseudomonas aeruginosa Infection Amol Suryawanshi1, Zhiyi Cao1, Tanveer S. Zaidi2, Noorjahann A. Panjwani2.
1New England Eye Center and Department of Ophthalmology, Tufts University School of Medicine, Boston, MA; 2Department of Medicine, Brigham and Women’s Hospital, Boston, MA.

Purpose: Although, past studies have indicated the critical role of CD4+ T cells, particularly the Th1 cells, in P. aeruginosa keratitis, the relative contribution of recently discovered Th17 and Treg cells is unknown. The goal of this study was to define the role of different CD4+ T cells subsets in P. aeruginosa-induced corneal immunopathology, and to characterize the role of a carbohydrate-binding protein, galectin-1 (Gal-1), in modulating the balance of various CD4+ T cells subsets and the associated P. aeruginosa-induced corneal pathology.

Methods: Corneal P. aeruginosa infected B6 mice corneas and local draining lymph nodes (DLN) were analyzed on day 5 and day 8 post infection (pi) for CD4+ T cells, Th1, Th17 and Treg cell composition by flow cytometry. In another set of experiments, infected B6 mice were treated with recombinant galectin-1 (rGal-1) or control vehicle by sub-conjunctival injections every alternate day starting from day 1 until day 11 pi. On day 12 pi, corneas and DLN were collected to analyze the effect of rGal-1 treatment on various CD4+ T cell subsets by flow cytometry and cytokine levels for IL-17A, IL-10 and IL-4 in the cornea and DLN by ELISA.

Results: Corneal P. aeruginosa infection induced a strong Th17 cell response as compared to Th1 response in the cornea and DLN at all tested days pi. Furthermore, there was induction of Treg response in the cornea and DLN, however, the relative proportion of Treg over Th17 diminished significantly as infection progressed to a more severe form. Administration of rGal-1 significantly diminished corneal pathology through multiple mechanisms. Accordingly, rGal-1 treated group showed significantly reduced infiltration of total leukocytes, neutrophils and CD4+ T cells in the cornea. Moreover, rGal-1 treatment significantly diminished proinflammatory Th17 response, whereas promoted antiinflammatory Treg and Th2 response in the cornea as well as DLN when compared to control vehicle treated mice.

Conclusions: Corneal P. aeruginosa infection induces both Teffector (Th17 dominant) and Treg response in the cornea. Moreover, increasing representation of antiinflammatory Treg and Th2 cells over Th17 cells using endogenously derived molecules such as Gal-1 diminishes corneal pathology and represents a novel therapeutic approach to control bacterial keratitis, a common cause of vision loss and blindness in humans worldwide.

Commercial Relationships: Amol Suryawanshi, None; Zhiyi Cao, None; Tanveer S. Zaidi, None; Noorjahann A. Panjwani, None
Support: NIH Grant EY007088 (NP), Mass Lions Research Fund, Research to Prevent Blindness and New England Corneal Transplant Fund

Program Number: 5212 Poster Board Number: C0131
Presentation Time: 2:45 PM - 4:30 PM

In Vivo Efficacy of Keratin-Derived Antimicrobial Peptides (KDAMPs) in Corneal Defense Against Pseudomonas aeruginosa Connie Tam. School of Optometry, Univ of California, Berkeley, CA.

Purpose: We have shown that KDAMPs are novel antimicrobials constitutively expressed by corneal epithelial cells. Synthetic analogs of KDAMPs kill various bacterial pathogens of both Gram types in vitro. Specifically, they are rapidly bactericidal and cytoprotective against P. aeruginosa. In contrast to most known antimicrobial peptides, KDAMPs are unaffected by physiological salt concentration. As knockdown of keratin 6A significantly increases bacterial growth in human corneal epithelial cell lysates and bacterial adherence to intact mouse corneas, here we tested the hypothesis that exogenous addition of KDAMPs can protect corneas against P. aeruginosa in vivo.

Methods: Corneas of anesthetized C57BL/6 MyD88 knockout mice (known to be impaired in epithelial defense) were blotted with tissue paper to enhance susceptibility to bacterial adhesion, rinsed with physiological saline (0.9% NaCl), then inoculated with 10⁵ cfu in 5 μl GFF expressing P. aeruginosa PAO1. Eyes were rinsed with saline 2 h post-inoculation to remove non-adherent bacteria, followed by administration (once every 2 h) of 5 μl saline eye drops containing 200 μg/ml synthetic 19mer of KDAMPs or its scrambled control peptide. Animals were sacrificed 5 h post peptide treatment. Enculeated eyes were rinsed with PBS and unprocessed corneas/bacteria were imaged by confocal microscopy using 633 nm (red) and 488 nm (green) respectively. Z stacks images (entire corneal thickness, 1 μm steps) were collected from >10 random fields and reconstructed in 3-D by Image J.

Results: Blotted and inoculated mouse eyes instilled with scrambled 19mer control peptide or vehicle control (saline) in vivo were found to have massive P. aeruginosa colonization. In contrast, those instilled with synthetic 19mer KDAMP had significantly reduced bacterial adhesion on the cornea (Fig 1).

Conclusions: KDAMPs (i.e. the 19mer) are anti-pseudomonal not only in vitro but also in vivo. Further studies will be needed to understand the extent of their contributions to innate defense of the cornea, and if they can protect against infection once ongoing. Such studies could lead to novel therapeutic agents to prevent and/or treat infections of the cornea and other sites.
The 19mer KDAMP, but not scrambled control peptide, was effective in killing *P. aeruginosa* (green) colonized on the cornea (red) and protecting it against bacterial adhesion in vivo.

**Commercial Relationships:** Connie Tam, “Antimicrobial Peptides and Methods of Use Thereof” (P)
**Support:** NIH Grant 1R01EY023000-01

**Program Number:** 5213 Poster Board Number: C0132
**Presentation Time:** 2:45 PM - 4:30 PM

**Proteomic Analysis of the Keratitis Associated *Pseudomonas aeruginosa***


**Purpose:** To compare the proteomic profile of a clinical isolate of *Pseudomonas aeruginosa* (*P. aeruginosa*) obtained from an infected cornea of a contact lens wearer (corneal strain) and a non-corneal strain, ATCC 10145.

**Methods:** Phenotypic assays of *P. aeruginosa* such as twitching motility, biofilm formation and antibiotic sensitivity tests were performed using standard methods. Whole protein lysates were analyzed by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) in triplicate and relative protein abundances were determined by spectral counting. G test followed by post hoc Holm Sidak was used for statistical analyses to determine significance in the differential expression of proteins between the two strains.

**Results:** LC-MS/MS revealed significant differences in protein composition between the two strains. A total of 585 proteins were detected. Among these 73 were up or down regulated in the corneal strain compared to ATCC10145 and 44 were detected only in the corneal strain. Proteins that were detected explicitly in the corneal strain were involved in different functional groups including cell motility and secretion, lipid metabolism, cell envelope biogenesis and ion transport and metabolism. In addition to a number of hypothetical proteins which had similarities to proteins involved in iron storage, type IV secretion associated lipoproteins, proteases and secreted cytotoxins. Proteins that were significantly upregulated in the corneal strain compared to the non-corneal strain included those involved in motility (flagellin type B, 21fold, p=0.0003), polyhydroxyalkanoate synthesis (PhaF, 11fold, p=0.02), chorismate biosynthesis (chorismate synthase, 8fold, p=0.007) and virulence (lipotoxin F, 3fold, p=0.004). The non ribosomal peptide synthetases (NRPS) were the fourth most abundant proteins in the corneal strain.

**Conclusions:** Results from this study confirm that the keratitis associated *P. aeruginosa* strain is pathogenic and expresses a unique protein profile indicative of phenotypic adaptations to its environment. Identification of the protein profile of the corneal strain of *P. aeruginosa* in this study will aid in the elucidation of novel intervention strategies to reduce the burden of *P. aeruginosa* infection in keratitis.

**Commercial Relationships:** Abby L. Sewell, None; Jeffrey J. Dunmire, None; Michael Wehmann, None; Rachida Bouhenni, None
**Support:** non

**Program Number:** 5214 Poster Board Number: C0253
**Presentation Time:** 2:45 PM - 4:30 PM

**Pseudomonas aeruginosa** uses type three secretion-dependent and -independent mechanisms for traversal of multilayered corneal epithelia

Aaron B. Sullivan1, Victoria Hritonenko2, Connie Tam1, David J. Evans2, Suzanne M. Fleiszitz1, 1School of Optometry, UC Berkeley, Berkeley, CA; 2College of Pharmacy, Touro University- California, Vellejo, CA.

**Purpose:** We previously reported that the type 3 secretion system (T3SS) of *Pseudomonas aeruginosa* can mediate corneal epithelial traversal by invasive strain PAO1 in susceptible mouse corneas. In vitro, we showed that cytotoxic strain PA14 uses its T3SS (specifically the toxin ExoU) to traverse human corneal epithelial cells. Here, we crossed over the models and examined invasive strain PAK, which hyper-expresses the T3SS in *vitro*.

**Methods:** In *vitro* traversal capacity was examined using air-lifted, filter-grown multilayered human corneal epithelial cells. Bacteria were added apically chamber and bacteria traversing to the basal chamber were collected and counted. For *in vivo* experiments, mouse corneas were PBS rinsed, blotted with tissue paper, and treated with 100 mM EGTA for 1 h at 37 °C to enable bacterial traversal. Corneas were inoculated with GFP-labeled *P. aeruginosa* (200 μl ~109 CFU/ml) strain PAK, PA14, or PA14 T3SS mutants including mutants lacking ExoU, all of its three known effectors, or the needle required for effector secretion) for 1.5, 3 or 6 h at 37°C. After rinsing with PBS, corneas were imaged using 2-photon (NADPH autofluorescence), and confocal reflection microscopy. Epithelial thickness and bacterial traversal were evaluated.

**Results:** In *vitro* assays with invasive strain PAO1 showed that the role of the T3SS decreased with incubation time: 4 h, 29 fold (p=2.32E-06); 6 h, 14 fold (p=1.58E-04); 8 h, 4 fold (p=6.53E-03). In *in vivo*, cytotoxic strain PA14 required the T3SS (specifically ExoU) to traverse at 1.5 and 3 h, but not by 6 h. Surprisingly, a wild-type invasive strain that hyper-secretes T3SS effectors in *vitro* (PAK), did not traverse at all in *vitro*, contrasting with the efficient T3SS dependent traversal capacity we have reported for a different invasive strain (PAO1) that secretes the same T3SS effectors much less well in *vitro*.

**Conclusions:** The data continue to support a role for T3SS effectors (e.g. ExoU) in *P. aeruginosa* traversal, but they also show that T3SS-independent mechanisms can mediate traversal at longer time points both *in vivo* (mouse) and *in vitro* (human cells) and for both cytotoxic and invasive strains. Why PAK does not traverse the corneal epithelium is unclear considering it is a T3SS hyper-secretor in *vitro*; possibilities include a different capacity for secretion in *vitro* or a lack of essential co-factors.

**Commercial Relationships:** Aaron B. Sullivan, None; Victoria Hritonenko, None; Connie Tam, "Antimicrobial Peptides and Methods of Use Thereof" (P); David J. Evans, U.S. Provisional Patent Application No. 61/479,507. (P), U.S. Issued Patent 7,332,470 B2 (P); Suzanne M. Fleiszitz, Allergan (C), Allergan (F), New methods for preventing infection (P)
**Support:** NEI RO1-EY011221, RO1-All079192, AAO Ezell Fellowship

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ExoS, a type III secreted toxin, is produced intracellularly by P. aeruginosa after corneal epithelial cell invasion, and is triggered by exposure to cell lysates

Victoria Hritonenko, Amber L. Jolly, Courtney Maloney, Allison Farfel, David J. Evans, Suzanne M. Fleischig, School of Optometry, University of California, Berkeley, CA; \(^1\)Dept. of Environmental Science, Policy, and Management, University of California, Berkeley, AR; \(^2\)Dept. of Molecular & Cell Biology, University of California, Berkeley, CA; \(^3\)College of Pharmacy, Touro University California, Vallejo, CA.

Purpose: We have previously shown that the type 3 secretion system (T3SS) of Pseudomonas aeruginosa contributes to the pathogenesis of P. aeruginosa keratitis. We have also shown that ExoS, one of the known T3SS effectors, is required for intracellular survival by P. aeruginosa. Known triggers for T3SS expression include low calcium conditions and contact with live mammalian cells. Here, we tested the hypothesis that the environment inside epithelial cells can trigger effector expression.

Methods: To localize ExoS using immunofluorescence microscopy, corneal epithelial cells were grown on glass coverslips, inoculated with \(10^7\) CFU/mL PAO1ΔΔΔexoSSTY + pUCpexoS-HA or a translocon mutant (PAO1ΔΔΔpUCpexoS-STY + pUCpexoS-HA) for 3 h, followed by gentamicin solution (4 h) to kill extracellular bacteria. ExoS was localized in samples using antibody to HA (tagged to ExoS). In other experiments, bacteria were exposed to epithelial cell lysates and ExoS expression measured by Western blot. Lysates were prepared using freeze-thaw cycles, and after removal of cell debris, were inoculated with \(\sim 10^7\) CFU/mL PAO1 for 12 h. Bacteria were then removed by centrifugation, and supernatants examined and compared to uninoculated control lysate. The impact of challenge with bacterial supernatant (containing bacterial antigens) prior to lysate preparation was also explored.

Results: ExoS was found in the host cytosol of P. aeruginosa infected cells, even with a translocon mutant that cannot inject effectors across host cell membranes. ExoS expression was induced by exposure to cell lysates prepared in 300 µl PBS (20 ± 9.7%) compared to low calcium inducing conditions, but not when the lysate was diluted 3-fold. ExoS was found associated with lysate supernatants, not bacterial pellets. Intriguingly, the size of the band recognized by the ExoS antibody increased (by ~ 4 kDa) when lysates were prepared from cells pre-exposed to bacterial antigens.

Conclusions: The data show that P. aeruginosa expresses ExoS intracellularly after it invades corneal epithelial cells. The induction of ExoS expression by epithelial cell lysates suggests biochemical triggers for expression, which can now be identified. The significance of the increased size of ExoS when triggered by lysates from cells pre-exposed to bacterial antigens is to be determined.

Commercial Relationships: Victoria Hritonenko; None; Amber L. Jolly; None; Courtney Maloney; None; Allison Farfel; None; David J. Evans; U.S. Provisional Patent Application No. 61/479,507. (P), U.S. Issued Patent 7,332,470 B2 (P); Suzanne M. Fleischig, Allergan (C), Allergan (F), New methods for preventing infection (P)

Support: NIH Grant 1F32EY020111 to VH, NIH Grant AI079192 to SMJF

Program Number: 5216 Poster Board Number: C0135
Presentation Time: 2:45 PM - 4:30 PM
VIP Treatment of Bacterial Keratitis Against Multiple Pseudomonas Strains

Elizabeth A. Berger, Linda D. Hazlett, Anatomy & Cell Biology, Wayne State Univ Sch of Med, Detroit, MI.

Purpose: Studies from our laboratory have demonstrated the efficacy of vasoactive intestinal peptide (VIP) treatment in regulating inflammation following bacterial keratitis induced by P. aeruginosa (PA) ATCC strain 19660. However, we assessed whether these effects are specific to 19660 (a cytotoxic strain) or if VIP treatment would be just as effective against multiple strains of PA. As such, two additional strains were tested - PAO1 (ATCC 15692), an invasive strain and KEI 1025, a clinical isolate.

Methods: C57BL/6 (B6) mice received daily IP injections of VIP (5 nM in 100 µL) from -1 through 7 days p.i. Control mice were similarly injected with PBS. Mice were infected with PA 19660, PAO1 or KEI 1025. Whole corneas were graded by clinical score and disease response was documented using a slit-lamp. Real-time RT-PCR and ELISA were used to assess the effects of VIP treatment on cytokine/chemokine production and enzymes associated with specialized pro-resolving mediator (SPM) production. Bacterial plate counts, MPO and Greiss assays were performed to examine host inflammatory cell function.

Results: VIP treatment converted the susceptible response to resistant for all three strains of PA tested. Clinical scores were significantly improved and corneal perforation was averted. Further analysis revealed that corneas of VIP treated mice had significantly increased levels for TGF-β and IL-10; while pro-inflammatory molecules (IL-1β, TNF-α and CXCL2) were significantly down-regulated when compared to controls. Furthermore, enzymes associated with SPM formation (12-LOX, 15-LOX, COX-2) were disparately expressed after VIP treatment against PA 19660, PAO1 and KEI 1025 compared to PBS treated animals.

Conclusions: VIP treatment is effective at ameliorating disease pathogenesis for all three PA strains tested as indicated by cytokine/chemokine expression and host inflammatory cell function. In addition, this study is the first to indicate a possible role for VIP in driving SPM expression and subsequent disease resolution. In summary, the data from this study further strengthen the preclinical development of VIP as a therapeutic for ocular infectious disease.

Commercial Relationships: Elizabeth A. Berger; None; Linda D. Hazlett; None
Support: NIH Grants R01EY02986 and P30EY004068

Program Number: 5217 Poster Board Number: C0136
Presentation Time: 2:45 PM - 4:30 PM
IL-1R and TLR-5 mediate corneal epithelial defense against Pseudomonas aeruginosa colonization and traversal respectively

David J. Evans, Connie Tam, Suzanne M. Fleischig, College of Pharmacy, Touro University California, Vallejo, CA; \(^2\)School of Optometry, UC Berkeley, Berkeley, CA.

Purpose: We previously reported that corneal epithelial defense against P. aeruginosa traversal is MyD88-dependent. MyD88 is an adaptor molecule required for many of the signaling events mediated by Toll-like receptors (TLRs) and the Interleukin-1 receptor (IL-1R). To decipher the molecular mechanisms involved in MyD88-dependent protective activity, we tested the hypothesis that one or more of these receptors is critical for host defense against bacterial traversal.

Methods: Ex vivo whole eyeballs of C57BL/6 wild-type (control) and single gene (IL-1R, TLR-5 or TLR-7) knockout mice were rinsed with PBS, blotted with tissue paper on the corneal surface to enable susceptibility to bacterial adhesion (or were not blotted), followed by 6 h incubation at 35 °C in \(10^7\) cfu/mL GFP-expressing P. aeruginosa PAO1 then imaged by confocal microscopy. Corneal cells of the unprocessed whole eyeballs and bacteria were visualized using reflection of 633 nm and 488 nm confocal lasers respectively. Z stacks (entire corneal epithelial thickness, 1.0 µm steps) were made.
collected from ≥3 random fields/eye. 3-D image reconstruction was performed by Image-J.

**Results:** Bacteria did not adhere to wild-type or TLR-7 knockout mouse corneas unless they were blotted prior to inoculation. In contrast, bacteria bound to non-blotted IL-1R knockout corneas, with partial penetration into the cornea also occurring if corneas were blotted. Blotted TLR-5 knockout mouse corneas showed deep bacterial traversal through to the basal lamina. Non-blotted TLR-5 knockout mouse corneas showed little or no bacterial adherence.

**Conclusions:** The data suggest that the IL-1R is involved in preventing *P. aeruginosa* adhesion to the intact corneal epithelium, while TLR-5 primarily contributes to host defenses against bacterial traversal after adhesion. In contrast, TLR-7, an intracellular receptor known to recognize single stranded viral RNA, is not required for preventing bacterial colonization or traversal. The effectors downstream of the IL-1R and TLR-5 involved in protecting the healthy mouse corneal epithelium against *P. aeruginosa* are to be determined.

**Commercial Relationships:** Ahmad Elsahn, None; Parvez Hossain, None; Myron Christodoulides, None

**Support:** British Council for the Prevention of Blindness

**Program Number:** 5219 Poster Board Number: C0137
**Presentation Time:** 2:45 PM - 4:30 PM

**Cystic Fibrosis Transmembrane conductance Regulator (CFTR) competes with *Pseudomonas aeruginosa* Type 3 Secretion System (T3SS) to direct the fate of intracellular bacteria**


**Purpose:** *Pseudomonas aeruginosa*, a leading cause of corneal infections, is able to enter and replicate within corneal epithelial cells. The T3SS of *P. aeruginosa* is necessary for intracellular bacterial survival within membrane blebs. CFTR is a receptor for *P. aeruginosa*; mutations in CFTR result in defective internalization and are highly correlated with chronic *Pseudomonas* lung infections in Cystic Fibrosis (CF). Here, we test the hypothesis that CFTR functions to promote intracellular survival.

**Methods:** Telomerase-immortalized human corneal epithelial cells treated with DMSO or CFTR inhibitor-172 to block chloride channel function were infected with *P. aeruginosa* invasive strain PAO1. Telomerase-immortalized human bronchial epithelial cells from a healthy patient and a CF patient (AF508/ AF508 CFTR) were compared before and after infection with wild-type PAO1 and T3SS mutants. Infected cells were analyzed for bleb formation, and LysoTracker® dye used to selectively label acidic organelles. Cell death and apoptotic status were determined using propidium iodide staining and an annexin V FITC conjugate, respectively. Gentamicin exclusion assays were used to determine bacterial internalization and survival.

**Results:** CFTR-deficient infected bronchial epithelia displayed an enhanced blebbing phenotype (median bleb area is 440 µm² in CFTR-deficient cells versus 138 µm² in controls, p < 0.0001). Similar results were observed in corneal epithelial cells following treatment with CFTR inhibitor. CFTR-deficient cells also showed ~2 fold host cell death and innate responses were analysed by LDH release and cytokine measurement.

**Commercial Relationships:** Ahmad Elsahn, None; Parvez Hossain, None; Myron Christodoulides, None

**Support:** British Council for the Prevention of Blindness

**Program Number:** 5219 Poster Board Number: C0137
**Presentation Time:** 2:45 PM - 4:30 PM

**Microbiological and electron microscopic assessment of *Pseudomonas aeruginosa* infection of primary human corneal fibroblasts and epithelial cells in-vitro**

*Ahmad Elsahn*1,2, Parvez Hossain1, Myron Christodoulides1

1Molecular Microbiology, University of Southampton, Southampton, United Kingdom; 2Southampton Eye Unit, University Hospitals Southampton NHS Foundation Trust, Southampton, United Kingdom.

**Purpose:** To test the hypothesis that *Pseudomonas aeruginosa* sp. invasion of human corneal epithelial cells (HCEC) and fibroblasts (CF) contributes to the pathology of microbial keratitis.

**Methods:** Primary HCEC and CF were extracted from clinical samples from several patients, cultured separately to confluence in vitro and infected with various doses of *Pseudomonas aeruginosa* strain PAO-1. Total bacterial association was quantified at 15 and 30 min and at 1, 3, 6 and 9 h using the saponin-lysis and viable counting method. Invasion was quantified using the standard gentamicin exclusion assay. Scanning and transmission electron microscopy (TEM/SEM) were used to confirm bacterial attachment and invasion. Pathogen-induced

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greater intracellular bacterial replication over 2-3 h compared to healthy epithelial cells (p < 0.02). Increased blebbing and bacterial survival in CFTR-deficient cells required the T3SS. CFTR-deficient cells displayed similar lysosome morphology and pH to control cells. However, 78% of blebbing cells were devoid of acidic vacuoles, independent of CFTR status, suggesting a relationship between bleb formation and bacterial modification of lysosomes. Blebbing cells lacked apoptotic markers and resisted infection-induced cell death. Conclusions: CFTR functions to inhibit P. aeruginosa intracellular survival, independent of a role for CFTR in lysosome acidification. Rather, enhanced intracellular survival in CFTR-defective cells appears to be related to the T3SS-induced blebbing ability in these cells.

Commercial Relationships: Amber L. Jolly, None; Sarah A. Whiteside, None; David J. Evans, U.S. Provisional Patent Application No. 61/479,507, (P), U.S. Issued Patent 7,332,470 B2 (P); Suzanne M. Fleischig, Allergan (C), Allergan (F), New methods for preventing infection (P)

Support: R01-A1079192

Program Number: 5220 Poster Board Number: C0139

Presentation Time: 2:45 PM - 4:30 PM

Impediment of corneal cell migration by a Serratia marcescens factor in an in vitro wound healing model
Kimberly Brothers, Nicholas A. Stella, Jes Klarlund, Robert M. Shanks. University of Pittsburgh, Pittsburgh, PA.

Purpose: To examine the effects of S. marcescens secreted factors on corneal wound healing.

Methods: Human corneal limbal epithelial (HCLE) cells were grown to confluence and subsequently stratified in 12-well plates with agarose strips. Bacterial secreted fractions (BSF) of S. marcescens, Escherichia coli and Pseudomonas aeruginosa were grown over night in LB medium, normalized by optical density, and filter sterilized. Upon removal of agarose strips to simulate a wound, HCLEs were recruited (0 cells). The other proteolytic fraction containing proteins of 34 and 30 kDa. The >170 kDa protein (0.6 µg/cornea) was filter sterilized and concentrated. ECM was each injected into rabbit corneal stromas, and corneal damage assessed at 8, 24, and 48 hours. ECM was tested for cytotoxicity of human corneal epithelial cells (HCECs), hemolysis of red blood cells, and protease activity against casein. This ECM was subjected to anion exchange and gel filtration chromatography, and fractions were tested for protease activity, corneal damage, and induction of neutrophil recruitment. All proteins were detected by SDS-PAGE and silver staining.

Results: Forty µg of ECM from K1263APLY, but not heat-treated ECM, produced protease activity in vitro, corneal erosions in vivo by 8 hours, and corneal opacity by 48 hours. Cytotoxicity and hemolytic activity were not detected in vitro. Unbound material from anion exchange chromatography produced protease activity, corneal erosions, opacity, and neutrophil recruitment equivalent to 9,860 neutrophils/mL. Heat treatment abolished all activity and resulted in recruitment of 6,826 neutrophils/mL. Gel filtration produced two fractions with protease activity and ability to form corneal erosions at concentrations of 0.6 µg and 1.9 µg per cornea, which were abolished by heat treatment. One of these fractions contained one detectable protein of >170 kDa and the other fraction contained two detectable proteins of 34 and 30 kDa. The >170 kDa protein (0.6 µg/cornea) caused neutrophil recruitment (9,100 cells/mL), which was abolished by heat treatment (0 cells). The other proteolytic fraction containing the 34 and 30 kDa proteins (1.9 µg/cornea) did not have the ability to recruit neutrophils (0 cells).

Conclusions: S. pneumoniae produces at least two previously unreported proteases that cause corneal erosions. One of these proteases can also recruit neutrophils to the cornea and cause opacity. Future work will entail identification of these novel corneal virulence factors.

Commercial Relationships: Mary E. Marquart, None; Sidney D. Taylor, None

Support: NIH Grant EY016195

Program Number: 5222 Poster Board Number: C0141

Presentation Time: 2:45 PM - 4:30 PM

Isolation of Two Proteases from Streptococcus pneumoniae that Cause Corneal Erosions
Mary E. Marquart, Sidney D. Taylor. Microbiology, Univ of Mississippi Med Ctr, Jackson, MS.

Purpose: Previous work has shown that pneumolysin-deficient strains of Streptococcus pneumoniae retain virulence in the cornea. This study was undertaken to isolate other virulence factors involved in this disease.

Methods: S. pneumoniae K1263APLY, a pneumolysin-deficient strain, was used in this study and grown in Todd Hewitt broth containing yeast extract (THY). Bacterial cells grown overnight were removed by centrifugation, and the resulting extracellular milieu (ECM) was filter-sterilized and concentrated. ECM and heat-treated ECM were each injected into rabbit corneal stromas, and corneal damage assessed at 8, 24, and 48 hours. ECM was tested for cytotoxicity of human corneal epithelial cells (HCECs), hemolysis of red blood cells, and protease activity against casein. This ECM was subjected to anion exchange and gel filtration chromatography, and fractions were tested for protease activity, corneal damage, and induction of neutrophil recruitment. All proteins were detected by SDS-PAGE and silver staining.

Results: Forty µg of ECM from K1263APLY, but not heat-treated ECM, produced protease activity in vitro, corneal erosions in vivo by 8 hours, and corneal opacity by 48 hours. Cytotoxicity and hemolytic activity were not detected in vitro. Unbound material from anion exchange chromatography produced protease activity, corneal erosions, opacity, and neutrophil recruitment equivalent to 9,860 neutrophils/mL. Heat treatment abolished all activity and resulted in recruitment of 6,826 neutrophils/mL. Gel filtration produced two fractions with protease activity and ability to form corneal erosions at concentrations of 0.6 µg and 1.9 µg per cornea, which were abolished by heat treatment. One of these fractions contained one detectable protein of >170 kDa and the other fraction contained two detectable proteins of 34 and 30 kDa. The >170 kDa protein (0.6 µg/cornea) caused neutrophil recruitment (9,100 cells/mL), which was abolished by heat treatment (0 cells). The other proteolytic fraction containing the 34 and 30 kDa proteins (1.9 µg/cornea) did not have the ability to recruit neutrophils (0 cells).

Conclusions: S. pneumoniae produces at least two previously unreported proteases that cause corneal erosions. One of these proteases can also recruit neutrophils to the cornea and cause opacity. Future work will entail identification of these novel corneal virulence factors.

Commercial Relationships: Mary E. Marquart, None; Sidney D. Taylor, None

Support: NIH Grant EY016195

Program Number: 5222 Poster Board Number: C0141

Presentation Time: 2:45 PM - 4:30 PM

Genomics of the Conjunctival Pathogen Streptococcus pneumoniae
Michael Valentino1, Wolfgang Haas2, Christine M. Sanfilippo2, Jason W. Rosch3, Elaine I. Tuomanen1, Timothy W. Morris4, Michael S. Gilmore1. 1Harvard Medical School / Mass. Eye & Ear Infirmary, Boston, MA; 2Bausch & Lomb, Inc., Rochester, NY; 3St. Jude Children's Research Hospital, Memphis, TN.

Purpose: Purpose: Streptococcus pneumoniae is a prevalent cause of bacterial conjunctivitis, especially in children. One bacterial lineage in particular, ST448, has caused multiple outbreaks of conjunctivitis and was recently shown by our group to be a major contributor to non-outbreak related ocular infections throughout the US. These findings suggest that some lineages of S. pneumoniae possess a unique ocular tropism and are capable of spawning epidemics. In the

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Methods: Genomes of 6 S. pneumoniae isolates from conjunctival infection (two each from outbreaks in New Hampshire, Maine and Minnesota) were sequenced, assembled and compared to the genomes of other S. pneumoniae from other types of infection.

Results: Comparitive analysis shows that ST448 strains possess only about 80% of the gene content of TIGR4. Absent from ST448 are genes for capsular biosynthesis and surrounding genetic elements, as well as ~ 25kb of the TIGR4 genome putatively encoding a cell wall surface anchor protein, sortases, a transcriptional regulator and cellobiose biosynthesis machinery. Metabolic reconstruction of the ST448 draft genome and comparison to TIGR4 indicates that various metabolic subsystems do not occur in ST448. Additionally, several bacteriocin operons also were not found. ST448 includes an additional 360kb of sequence, with about 1/3 of that related to more distant S. pneumoniae strains, and the remainder being derived from either non-pneumococcal species or lacking nucleotide homology to any other genes in GenBank.

Conclusions: Conclusion: The genome landscape content of an ST448 strain is substantially different from even the nearest strain, and possesses a number of genes with the potential to contribute to its prevalence in outbreak and non-outbreak infections and tropism for the ocular surface of sequence type 448 S. pneumoniae.

Commercial Relationships: Michael Valentin0, Bausch & Lomb (F); Wolfgang Haas, Bausch & Lomb, Inc. (E); Christine M. Sanfilippo, Bausch & Lomb, Inc. (E); Jason W. Rosch, None; Elaine I. Tuomanen, None; Timothy W. Morris, Bausch & Lomb, Inc. (E); Michael S. Gilmore, Bausch & Lomb (F)

503 Posterior Segment Inflammation II
Thursday, May 09, 2013 8:30 AM-10:15 AM
Exhibit Hall Poster Session
Program #/Board # Range: 5368-5398/A0001-A0031
Organizing Section: Immunology/Microbiology
Contributing Section(s): Biochemistry/Molecular Biology

Program Number: 5368 Poster Board Number: A0001
Presentation Time: 8:30 AM - 10:15 AM
Pivotal roles of EB13 for the initiation and maintenance of experimental autoimmune uveitis
Atsunobu Takeda1, Takeru Yoshimura1, Eiichi Hasegawa1, Sayaka Hirakawa1, Toshio Hisatomi1, Koh-Hei Sonoda1, Tatsuro Ishibashi2
1Ophthalmology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; 2Ophthalmology, Graduate School of Medicine, Yamaguchi University, Ube, Japan.

Purpose: Epstein Barr induced gene 3 (EB13), one of the components of IL-27, is a heterodimeric inflammatory cytokine. Recently, EB13 also has been reported to be one of the components of IL-35 which mediates proliferation of CD4+CD25+ regulatory T cells (Tregs). We have reported that IL-27 Receptor plays important roles in Th1 induction in EAU. In this study, we investigated the role of EB13 in experimental autoimmune uveitis (EAU).

Methods: Mice (either wild-type (WT) or EB13-deficient (EB13 KO)) were immunized with interphotoreceptor retinoid-binding protein (IRBP) peptide 1-20 previously described. Severity of EAU was evaluated clinically and histopathologically. The induction of IRBP-specific cytokines in the draining lymph node was assessed by ELISA and flow cytometry. The frequency of Tregs was examined by flow cytometry.

Results: The clinical score of EAU in EB13 KO in the early phase was diminished as compared with that in WT. Consistently, histological analysis revealed that significant reduction of cellular infiltration into the retina was seen in EB13 KO. The induction of IFN-γ by draining lymph node cells from EB13 KO on day9 was less than that from WT, suggesting that EB13 plays important roles in the induction of Th1. However, compared with WT mice, the score reached to the same level in EB13 KO on day 22 and comparably diminished. The production of IL-17 by draining lymph node cells from EB13 KO on day9 was the same as compared with that in WT, but IL-17 production in EB13 KO on day16 was increased as compared with that in WT. There was no difference of IL-10 and Foxp3 expression, which is concerned with CD4+ Tregs, between WT and EB13 KO.

Conclusions: These results indicate that EB13 may affect Th1 response in the initiation phase and that EB13 may suppress Th17 in the late phase independently of CD4+ Tregs.

Commercial Relationships: Atsunobu Takeda, None; Takeru Yoshimura, None; Eiichi Hasegawa, None; Sayaka Hirakawa, None; Toshio Hisatomi, None; Koh-Hei Sonoda, None; Tatsuro Ishibashi, None
Support: JSPS KAKENHI Grant Number 23689071 (grant-in-aid for Young Scientists (A)

Program Number: 5369 Poster Board Number: A0002
Presentation Time: 8:30 AM - 10:15 AM
The effect of light on retinal structure and the development of EAU in the transgenic spontaneous model of uveitis
Clare L. Corbett, Elizabeth Muckersie, John V. Forrester
Ophthalmology, University of Aberdeen, Aberdeen, United Kingdom.

Purpose: The transgenic model of spontaneous experimental uveitis (EAU) develops retinal inflammation characterized by infiltration of the retina by T-cells, macrophages and dendritic cells. This model was generated by crossing IRBP-HELhi single transgenic mice on a B10.BR background, which express the protein hen egg lysozyme (HEL) as a novel self-antigen in the eye, with the 3A9 strain of mice that express HEL-specific T-cell receptors. The single and double Tg mice both display unusual clinical and histological retinal degenerative disease that includes patchy chorioretinal atrophic lesions, occasional occluded blood vessels and extensive photoreceptor loss in the inferior retina. The mice are negative for the retinal degeneration genes rd1, rd8 and rd11. The purpose of this study was to investigate the role of light damage in this retinal degeneration.

Methods: To study the effect of ambient lighting conditions, non-transgenic, 3A9, IRBP-HELhi single and double Tg mice were maintained for 30 and 60 days in high lighting (150 lux) or low lighting (5 lux) conditions. Eye health and EAU development were assessed for each genotype and time-point by clinical fundoscopic grading (n=6). Eyes were harvested, SNAP frozen in OCT and 8-μm sections stained for the presence of macrophages, dendritic cells, B-cells and CD4+ T-cells. Sections were counterstained with haematoxylin and disease severity graded histologically.

Results: Both single IRBP-HELhi Tg and double Tg mice demonstrated inferior retina photoreceptor loss to a similar degree in both the high light and low light conditions. No photoreceptor loss was observed in the non-transgenic or 3A9 mice strains. The clinical EAU scores of the double Tg mice showed no difference between the high and low light conditions at 30 days with active inflammation in both the inferior and superior retina. Inflammatory cells were not observed in the eyes of single IRBP-HELhi Tg mice in any

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Conclusions: Photoreceptor loss is a feature in single and double Tg mice in both high and low light conditions. In the IRBP-HELh strain single Tg mice photoreceptor loss is not a consequence of immune cell induced damage. As ten percent of transgenic animals have altered phenotypes associated with transgene insertion, the insertion site of the transgene construct in the IRBP-HELh strain genome may have disrupted genes involved in retinal structure.

Commercial Relationships: None; Elizabeth Muckersie, None; John V. Forrester, None

Support: Saving Sight in Grampian, Development Trust, University of Aberdeen

Program Number: 5370 Poster Board Number: A0003
Presentation Time: 8:30 AM - 10:15 AM
Comparison of Pharmacokinetic profiles of Sirolimus following Subconjunctival or Intravitreal Administrations in Rabbits and Humans
Masaaki Kageyama1, Joel Naor2, Hitomi Takanaga1, Laura J. Wilson2, Nenina Ihekoromadu4, Afsheen A. Khwaja5, Sri Mudumba2.
1Research & Development, Santen Inc, Emeryville, CA; 2Global Clinical Development & Medical Affairs, Santen Inc, Emeryville, CA.

Purpose: To compare pharmacokinetic (PK) profiles of a proprietary sirolimus depot-forming ocular formulation (DE-109; currently in clinical trials for Posterior Uveitis. www.clinicaltrials.gov; NCT01358266) following subconjunctival (SCJ) or intravitreal (IVT) injection in rabbits and humans.

Methods: New Zealand White (NZW) rabbits were injected SCJ or IVT in both eyes with the formulation. Each group of 3 animals received one SCJ injection of 66, 220, or 660 μg/eye vs. an IVT injection of 22, 66, or 220 μg/eye, in 2 separate studies. Ocular tissues and whole blood (WB) were taken at multiple time points post-injection. Eyes were enucleated, frozen, and dissected to separate ocular tissues. WB samples were obtained at each time point prior to euthanasia. sirolimus concentrations were measured using LC/MS/MS. In a separate human study of Age-related Macular Degeneration (ARMD), 10 patients received three SCJ (1320 μg) or IVT (352 μg) injection in one eye bimonthly (NCT 00712491). WB samples were collected at similar time points for quantification of sirolimus concentrations using LC/MS/MS.

Results: SCJ delivery of sirolimus in NZW rabbits following a single injection was characterized by a distribution gradient of sirolimus concentration order: Sclera > Retina/Choroid > VH > WB. In contrast, IVT delivery showed ocular distribution order: VH > Retina/Choroid > Scera > WB. 3-days post-SCJ injection of 660 μg/eye (1320 μg total), sirolimus concentrations of 14, 0.37, 0.014 and 0.01 μg/g or mL) were detected in Sclera, Retina/Choroid, VH, and WB, respectively. 3-days post-IVT injection of 220 μg/eye (440 μg total), sirolimus concentrations of 390, 1.6, 0.01 and 0.004 μg/g or mL) were detected in VH, Retina/Choroid, Sclera, and WB, respectively.

In ARMD study, the highest sirolimus blood levels Cmax were (IVT: <2 ng/mL vs. SCJ: <10 ng/mL) at Day 2 and half-life t1/2 (IVT: 8-9 days vs. SCJ: 3-4 days).

Conclusions: Compared with SCJ delivery, the IVT route of administration showed an ocular distribution gradient more consistent with the intended preferential release of the drug from the depot to the retina. Both routes showed low systemic exposure.

Commercial Relationships: Masaaki Kageyama, Santen Inc (E); Joel Naor, Santen Inc (E); Hitomi Takanaga, Santen Inc (E); Laura J. Wilson, Santen Inc (E); Nenina Ihekoromadu, Santen Inc. (E); Afsheen A. Khwaja, Santen Inc. (E); Sri Mudumba, Santen, Inc (E).

Clinical Trial: NCT 00712491

Program Number: 5371 Poster Board Number: A0004
Presentation Time: 8:30 AM - 10:15 AM
Regulatory Effect of Interleukin 37 in Behçet’s disease
Zi Ye1, Chaokui Wang1, Aize Kijlstra1, Peizeng Yang1. 1The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Lab of Ophthalmology, Chongqing Eye Institute, Chongqing, China; 2University Eye Clinic Maastricht, Maastricht, Netherlands.

Purpose: Interleukin 37 has been found to have significant regulatory role in innate immune response. This study was to determine its role in the pathogenesis of Behcet’s disease (BD).

Methods: IL-37 mRNA expression in peripheral blood mononuclear cells (PBMCs) from BD patients and normal controls were measured by RT-PCR. PBMCs and monocyte-derived Dendritic Cells (DCs) were cultured with or without IL-37. The levels of cytokines in the supernatants of PBMCs and DCs were measured by ELISA. The IL-37R (IL-18Rα) expression, DCs surface markers, reactive oxygen species (ROS) production and mitogen-activated protein kinase (MAPK) activation were measured by flow cytometry. The effect of IL-37-treated DCs on the development of CD4+ T cells was measured by ELISA and flow cytometry.

Results: IL-37 mRNA expression was significantly decreased in PBMCs from active BD patients compared with inactive BD patients and normal controls. PBMCs and DCs stimulated with IL-37 showed a decreased expression of IL-6, IL-1β and TNF-α, and a higher production of IL-27. rIL-37 inhibited the production of ROS by DCs and reduced the activation of ERK1/2, JNK and P38 MAPK in DCs. rIL-37-treated DCs inhibited Th1 and Th1 cell response as compared with control DCs. However, rIL-37 did not have any influence on DCs surface markers (CD40, CD86, CD80 and HLA-DR) and IL-10 production by PBMCs or DCs.

Conclusions: This study showed that decreased IL-37 expression in active BD patients could trigger the production of pro-inflammatory cytokine and ROS in association with activation of Th1 and Th17 cells by DCs. These results collectively suggest that down-regulated IL-37 may be involved in the pathogenesis of BD.

Commercial Relationships: Zi Ye, None; Chaokui Wang, None; Aize Kijlstra, None; Peizeng Yang, None

Support: National Basic Research Program of China (973 Program) (2011CB510200)

Program Number: 5372 Poster Board Number: A0005
Presentation Time: 8:30 AM - 10:15 AM
Efficacy of Rebamipide in an Endotoxin-Induced Uveitis Model
Akira Takamaya, Harumasa Yokota, Akito Shimouchi, Akitoshi Yoshida. Ophthalmology, Ashihara Medical University, Ashiawa, Japan.

Purpose: To determine if rebamipide treatment suppresses the retinal inflammatory response in an endotoxin-induced uveitis (EIU) murine model by injection of lipopolysaccharide (LPS).

Methods: Uveitis was induced by intraperitoneal injection of LPS (Sigma Aldrich) in adult wild-type mice 24 hours after a sub-Tenon injection of 2% rebamipide ophthalmic suspension (rebamipide group) or phosphate buffered saline (control group). Retinal cross-sections were prepared on days 1, 3, and 7 after LPS treatment, and immunohistochemistry was performed using anti-glial fibrillary acidic protein (GFAP) antibody, anti-phosphorylated ERK antibody, and anti-phosphorylated STAT3 antibody to histologically determine the efficacy of rebamipide in the EIU model.

Results: The GFAP expression increased in both groups on days 1
and 3 after LPS treatment, but the expression in the rebamipide group was suppressed in the retina compared with that in the control group. However, phosphorylation of EPK and STAT3 in the retina was seen in both groups. The phosphorylation was also suppressed in the rebamipide group compared to the control group.

**Conclusions:** Our results suggested that rebamipide may play a role in suppression of the inflammatory response in the retina in an EIU murine model.

**Commercial Relationships:** Akira Takamiya, None; Harumasa Yokota, None; Akitoshi Yoshida, None

**Program Number:** 5373 Poster Board Number: A0006
**Presentation Time:** 8:30 AM - 10:15 AM

**Evidence-Based Analysis for the Medical Treatment of Behçet’s Disease**

**Philip I. Murray, Mohammed Mubin, Henry Knott, Bharat Markandey, Nikita Joji, Rahul Malhotra, Alastair K. Denniston.**

Academic Unit of Ophthalmology, University of Birmingham, Birmingham, United Kingdom.

**Purpose:** Behçet’s disease (BD) is a multisystem, remitting-relapsing, potentially blinding disease of unknown aetiology. The mainstay of treatment is systemic corticosteroid and immunosuppression. We wished to quantify the available levels of evidence for the treatment of BD with regards to the different systems involved.

**Methods:** We performed a Medline, EMBASE and CENTRAL literature search between 1975 - 2010 for papers on the treatment of BD. Inclusion criteria were: written in English, meta analyses, systematic reviews of RCTs, RCTs, cohort studies, case control studies, case series involving more than 20 patients, and expert opinion. Assessment of eligible studies included system involved, number of patients in study, level of evidence according to SIGN (Scottish Intercollegiate Group Network) criteria, and therapy used.

**Results:** From an initial scope of 2892 papers, 93 papers fulfilled the inclusion criteria. Only 25% were graded as SIGN 1 (meta-analyses, systematic reviews of RCTs, and RCTs). Treatments included corticosteroids, immunosuppressants and biologics. Many RCTs were poorly designed with small patient numbers and short follow up times. Just under 50% of the 93 studies included patients with ocular disease. Patients with orogenital ulceration and skin lesions comprised 31% and 23% of the 93 studies, respectively. Patients with musculoskeletal and vascular manifestations were each mentioned in about 15% of studies, with a paucity of studies on CNS disease.

**Conclusions:** BD has potentially sight and life threatening complications but the quality of current evidence for therapy is poor. A myriad of different treatments are being employed for numerous systemic manifestations.

**Commercial Relationships:** Philip I. Murray, None; Mohammed Mubin, None; Henry Knott, None; Bharat Markandey, None; Nikita Joji, None; Rahul Malhotra, None; Alastair K. Denniston, None

**Program Number:** 5374 Poster Board Number: A0007
**Presentation Time:** 8:30 AM - 10:15 AM

**Activation of the aryl hydrocarbon receptor (AhR) inhibits Th1 and Th17 cell immune response in Behçet’s disease**

**Peizeng Yang1, Chaokui Wang1, Zi Ye1, Aize Kijlstra2.**

1Ophthalm, The 1st Hosp, Chongqing Medical University, Chongqing, China; 2University Eye Clinic Maastricht, Maastricht, Netherlands.

**Purpose:** Recent studies show that AhR is involved in immune response. AhR exerts its role via interaction with its ligands, such as 6-formyldihydoro[3,2-b]carbazole (FICZ), and 2-(1’Hindole-3’-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE). In this study, we investigated the role of AhR activation by its endogenous ligands in the aberrant immune response of Behcet’s disease and the possible immunologic mechanisms involved.

**Methods:** AhR mRNA and protein expression in peripheral blood mononuclear cells (PBMCs) from active BD patients, inactive BD patients and normal controls was examined using RT-PCR and flow cytometry. The effect of FICZ and ITE on PBMCs, CD4+ T cells, DCs, retinal pigment epithelium (RPE) was detected by ELISA and flow cytometry. Flow cytometry was performed to study the mechanisms involved in the effect of FICZ and ITE on CD4+ T cells and DCs.

**Results:** The expression of AhR was significantly decreased in active BD patients as compared to inactive BD patients and normal controls. Both FICZ and ITE inhibited Th1 and Th17 polarization, and induced the expression of IL-22 by PBMCs and CD4+ T cells. The effect of FICZ and ITE on CD4+ T cells was associated with a decreased expression of RORC, IL-17, IL-23R, CCR6, decreased phosphorylation of STAT3, and increased phosphorylation of STAT5. FICZ- or ITE-treated DCs showed a decreased expression of co-stimulatory molecules including HLA-DR, CD80 and CD86, lower production of pro-inflammatory cytokines such as IL-1β, IL-6, IL-23 and TNF-α, and higher production of IL-10, in association with lower stimulation on Th1 and Th17 cell polarization. The inhibition of both ligands on DCs was mediated via suppressing the phosphorylation of P38 and JNK. AhR was also found to be expressed by RPE. Both FICZ and ITE could inhibit the IL-8 and IL-6 secretion by the RPE stimulated with TNF-α or LPS.

**Conclusions:** The present study suggests that a decreased AhR expression is associated with disease activity in BD patients. The activation of AhR either by FICZ or ITE showed a negative regulation role in the pathogenesis of BD as evidenced by its inhibition on Th1 and Th17 cell polarization, the maturation, differentiation and function of DCs, as well as the inflammatory cytokine secretion by RPE.

**Commercial Relationships:** Peizeng Yang, None; Chaokui Wang, None; Zi Ye, None; Aize Kijlstra, None

**Support:** Key Project of Natural Science Foundation 81130019

**Program Number:** 5375 Poster Board Number: A0008
**Presentation Time:** 8:30 AM - 10:15 AM

**Comparison of the Spectral Domain Optical Coherence Tomography Characteristics in Patients with Multifocal Choroiditis and Punctate Inner Choroidopathy**

**Roomasa Channa1, Jiangxia Wang2, Mohamed A. Ibrahim1, Jeong Lee1, Daniel Ferraz2, Yasir J. Sepahi1, Millena G. Bittencourt1, Raafay Sophie1, Elham Hafiz Naim1, Quan Dong Nguyen1.**

1Retinal Imaging Research and Reading Center, Johns Hopkins University, Wilmer Eye Institute, Baltimore, MD; 2Dana Center, Johns Hopkins University, Wilmer Eye Institute, Baltimore, MD.

**Purpose:** Multifocal choroiditis (MFC) and punctate inner choroidopathy (PIC) are posterior uveitic entities. Both diseases have retinal and choroidal changes on spectral domain optical coherence tomography (SD-OCT). In this study, we aimed to determine if PIC and MFC could be distinguished based on microstructural characteristics on SD-OCT.

**Methods:** Clinic charts and images of patients meeting the diagnosis of MFC or PIC were retrospectively reviewed in this cross-sectional study. Two masked graders independently evaluated lesions on SD-OCT for microstructural changes in retina or choroid, with arbitration by a senior grader in cases of disagreement. Retinal changes were identified in the retinal pigment epithelium (RPE) and photoreceptor inner-outer segment associated bands on SD-OCT (iSO). RPE
changes were classified as RPE elevation or RPE disruption. IS/OS changes were classified as IS/OS disruption or IS/OS intact. Generalized linear latent and mixed models were used to compare the different diagnoses and the occurrences of particular characteristics on SD-OCT. The models have the random effects of eyes nested within patients to take into consideration the possible correlation between different lesions in the same eye and fellow eye of the same subject.

**Results:** Twenty-six lesions from 8 eyes of 6 patients were included in the study. Sixteen lesions (62%) were from eyes with MFC; 10 lesions (38%) were from eyes with PIC. RPE changes were observed in 11 of 16 MFC lesions (69%) compared to 10 of 10 PIC lesions (100%). RPE elevation was identified in 6 of 10 PIC lesions (60%) compared to 3 of 16 MFC lesions (19%) (p = 0.063; OR= 7.09). RPE disruption was identified in 4 of 10 PIC lesions (40%) compared to 8 of 16 MFC lesions (50%) (p=0.53, OR=0.43). IS/OS disruption was identified in 4 of 10 PIC lesions (40%) compared to 15 of 16 MFC lesions (94%) (p = 0.029, OR= 0.04).

**Conclusions:** IS/OS disruption was significantly more frequently observed in MFC lesions versus PIC lesions. Our findings suggest that the two uveitic diseases may have distinctive characteristic on SD-OCT, which may help to distinguish between them. Additional studies to analyze more lesions from a larger number of patients are indicated to support our novel findings.

**Commercial Relationships:** Roomasa Channa, None; Jiangxia Wang, None; Mohamed A. Ibrahim, None; Jeong Lee, None; Daniel Ferraz, None; Yasir J. Sepah, None; Millena G. Bittencourt, None; Raafay Sophie, None; Elham Hatel Naimi, None; Quan Dong Nguyen, Genentech (F), Regeneron (F), Lux Biosciences (F), Abbott (F), GSK (F), Santen (F), Santen (C), Bausch and Lomb (C), Optos (F), Heidelberg Engineering (F)

**Support:** Wilmer research grant award; Wilmer Biostatistics Core Grant EY01765

**Program Number:** 5376 **Poster Board Number:** A0009
**Presentation Time:** 8:30 AM - 10:15 AM

**Fundus autofluorescence and birdshot chorioretinopathy: a study of 162 patients**

**Astrid Queant, Dominique Monnet, Antoine P. Brezin.** Cochin Hospital, Paris, France.

**Purpose:** To report the characteristics of fundus autofluorescence in birdshot chorioretinopathy (BCR).

**Methods:** Our study was based on the prospective follow-up of an open cohort of patients with BCR examined yearly in a standardized manner since 2002 as previously reported (Monnet et al., Am J Ophthalmol. 2006; 141:135-42). Autofluorescence (AF) imaging was performed as an additional test for patients examined in 2011. All patients had simultaneous acquisition of OCT and posterior pole AF imaging with the Heidelberg Retina Angiograph (HRA Spectralis®). The characteristics of AF were analyzed in the peripapillary area and at the posterior pole.

**Results:** 310 eyes of 162 patients (66 men (40.7%), mean age 59.1 ± 10.8 years) were included in the analysis. In 48 eyes AF images could not be obtained or were of insufficient quality to be analyzed. The average logMAR best corrected visual acuity (BCVA) was +0.18 ± 0.4 and the average central macular thickness was 290.7 ± 90 μm. In 137 (44.2%) eyes AF quality image was imperfect, mostly because of shadows caused by opacities anterior to the retinal plane [95 eyes, (30.6%)]. Abnormalities of AF images were observed in 295 (95.2%) eyes and peripapillary atrophy was the most common finding, seen in 256 (82.6%) eyes. Other findings were a hyperautofluorescent line at the border of the optic disc in 79 (25.5%) eyes, extended macular hypoafluorescence (73 eyes, 23.5%), heterogeneous macular AF (58 eyes, 18.7%), hypoafluorescent spots (134 eyes, 43.2%) with a prominent perivascular location (108 eyes, 34.8%) and vascular unsharpened edges (81 eyes, 26.1%). Abnormal macular AF was correlated with increased macular thickness (p=0.013) as analyzed by OCT and with decreased visual acuity (p<0.01). Perivascular AF showed many atrophic retinal pigment epithelium (RPE) lesions, which were linked to a history of vasculitis. In 63 (20.3%) eyes posterior pole (PP) hypoafluorescent spots did not coincide with the typical hypopigmented spots of BCR disease (seen in the PP in 94 (30.3%) eyes). Hypoafluorescent spots were located in areas of RPE atrophy as seen by fluorescein angiography in 100 (32.3%) eyes. Hypoafluorescent spots were located in areas of RPE atrophy as seen by fluorescein angiography in 100 (32.3%) eyes. Hypoafluorescent spots were located in areas of RPE atrophy as seen by fluorescein angiography in 100 (32.3%) eyes.

**Conclusions:** The heterogeneity of the clinical presentation of BCR was reflected by a spectrum of images observed by AF. AF may provide additional information useful for the monitoring of patients and may help to better understand the mechanisms of tissue damage in the disease.

**Commercial Relationships:** Astrid Queant, None; Dominique Monnet, None; Antoine P. Brezin, None

**Program Number:** 5377 **Poster Board Number:** A0010
**Presentation Time:** 8:30 AM - 10:15 AM

**Treatment Paradigms in Retinal Vasculitis**

**Jacqueline R. Busingye, Sergio Schwartzman, Thomas Flynn.** Ophthalmology, Mount Sinai School of Medicine, New York, NY; Rheumatology, Hospital for Special Surgery, New York, NY; Rheumatology, Weill Medical College of Cornell University, New York, NY.

**Purpose:** Currently there are no definitive treatment paradigms for retinal vasculitis complicated by vaso-obliterrative disease. Here we present 4 cases of patients exhibiting retinal vasculitis complicated by branch vein occlusion.

**Methods:** Retrospective chart review from 2009-2012 for diagnosis codes of retinal vasculitis. Charts of patients with this diagnosis were reviewed. Only those patients who had leakage of retinal blood vessels in addition to occlusive disease as manifested by angiographic and clinical evidence of venous occlusion were included in this analysis.

**Results:** 4 out of 4 patients were found to have an associated underlying systemic condition. One patient was diagnosed with SLE, one patient with sarcoid, one patient with psoriasis, and one patient with anklyosing spondylitis. All patients required treatment with prednisone and anti-metabolite therapy. 2 of the 4 patients were noted to have progressive disease activity despite prednisone and anti-metabolite therapy as manifested by new occlusive activity. In those patients, anti-TNF-alpha (infliximab) therapy was initiated. Following the change in medication regimen, those patients were stable for over a year without evidence of recurrent disease activity. All patients have retained stable or improved visual acuity from baseline without significant medication side effects.

**Conclusions:** Our small case series suggests a significantly higher rate of underlying systemic disease in patients presenting with retinal vasculitis complicated by vaso-occlusive disease than what has been reported in previous studies. Patients presenting with this clinical picture require aggressive immunosuppressive agents. Initial therapy may not offer optimal control in all patients. In this study, one patient with sarcoid, one patient with psoriasis, and one patient with anklyosing spondylitis. All patients required tr

**Commercial Relationships:** Jacqueline R. Busingye, None; Sergio Schwartzman, Abbott (C), Janssen (C), Pfizer (C), UCB (C), Genentech (C), Amgen (C); Jessica M. Ackert, None; Thomas Flynn, None

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Purpose: To determine quantitatively the thickness and volume of retinal and choroidal layers in fovea and macula in a series of Punctate Inner Choroidopathy (PIC) patients and determine their relationship with clinical data collected from medical records.

Methods: Enhanced depth imaging OCT images from patients diagnosed with PIC and quiescent status of disease were collected in two tertiary referral uveitis clinics at Moorfields Eye Hospital for 5 months. Quantitative analysis included retinal, choroidal, Sattler medium vessel layer and Haller large vessel layer thickness and volume determination with a custom OCT analysis software (OCTOR). Clinical data collected included age, gender, refractive status, type of disease (typical vs atypical), duration of disease and BCVA.

Results: 224 uveitic patients were screened, 46 patients diagnosed with PIC were identified, and 35 eyes (35 patients) had EDI-OCT images that met the inclusion criteria. Mean retinal thickness and volume was 256.04±32.9 µm and 10.16±1.41 mm³, mean choroidal thickness and volume was 210.52±103.44 µm and 8.13±3.60 mm³. High myopic PIC eyes presented significantly thinner choroid, Haller’s, and Sattler’s layers thickness and volume than low myopic eyes (p<0.001).

Refration was significantly correlated with both foveal and macular choroidal thickness (r:0.72, p<0.001; r:0.73, p<0.001) and choroid than atypical PIC (249.42±95.10 vs 177.77±100.98 µm, p:0.039). High myopic PIC eyes presented significantly thinner choroid, Haller’s, and Sattler’s layers thickness and volume than low myopic eyes (p<0.001). Refraction was significantly correlated with both foveal and macular choroidal thickness (r:0.72, p<0.001; r:0.73, p<0.001) and volume (r:0.72, p<0.001; r:0.60, p<0.001). In subgroup analysis, duration of disease was significantly correlated to retinal thickness and volume (r:-0.53, p:0.018; r:-0.47, p:0.040) in non-high myopic eyes. A strong significant correlation was observed between BCVA and retinal thickness (r:0.72, p<0.001; r:0.73, p<0.001) and volume (r:-0.40, p:0.030) and volume (r:-0.40, p:0.027) in fovea of PIC eyes

Conclusions: Typical PIC patients presented thicker retina and choroid than atypical cases. Refractive status appears as the main predictor of choroidal thickness and volume measures, and retinal values are correlated with BCVA and with duration of disease in non-highly myopic PIC eyes

Commercial Relationships: Noa Fernandez Ledo, None; Javier Zarranz-Ventura, None; Dawn A. Sim, None; Pearse A. Keane, None; Catherine A. Egan, Bayer (S), Oculogics (S), Novartis (S), Allergan (S), Novartis (F), Praveen J. Patel, Allergan (R), Bayer (C), Novartis UK (C), Heidelberg UK (R), Topcon UK (R), Thrombogenics (C); Mark C. Westcott, None; Richard W. Lee, Genentech (C); Adnan Tufail, Allergan (C), Bayer (C), GSK (C), Oculogics (C), Pfizer (C), Thrombogenics (C), Amakem (C), Heidelberg Engineering (R), Novartis/Alcon (C), Sanofi/Genzyme (C); Carlos E. Pavesio, None

Program Number: 5378 Poster Board Number: A0012
Presentation Time: 8:30 AM - 10:15 AM
Clinical course of a cohort of patients with Punctate Inner Choroidopathy managed with different modalities of treatment in North Scotland
Shiau Wei Wong1, P. Tyagi2, K. Khan1, S. Hewick1, Lucia Kuffova1.
1Aberdeen Royal Infirmary, NHS, Aberdeen, United Kingdom;
2Raigmore Hospital, NHS, Inverness, United Kingdom.

Purpose: The purpose of this study was to describe the clinical course of patients with PIC who received different combinations of treatments including observation, immunosuppression, photodynamic therapy (PDT), argon laser photoagulation and intravitreal anti-vascular endothelial growth factor (anti-VEGF) injections.

Methods: A total of 21 patients (24 eyes) with PIC were included in this study. Patients with clinical features suggesting neovascular membrane related to age related macular degeneration or high myopia (-6.00 diopter in affected eye) and with previous other ocular diagnoses were excluded. The following data was collected retrospectively which includes patient demographics (age, gender), presence of myopia, clinical presentation, ocular findings on slit-lamp examinations, episodes of occurrence of choroidal neovascular membrane (CNVM) in affected eye and different modalities of treatment patients received. The rate of recurrence of CNVM, baseline visual acuity and final visual acuity on the last follow up appointment were determined.

Results: Of 21 patients (24 eyes), the mean age at diagnosis was 45 years. 19 (90.5%) female and 2 (9.5%) were male. 12 patients (57.1%) were myopic. Mean duration of follow-up was 70 (range 1 to 285) months. Treatment received- 1) None- 2 eyes (8.3%), 2) Immunosuppression only- 4 (16.7%) eyes, 3) Immunosuppression + Anti-VEGF- 1 eye (4.2%), 4) Immunosuppression + PDT- 7 eyes (29.2%), 5) Immunosuppression + laser- 2 eyes (8.3%), 6) Immunosuppression + Anti-VEGF + Photodynamic Therapy- 8 eyes (33.3%). 21 eyes (87.5%) developed CNVM and 19 eyes (79.2%) developed recurrence of CNVM. Throughout the follow up period, 5 eyes (20.8%) maintained vision and 7 eyes (29.2%) showed improvement of vision.

Conclusions: Due to variable course of PIC and availability of
treatments, different combinations were given to achieve optimum outcome for patients in this study.

Commercial Relationships: Shiao Wei Wong, None; P. Tyagi, None; K. Khan, None; S. Hewick, None; Lucia Kuffova, None

Program Number: 5380 Poster Board Number: A0013
Presentation Time: 8:30 AM - 10:15 AM
Clinical Characteristics of Vogt-Koyanagi-Harada Disease with Choroidal Folds
Kouhei Hashizume¹, Yutaka Imamura², Takamitsu Fujiwara¹, Shigeki Machida², Masahiro Ishida², Daijiro Kurosaka², ³Ophthalmology, Iwate Medical University, Morioka, Japan; ²Ophthalmology, Teikyo University School of Medicine, Mizonokuchi Hospital, Kawasaki, Japan.

Purpose: To investigate the clinical characters of Vogt-Koyanagi-Harada disease (VKH) patients with choroidal folds.

Methods: Choroidal folds in eyes with VKH were observed using enhanced depth imaging optical coherence tomography. Clinical characteristics associated with the presence of choroidal folds were investigated.

Results: Among 30 patients with VKH (mean age: 49 years), 15 patients showed choroidal folds at acute phase. The age at onset of the patients with choroidal folds was significantly higher than those without choroidal folds (57.5 ± 11.7 years vs. 38.6 ± 12.9 years; t-test: p = 0.001). Choroidal thickness of the patients with choroidal folds was significantly larger than those without choroidal folds before high-dose steroid therapy (936 ± 160 μm vs. 811 ± 207 μm; Mann-Whitney U test: p = 0.004). The patients with choroidal folds had significantly thinner choroidal thickness at two weeks and one month after high-dose steroid therapy than those without choroidal folds. (362 ± 131 μm vs. 547 ± 219 μm; Mann-Whitney U test: p = 0.001, 330 ± 101 μm vs. 428 ± 143 μm; Mann-Whitney U test: p = 0.007, respectively).

Conclusions: VKH patients showing choroidal folds at acute phase appear older and to show thicker choroids than those without.

Commercial Relationships: Kouhei Hashizume, None; Yutaka Imamura, None; Takamitsu Fujiwara, None; Shigeki Machida, None; Masahiro Ishida, None; Daijiro Kurosaka, None

Program Number: 5381 Poster Board Number: A0014
Presentation Time: 8:30 AM - 10:15 AM
Clinical Outcomes of Vogt-Koyanagi-Harada Disease at a Tertiary Center in 194 Patients: The KKESH Uveitis Survey
Study Group
Abdulaziz A. Alrhushood¹ ² ³ 4, J. Fernando Arevalo¹ ² 3, Hassan A. Al Dhiabi¹, Yahya A. Al-Zahrani¹, Vishali Gupta¹, sulaiman M. Alsulaiman¹, Andres F. Lasave¹, Hanan N. Al-Shamsi¹, ³Retina and Vitreous, KKESH, Riyadh, Saudi Arabia; ²Retina and Vitreous, Wilmer Eye Institute, Baltimore, MD; ³Retina and Vitreous, Clínica de Oftalmología de Cali and Hospital Universitario del Valle, cali, Colombia; ⁴ophthalmology, Dammam university, dammam, Saudi Arabia.

Purpose: To describe ocular clinical characteristics, complications, surgical outcomes and treatment among patients with Vogt-Koyanagi-Harada (VKH) disease in a tertiary center over a 25-year period.

Methods: We retrospectively analyzed 194 patients (382 eyes) diagnosed with VKH disease in a tertiary center from January 1986 through December 2011.

Results: VKH disease was diagnosed at a median age of 35.1 ± 12.8 years (range 7 to 68 years), occurred in 135 (69.6%) females, and was bilateral in 188 (96.9%) patients. Mean baseline best-corrected visual acuity (BCVA) was 20/125 (logMAR 0.8 ± 0.72) in both eyes. Symptoms duration was short (< 3 months) in 110 (56.7%) patients. A single episode occurred in 87 (44.8%) patients, and recurrent episodes in 107 (54%) patients. The most common form of presentation was panuveitis in 151 (77.8%) eyes. Retinal detachment (RD) was present in 164 (42.9%) eyes, an exudative retinal detachment (ERD) was diagnosed in 143 (87.2%) eyes, and tractional retinal detachment (TRD) in 21 (12.8%) cases. Oral prednisone was the first line of treatment in 168 (86.6%) patients.

Immunosuppressive treatment with cyclosporine was employed in 87 (44.8%) patients, azathioprine in 58 (29.9%), intravenous steroid in 50 (25.8%), mycophenolate mofetil in 18 (9.3%), and methotrexate in 12 (6.2%) patients. During the 25 years of this study, 89 (45.9%) patients (136 eyes) underwent surgery. Visual acuity was better than 20/50 in 240 (62.8%) affected eyes and 20/200 or worse in 72 (18.8%) affected eyes at the last visit. More common complications at the last visit were glaucoma in 135 (35.3%) eyes, followed by posterior synchia 96 (25.1%), cataract in 25 (6.5%), and choroidal neovascularization (CNV) in 21 (5.5%) eyes.

Conclusions: In Saudi Arabia, VKH uveitis affects predominantly young women. Bilateral panuveitis is the most common ocular manifestation. At presentation, retinal detachment is present in 164 (42.9%) eyes. Oral prednisone is the first line of treatment in these patients. More than 60% of eyes maintain a visual acuity of 20/50 or better.

Commercial Relationships: Abdulaziz A. Alrhushood, None; J. Fernando Arevalo, None; Hassan A. Al Dhiabi, None; Yahya A. Al-Zahrani, None; Vishali Gupta, Allergan (R); sulaiman M. Alsulaiman, None; Andres F. Lasave, None; Hanan N. Al-Shamsi, None

Program Number: 5382 Poster Board Number: A0015
Presentation Time: 8:30 AM - 10:15 AM
Choroidal Bulging in Patients with Vogt-Koyanagi-Harada and Long-standing Disease
Viviane M. Sakata¹, Felipe G. da Silva¹, Carlos Hirata¹, Edilberto Olivalves¹, Walter Y. Takahashi¹, Rogerio A. Costa² ³ ⁴ ⁵, Joyce H. Yamamoto¹ ³ ⁴ ⁵. Ophthalmology, Hospital das Clinicas da Faculdade de Medicina da Universidade São Paulo, São Paulo, Brazil; ²Division of Macula: Imaging & Treatment, Centro Brasileiro de Ciencias Visuais, Belo Horizonte, Brazil; ³Ophthalmology, Faculdade de Medicina da Universidade de Ribeirão Preto, Ribeirão Preto, Brazil. 

Purpose: To describe a new finding on spectral optical coherence tomography (SOCT) in patients with Vogt-Koyanagi-Harada (VKH) and long-standing disease, the “choroidal bulging”, believed to represent a sign of disease-related inflammation.

Methods: Retrospective review of clinical and imaging data from all eyes noted to have the “choroidal bulging” on SOCT performed as part of the follow-up protocol in an ongoing longitudinal VKH
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disease study, which is being conducted at the Uveitis Section, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil. The study protocol followed the statements of the Declaration of Helsinki, and was approved by local Institutional Review Board. Fundus photography was performed using a conventional fundus camera system. Angiographic studies (fluorescein, FA, and indocyanine green, ICGA) and SOCT imaging were performed using Spectralis® HRA+OCT. For the SOCT evaluations, the “high-resolution” mode with the automatic real time (ART) mean module set at 25 frames and the AutoRescan® feature (i.e., automatic follow-up scan placement) were utilized.

**Results:** A localized thickening of the choroid that assumes a convex appearance with consequent bulging of the adjacent retina (i.e., “choroidal bulging”) was identified in at least one follow-up visit in four eyes of three patients. A focal increase of the choroidal thickness of at least 40% as compared with the baseline was observed in all eyes at the bulging region. No retinal lesion was observed within the choroidal bulging region. Signs of ongoing disease-related inflammation/activity, such as anterior chamber cells on clinical exam and/or optic disc hyperfluorescence on FA were observed in all four eyes by the time choroidal bulging was identified. Furthermore, increased dark dots on ICGA ran in parallel to the appearance of choroidal bulging. Choroidal bulging resolved as these clinical or angiographical signs of disease-related inflammation/activity improved.

**Conclusions:** Although the diffuse thickening of the choroid has been described in several retinal diseases (including VKH), this is the first time that a localized increase in choroidal thickness has been described. We propose that this finding can be a new sign of disease-related activity in patients with VKH and long-standing disease. Choroidal bulging can be observed non-invasively and may help monitoring VKH patients.

**Commercial Relationships:** Viviane M. Sakata, None; Felipe G. da Silva, None; Carlos Hirata, None; Edilberto Olivalves, None; Walter Y. Takahashi, Novartis (C); Bayer (C); (Bayer (R)); Rogerio A. Costa, None; Joyce H. Yamamoto, None

**Support:** Fapesp grant # 07 57155-S; Fapesp scholarship 19194-4

**Program Number:** 5383 Poster Board Number: A0016

**Presentation Time:** 8:30 AM - 10:15 AM

**Sympathetic ophthalmia: Histopathological and Immunohistochemical Characteristics**

**Purpose:** To evaluate histopathological and immunohistochemical characteristics in enucleated globes with sympathetic ophthalmia.

**Methods:** Reevaluation of specimens of enucleated inciting eye of 16 patients clinically diagnosed with sympathetic ophthalmia between 1987 and 2009. Specimens were considered histologically confirmed cases and may serve as a useful marker for sympathetic ophthalmia. Histological findings are likely altered histopathologically confirmed cases and may serve as a useful marker for sympathetic ophthalmia.

**Results:** A localized thickening of the choroid that assumes a convex appearance with consequent bulging of the adjacent retina (i.e., “choroidal bulging”) was identified in at least one follow-up visit in four eyes of three patients. A focal increase of the choroidal thickness of at least 40% as compared with the baseline was observed in all eyes at the bulging region. No retinal lesion was observed within the choroidal bulging region. Signs of ongoing disease-related inflammation/activity, such as anterior chamber cells on clinical exam and/or optic disc hyperfluorescence on FA were observed in all four eyes by the time choroidal bulging was identified. Furthermore, increased dark dots on ICGA ran in parallel to the appearance of choroidal bulging. Choroidal bulging resolved as these clinical or angiographical signs of disease-related inflammation/activity improved.

**Conclusions:** Although the diffuse thickening of the choroid has been described in several retinal diseases (including VKH), this is the first time that a localized increase in choroidal thickness has been described. We propose that this finding can be a new sign of disease-related activity in patients with VKH and long-standing disease. Choroidal bulging can be observed non-invasively and may help monitoring VKH patients.

**Commercial Relationships:** Viviane M. Sakata, None; Felipe G. da Silva, None; Carlos Hirata, None; Edilberto Olivalves, None; Walter Y. Takahashi, Novartis (C); Bayer (C); (Bayer (R)); Rogerio A. Costa, None; Joyce H. Yamamoto, None

**Support:** Fapesp grant # 07 57155-S; Fapesp scholarship 19194-4

**Program Number:** 5383 Poster Board Number: A0016

**Presentation Time:** 8:30 AM - 10:15 AM

**Sympathetic ophthalmia: Histopathological and Immunohistochemical Characteristics**

**Hassan Aziz, Harry W. Flynn, Ryan C. Young, Janet L. Davis, Sander R. Dubovy, Bascom Palmer Eye Institute, Miami, FL

**Purpose:** To evaluate histopathological and immunohistochemical characteristics in enucleated globes with sympathetic ophthalmia.

**Methods:** Reevaluation of specimens of enucleated inciting eye of 16 patients clinically diagnosed with sympathetic ophthalmia between 1987 and 2009. Specimens were considered histologically confirmed if there was diffuse granulomatous inflammation. Clinical data collected included prior treatment with immunosuppressive drugs or corticosteroids and time to enucleation after onset of symptoms. An average of 30 stored paraffin sections were obtained from the Florida Lions Ocular Pathology Laboratory and reassessed for histologic characteristics. Immunohistochemical stains included cell lineage markers CD-3, CD-20, CD-68; and cytokine receptors TNF-alpha, IL-4, INF-g, IL-17. A 200px 200px format was used to compare the intensity of the immunohistochemical stains to a positive control slide graded 3+ supplied by the manufacturer.

**Results:** Histopathologic evaluation of the 16 inciting eyes disclosed that 9 of 16 were diagnostic for sympathetic ophthalmia. In these 9 eyes Daulen-Fuchs nodules were present in 5 of 9 eyes and cosinophils were present in 9 of 9 globes. All patients with negative histology were receiving corticosteroids at the time of enucleation. The infiltrate showed an average of 3+ CD68 (macrophages), 2+ CD20 (B cells) and 1+ CD3 (T cells). There was no consistent pattern of cytokine receptor staining.

**Conclusions:** B cells and macrophages were more prevalent than T cells. Presence of eosinophils was a constant finding in histopathologically confirmed cases and may serve as a useful marker for sympathetic ophthalmia. Histological findings are likely altered histopathologically confirmed cases and may serve as a useful marker for sympathetic ophthalmia.

**Commercial Relationships:** Hassan Aziz, None; Harry W. Flynn, None; Ryan C. Young, None; Janet L. Davis, None; Sander R. Dubovy, None

**Program Number:** 5384 Poster Board Number: A0017

**Presentation Time:** 8:30 AM - 10:15 AM

**Natural history of Multiple Evanescent White Dot Syndrome: a multimodality imaging study**

**Mafalda Macedo1,2, Sara Vaz-Pereira1,3, Gabriella De Salvo1,2, Bishwanath Pal1,2, Medical Retina Department, Moorfields Eye Hospital, London, United Kingdom; 1Department of Ophthalmology, Hospital de Santo Antonio-CHP, Porto, Portugal; 2Department of Ophthalmology, Hospital de Santa Maria, Lisbon, Portugal.

**Purpose:** To describe the natural history and imaging features of a case series of multiple evanescent white dot syndrome (MEWDS).

**Methods:** Retrospective review of three patients with MEWDS followed at the Medical Retina Department of Moorfields Eye Hospital (MEH). We performed a comprehensive ophthalmic examination, including slitlamp biomicroscopy, funduscopy, evaluation with Amsler grid, visual fields, fundus photographs, red-free images, Spectralis® optical coherence tomography (Spectralis® OCT), fundus autofluorescence (FAF), fluorescein angiography (FA) and indocyanine green angiography (ICGA).

**Results:** Three previously healthy female patients with an average age of 26 years old presented to MEH with complaints of unilateral acute visual loss, photopsias and central/paracentral scotomas. The average visual acuity (VA) at presentation was 6/11. Fundus examination included multiple yellow-white dots extending from the posterior pole out to the mid-peripheral retina associated with an orange granular appearance of the fovea and a swollen hyperemic optic disc. FAF demonstrated hyperautofluorescence in the distribution corresponding to altered retinal pigment epithelium (RPE) pigmentation. Spectralis® OCT showed pathologic disruption of the outer retina with possible RPE involvement. FA and ICGA were also consistent with the diagnosis. In all patients the prognosis was good with complete spontaneous recovery of vision and resolution of fundus abnormal findings within several weeks. The disrupted outer retina in the acute phase was restored in the convalescent phase in all patients. One patient had a recurrence of MEWDS in the same eye twenty months after the first episode. In this patient, functional and structural abnormalities returned to normal within seven weeks.

**Conclusions:** The correct diagnosis of MEWDS can be a challenge due to the subtle and evanescent nature of fundus abnormalities. However, a detailed medical history along with several morphological and functional examinations are essential in the diagnosis and follow-up of these patients and may allow a deeper insight in the pathophysiology of this entity. MEWDS appears to involve the outer retina and the RPE/choriocapillaris. It is usually a self-limiting condition with complete visual recovery within a few weeks after the onset, even though it may rarely recur.

**Commercial Relationships:** Mafalda Macedo, None; Sara Vaz-Pereira, None; Gabriella De Salvo, None; Bishwanath Pal, None

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A retrospective analysis of the natural history and management of serpiginous choroidopathy (SC) in Australia and New Zealand

**Purpose:** To examine the prevalence of serpiginous choroidopathy (SC) in a predominantly Caucasian community, to examine associations between SC and other systemic diseases, and to determine the effect of immunosuppression on the long-term relapse rate of SC.

**Methods:** Retrospective cohort study of SC patients using the Australian and New Zealand Ophthalmic Surveillance Unit. Data collected included patient demographics, clinical features, associated systemic diseases, initial and maintenance treatments administered and dates of relapse. Three maintenance treatment groups were identified: No maintenance treatment, maintenance treatment with prednisolone monotherapy and maintenance treatment with combination prednisolone and immunosuppressant therapy. Negative binomial regression was used to calculate incidence rate ratios for patient relapse depending on which maintenance treatment category the patient fell into at the time of relapse.

**Results:** 18 patients (9 male, 9 female, mean age 48 yrs at baseline) were identified. One patient was seen only once. For the remaining 17 patients, median follow up was 69 months (5.8 years, range 0.4-29.7 years). The prevalence of SC in Australia and NZ was between 1 in 702,000 and 1 in 1.5 million people. Five cases (28%) had a positive QuantiFeron and 1 of these had pulmonary tuberculosis. A total of 32 relapses were observed (1 per 4.8 patient years of followup); 14 whilst receiving no maintenance treatment, 11 on maintenance prednisolone and 7 on maintenance therapy with combined immunosuppressive therapy. Compared to the no treatment group, the incidence rate ratio for relapse on prednisolone monotherapy and combination therapy were 1.29 and 2.92 respectively (95% CI: 0.40-4.14 and 0.96-8.88).

**Conclusions:** SC is uncommon in Australia and New Zealand. Tuberculosis is associated with a significant minority of SC cases in Australian and New Zealand. There is a significant subpopulation of patients with SC who have a benign course. Whilst the confidence intervals indicate that the difference in relapse incidence rate ratios are not significant, these treatment results do not suggest a reduced incidence of relapses for patients receiving combination prednisolone and immunosuppressant therapy.

**Commercial Relationships:** Anthony J. Hall, None; Jason Tonio, None; Jo Sims, None; Samantha Fraser-Bell, None; Jane Khan, None; Christine Younan, None; Brian Kent-Smith, None; Stephanie Young, None; Eldho Paul, None; Lyndell Lim, None

**Support:** Eye Foundation

**MicroRNA-146a and Ets-1 gene polymorphisms in ocular Behcet’s disease and Vogt-Koyanagi-Harada syndrome qingyun zhou, Aize Kijistra.**

**Purpose:** MicroRNA-146a (miR-146a) is involved in certain immune-mediated diseases. Transcription factor Ets-1 strongly affects miR-146a promoter activity and directly regulates miR-146a expression. This study was performed to investigate the association of miR-146a and Ets-1 gene polymorphisms with Behcet’s disease (BD) and Vogt-Koyanagi-Harada (VKH) disease in a Chinese Han population.

**Methods:** A total of 809 BD patients, 613 VKH patients and 1132 normal controls were genotyped for miR-146a/rs2910164, rs57095329 and rs6864584, Ets-1/rs1128334 and rs10893872 using a PCR restriction fragment length polymorphism assay. miR-146a expression was examined in PBMCs by real-time PCR. Cytokine production by PBMCs were measured by ELISA.

**Results:** A significantly decreased frequency of the homozygous rs2910164 CC genotype and C allele was observed in BD patients compared with controls (pca=1.24x10-5, odds ratio (OR) 0.61; pca=1.33x10-4, OR 0.75, respectively). MiR-146a expression in GS was 2.4-fold and 1.99-fold respectively higher than that in CC cases and GC cases. There was no association of other four SNPs with BD. There was also no association of these five SNPs with its main clinical features. No associations were found with the five SNPs tested or with its clinical manifestations in VKH disease. IL-17, TNF-α and IL-1β production from rs2910164 CC cases was markedly lower than that in GG cases. No effect of genotype was observed on the IL-6 and MCP-1 production and IL-8 expression was slightly higher in CC cases.

**Conclusions:** Our study identified a strong association of rs2910164 of miR-146a with BD in a Chinese population and an decreased expression of miR-146a and certain proinflammatory cytokines in individuals carrying the CC genotype.

**Commercial Relationships:** qingyun zhou. None; Aize Kijistra. None

**Support:** Natural Science Foundation Major International (Regional) Joint Research Project (30910130912)
Involvement of Vitreous in Castleman's Disease

Sunja Park, Matthew S. Katz, David C. Gritz, Ophthalmology and Visual Sciences, AECOM/Montefiore Medical Center, Bronx, NY.

Purpose: To present the first reported patient with Castleman's Disease found to have involvement of the vitreous body.

Methods: Case report and review of literature.

Results: A 28-year-old Caribbean-born black woman with known Castleman's Disease presented to the emergency department complaining of frontal headaches for three days. She had been treated several years prior with two rounds of rituximab and her Castleman's Disease was thought to be in remission. Her medical history was also remarkable for recurrent interstitial lung disease and deep venous thromboses and pulmonary emboli necessitating systemic anticoagulation. The patient was evaluated by ophthalmology for a complaint of progressive decrease in vision bilaterally. On examination, best-corrected visual acuity was found to be 20/20 OU. Anterior segment examination was within normal limits. Dilation revealed anterior vitreous cell OU (Figure A) and rare snowballs in the far periphery OS (Figure B). Her posterior segments were otherwise anatomically within normal limits.

Conclusions: Castleman's Disease, or angiofollicular lymph node hyperplasia, is an uncommon and poorly understood lymphoproliferative disorder first described in 1954. This disease entity only rarely involves orbital and ocular structures and infrequently presents with ocular symptoms. Orbital or ocular involvement has been reported in both unicentric and multicentric variants. Previously reported cases have involved the eyelids, lacrimal gland, and the extraocular muscles. Ocular involvement previously reported includes infiltration of the optic disc, chorioretinal lesions, lesions of the retinal pigment epithelium (RPE) with accompanying serous retinal detachment. Ocular symptoms constitute a particularly rare presentation of Castleman's Disease. Patients may complain of diplopia, ptosis, lid swelling, or exopthalmos. Only four previously reported cases reported a decrease in vision. The patient described in this case is the first patient with Castleman's Disease reported to have involvement of the vitreous body.

Figure A: Anterior vitreous cell, right eye
Conclusions: Bromfenac may be an additional safe and effective modality of treatment in patients with uveitis induced macular edema.

Commercial Relationships: Joanna Saade, None; Marwan Abdulaal, None; Rola N. Hamam, None

Program Number: 5391 Poster Board Number: A0024
Presentation Time: 8:30 AM - 10:15 AM

Assessment of Changes in Quality of Life Among Patients in the SAVE Study - Sirolimus As Therapeutic Approach To UVEitis: A Randomized Study To Assess The Safety And Bioactivity Of Intravitreal And Subconjunctival Injections Of Sirolimus In Patients With Non-infectious Uveitis

Erin Vigil, Yasir J. Sepah, Owhofasa O. Agbedia, Anthony L. Watters, Mohammad A. sadiq, Mehreen Ansari, Millena G. Bittencourt, Mohamed A. Ibrahim, Quan Dong Nguyen. The Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD.

Purpose: To assess the change in quality of life (QOL) in patients with non-infectious posterior, intermediate, or panuveitis, treated with subconjunctival (SCJ) or intravitreal (IVT) sirolimus as an immunomodulatory therapeutic (IMT) agent, delivered subconjunctivally (SCJ) or intravitreally (IVT) (the SAVE Study).

Methods: The 25-question Visual Function questionnaire (VFQ-25) was administered at baseline, month 6, and month 12 visits. The survey measures self-reported vision health status for patients with chronic eye disease. The questionnaire assesses the effects of visual impairment on both task-oriented visual function and general health domains such as emotional well-being and social functioning. Each patient’s questionnaire was converted to a scaled score between 0 (worst) and 100 (best). Individual question scores were combined into 12 different subcategories.

Results: Thirty subjects were randomized in the SAVE study (SC1:IVT, 1:1). Among the 24 subjects who finished month 12, 18 completed all questions of the VFQ-25 at all three time points. Mean and median scores were calculated for each of the subcategories and for overall composite score at baseline, month 6, and month 12, using Stata 12 (Table 1). Wilcoxon signed-rank test was performed. Overall, patients showed a significant improvement in composite scores between BL and month 6 as well as BL and month 12. From BL to month 6, patients showed significant improvements in the subcategories of general vision, distance activities, vision-specific mental health, and vision-specific role difficulties. From BL to month 12, patients improved significantly in the subcategories of vision specific mental health and vision-specific role difficulties.

Conclusions: Patients with uveitis who have been treated with local delivery of sirolimus demonstrated significant improvement in their QOL during the 12-month course of therapy. Specifically, subjects have gained in vision health and function. Larger randomized control trials with sirolimus are indicated to validate this gain in QOL.

Table 1: VFQ-25 Scores and P Values: Baseline, Month 6, and Month 12

<table>
<thead>
<tr>
<th>Sub-category</th>
<th>Baseline (n=24)</th>
<th>Month 6 (n=18)</th>
<th>Month 12 (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General health</td>
<td>76.76 (SD 11.84)</td>
<td>81.80 (SD 11.68)</td>
<td>87.40 (SD 9.42)</td>
</tr>
<tr>
<td>Role-activities</td>
<td>66.72 (SD 11.24)</td>
<td>72.10 (SD 11.55)</td>
<td>80.30 (SD 10.16)</td>
</tr>
<tr>
<td>Emotional well-being</td>
<td>58.70 (SD 11.84)</td>
<td>68.70 (SD 11.24)</td>
<td>72.90 (SD 10.16)</td>
</tr>
<tr>
<td>Vision specific role difficulties</td>
<td>50.89 (SD 11.10)</td>
<td>55.80 (SD 11.10)</td>
<td>60.80 (SD 11.10)</td>
</tr>
<tr>
<td>Vision specific mental health</td>
<td>61.30 (SD 11.10)</td>
<td>68.70 (SD 11.24)</td>
<td>72.10 (SD 11.55)</td>
</tr>
<tr>
<td>Vision specific activities</td>
<td>69.20 (SD 11.24)</td>
<td>75.80 (SD 11.68)</td>
<td>83.40 (SD 9.42)</td>
</tr>
<tr>
<td>Social functioning</td>
<td>69.80 (SD 11.24)</td>
<td>75.30 (SD 11.68)</td>
<td>82.90 (SD 9.42)</td>
</tr>
<tr>
<td>Society constraints</td>
<td>50.30 (SD 11.10)</td>
<td>55.80 (SD 11.10)</td>
<td>60.80 (SD 11.10)</td>
</tr>
<tr>
<td>Vision specific utilities</td>
<td>50.50 (SD 11.10)</td>
<td>55.30 (SD 11.10)</td>
<td>60.00 (SD 11.10)</td>
</tr>
<tr>
<td>Vision specific health</td>
<td>50.50 (SD 11.10)</td>
<td>55.30 (SD 11.10)</td>
<td>60.00 (SD 11.10)</td>
</tr>
<tr>
<td>Vision specific symptoms</td>
<td>50.50 (SD 11.10)</td>
<td>55.30 (SD 11.10)</td>
<td>60.00 (SD 11.10)</td>
</tr>
<tr>
<td>Vision specific QoL</td>
<td>50.50 (SD 11.10)</td>
<td>55.30 (SD 11.10)</td>
<td>60.00 (SD 11.10)</td>
</tr>
<tr>
<td>All subcategories</td>
<td>69.60 (SD 11.60)</td>
<td>75.80 (SD 11.68)</td>
<td>83.40 (SD 9.42)</td>
</tr>
</tbody>
</table>

Conclusions: Peripheral snowballs, left eye
L. Watters, None; Mohammad A. sadiq, None; Mehren Ansari, None; Milena G. Bittencourt, None; Mohamed A. Ibrahim, None; Quan Dong Nguyen, Genentech (F), Regeneron (F), Lux Biosciences (F), Abbott (F), GSK (F), Santen (F), Santen (C), Bausch and Lomb (C), Optos (F), Heidelberg Engineering (F)

Support: Uveitis Research Fund
Clinical Trial: NCT00908466

Program Number: 5392 Poster Board Number: A0025
Presentation Time: 8:30 AM - 10:15 AM

Myocophenolate Mofetil in Refractory Non-infectious Uveitis

Purpose: To evaluate the effectiveness of Myocophenolate Mofetil (MMF) as an immunosuppressive agent for refractory non-infectious uveitis.

Methods: Data from patients with non-infectious uveitis, followed in the Uveitis Service, Hospital das Clinicas, Universidade de Sao Paulo, Sao Paulo, Brazil, under MMF (1-3g/day) oral therapy during 2007-2012 period were retrospectively analyzed. Treatment duration was at least 6 months. Patients were evaluated at 6 (T6) and 12 months (T12) after maximum dosage was achieved. Primary outcomes were success in gaining complete control of inflammation, success in maintaining control of inflammation after tapering of prednisone to ≤10mg/day (at least for 28 days) and discontinuation of treatment. Secondary outcomes were partial improvement in inflammation and use of other immunosupressant after MMF therapy start. Clinical activity was evaluated according to SUN guidelines. Fluorescein angiography (FA) and optical coherence tomography (OCT) were analyzed when indicated. This study was approved by Institutional Ethics Committee Board (0621/11).

Results: Seventeen patients (Vogt-Koyanagi-Harada disease, 5; Behcet disease, 4; idiopathic retinal vasculitis, 3; intermediate uveitis, 3; HLA-B27 +ve anklyosing spondylitis,1; idiopathic diffuse uveitis,1) with a mean age of 40±15 years (14-59 y) were included. Nine patients (53%) were male. The mean duration of inflammation prior to achieving MMF maximum dosage was 85.9±73.6 mo (24-276mo). All patients at MMF start (T0) had previously taken other immunosuppressant/biologic agent (cyclosporine, 14; azathioprine, 13; methotrexate,2; chlorambucil, 3; cyclophosphamide,2; infliximab, 2; adalimumab,2). Complete control of inflammation was achieved in 7 (41.2%) and in 6 patients (37.5%) at T6 and T12, respectively. Four out of 10 patients (40%), who were taking prednisone >10mg/d at T0, were succeeded in reducing prednisone to ≤10mg/day (a decline of 60%) and in maintaining sustained inflammatory control during the follow up. One patient discontinued MMF due to side effects after 11mo. Partial control of inflammation was observed in 9 patients (52.9%) and in 8 patients (47.1%) at T6 and T12, respectively. 85% of those with complete control of inflammation had no additional immunosuppressant at T6 and T12.

Conclusions: Our results show that MMF is an effective immunosuppressant in patients with refractory non-infectious uveitis presenting few side effects.

Commercial Relationships: Manoel Gusmao Isidro, None; Viviane M. Sakata, None; Daniel C. Cavalcanti, None; Juliana Zaghetto, None; Edilberto Olivalves, None; Carlos Hirata, None; Joyce H. Yamamoto, None

Program Number: 5393 Poster Board Number: A0026
Presentation Time: 8:30 AM - 10:15 AM

STUDY OF THE PREVALENCE OF OPHTHALMOLOGY UVEITIS TERTIARY HOSPITAL IN TERESINA-PI
Leonardo Pinheiro Teixeira, Leticia Rosa Ribeiro Cunha, Caroline Dar'k Amorim Teles, Vitor Cortizo. "Faculdade de Saude Ciencias Humanas e Tecnologicas do Piau - NOVAFAPI, Teresina-PI, Brazil; "Universidade Federal do Piaui - UFPI, Teresina-PI, Brazil.

Purpose: To analyze the prevalence of uveitis in patients evaluated at a tertiary ophthalmic service in Teresina, PI.

Methods: A retrospective, cross-sectional, descriptive and epidemiological study was conducted based on the query of electronic medical records of patients treated at Hospital de Olhos Francisco Vilar (Eye Hospital Francisco Vilar) in the period from January 1st, 2006 to December 31st, 2011. There followed a search strategy on electronic medical records of the aforesaid hospital, looking up keywords that embraced “uveitis” and all its synonyms, besides the search for underlying diseases that could lead to it, being careful to insert the charts only once in the statistics. The following data were reported: gender, age, origin, underlying disease, and classifications related to the position, evolution, and clinical aspect. Each uveitis found was separated according to their underlying disease, then it was also created the groups “SDD” (without definite diagnosis) and “Other”. The group “SDD” was used to discriminate the uveitis that had only ocular manifestation without alterations that were systemic or that were in the complementary exams, and the group “Other” was used to lease diseases that appeared only once and that were not in the research protocol.

Results: 403 records were included, noting that 3.2 out of every 1000 patients treated there had uveitis. The average age of patients was 42 years old, both (50.6% females and 49.4% males) affected similarly, with 61.5% coming from the capital. The most common underlying disease was toxoplasmosis, followed by the idiopathic uveitis with ocular manifestations only. As to the anatomical classification, 49.6% of them were posterior and only 3.5% were intermediate. Regarding the clinical aspects, 64% were granulomatous, 24.8% non-granulomatous, and 11.2% were not classifiable. According to the clinical progression, 41.4% were acute, followed by 30.8% chronic ones, 14.4% recurrent cases, and 13.4% of the patients did not keep medical care.

Conclusions: This survey found a prevalence of 3.2 cases of uveitis for every 1000 patients evaluated, evidencing the relative frequency of uveitis found in this service, among which toxoplasmosis was the most prevalent followed by idiopathic uveitis with only ocular manifestations. Numerous other infectious and noninfectious causes with significant social impact as tuberculosis and syphilis were also identified.

Commercial Relationships: Leonardo Pinheiro Teixeira, None; Leticia Rosa Ribeiro Cunha, None; Caroline Dar’k Amorim Teles, None; Vitor Cortizo, None

Program Number: 5394 Poster Board Number: A0027
Presentation Time: 8:30 AM - 10:15 AM

Decline in Ocular Toxoplasmosis in the Midwestern United States
Asim Farooq, Joshua H. Hou, Sarju Patel, Howard Tessler, Debra A. Goldstein. "OphthalmoLOGY, Illinois Eye & Ear Infirmary, Chicago, IL; "OphthalmoLOGY, Weill Cornell Medical College, New York, NY; "OphthalmoLOGY, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Purpose: 1) To define epidemiologic trends in ocular toxoplasmosis from 1973 to 2012 in the Midwestern United States, based on patients seen at a tertiary referral uveitis practice. 2) To define the changing demographic profile of patients presenting with ocular toxoplasmosis in the same clinic.

Methods: A retrospective chart review of all new patients evaluated...
by the Uveitis Service at the Illinois Eye & Ear Infirmary 1973-2012 was performed for final diagnosis, self-reported racial background and country of origin. The percentage of new patients with a final diagnosis of ocular toxoplasmosis among all new patients was determined over successive five-year time-bands. Racial background, country of origin and gender were further analyzed for all patients with ocular toxoplasmosis.

Results: 328 out of 6,798 new patients (4.8%) were diagnosed with ocular toxoplasmosis. There was a progressive decline in the proportion of newly diagnosed toxoplasmosis (Figure 1). This was correlated with a declining proportion of toxoplasmosis among non-Hispanic Caucasians and African Americans (Figure 2). Over time, the proportion of toxoplasmosis cases in Hispanic patients increased, which correlated with an increase in Hispanic patients seen in the clinic. The majority of cases (85.4%) amongst the Hispanic population were in patients born outside of the US.

Conclusions: The diagnosis of ocular toxoplasmosis at a tertiary referral uveitis service in the Midwestern United States has declined over the past 40 years. This decrease is attributable to a decrease in disease seen in the non-Hispanic Caucasian and African American populations, which is consistent with published epidemiologic seroprevalence data. Over time, a higher proportion of toxoplasmosis was diagnosed in Hispanic patients, reflecting an increase in Hispanic patients born outside the US seen in our clinic.

Figure 1. From 1973 to 2012, there was a significant decline in the number of toxoplasmosis cases among uveitis patients.

Figure 2. A greater decline in the percentage of toxoplasmosis among uveitis patients was noted in the non-Hispanic Caucasian and African American populations.

Commercial Relationships: Asim Farooq, None; Joshua H. Hou, None; Sarju Patel, None; Howard Tessler, None; Debra A. Goldstein, Bausch and Lomb (C), Bausch and Lomb (R)

Program Number: 5395 Poster Board Number: A0028

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study according to the tenets of Declaration of Helsinki. The serum levels of angiotensin converting enzyme and lysozyme are analyzed by enzyme-linked immunosorbent assay. Krusal Wallis and Mann Whitney U tests are used for age distribution, and Chi Square test is used for sex distribution. Multivariate analysis of covariance is used to analyze the significance of differences in serum levels of angiotensin converting enzyme and lysozyme between the patient groups.

Results: The increase in serum angiotensin converting enzyme level is significant for patients with sarcoidosis with respect to HLAB27+, HLAB51+ and quantitative+ latent tuberculosis. (p<0.001) and quantitative+ latent tuberculosis. (p<0.015) The increase in serum lysozyme level is significant for patients with sarcoidosis with respect to HLAB27+ and quantitative+ latent tuberculosis. (p<0.001) However, there is no significant difference of increase serum lysozyme levels between patients with sarcoidosis and quantitative+ latent tuberculosis. (p=0.051)

Conclusions: The increase in serum angiotensin converting enzyme level is helpful for the diagnosis of sarcoidosis. The increase in serum lysozyme level is considered to be helpful for the diagnosis of both sarcoidosis and quantitative+ latent tuberculosis.

Commercial Relationships: Ozlem Gurses, None; Eda Karaismailoglu, None
Clinical Trial: 1576184

Program Number: 5397 Poster Board Number: A0030
Presentation Time: 8:30 AM - 10:15 AM
Inflammatory activity and visual outcomes in patients treated with cryotherapy for pars planitis
Benjamin C. Chaon, James C. Folk, Elliott H. Sohn. Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA.

Purpose: Most current treatments for visually disabling pars planitis have the potential for systemic and local toxicity. This study aims to determine the clinical outcomes of cryotherapy in a cohort of patients with vision loss from pars planitis.

Methods: A retrospective chart review of patients with pars planitis treated at the University of Iowa from 1973 to 2011 was conducted. Patients who underwent cryotherapy met inclusion criteria if they demonstrated evidence of intraocular inflammation with snowbanking and had a negative serologic workup for other causes of inflammation. LogMAR visual acuity, inflammatory activity, structural complications, and use of topical and/or systemic steroid and immunosuppressive agents were compared in the pre-cryotherapy period and at 9-24 months following cryotherapy.

Results: 38 cryotherapy-treated eyes of 27 patients with pars planitis were included in this study. The mean Snellen visual acuity of the eyes prior to cryotherapy was 20/70 (range 20/15 to “hand motion”). The mean Snellen visual acuity at an average of 18 months following cryotherapy was 20/37 (range 20/15 to 20/200). Visual acuity improved or remained unchanged in 76% of cryotherapy-treated eyes. The average change in logMAR acuity from baseline was 0.2637 log units, corresponding to a modest improvement in vision (p=0.0078). The average time from presentation to cryotherapy was 28 months. The mean number of cryotherapy treatments was 1.34 with 21% of eyes requiring more than 1 treatment. Cystoid macular edema was present in 56% of eyes at the time of cryotherapy and 17% of eyes at follow-up after cryotherapy.

Conclusions: Visual acuity improved or remained stable in the majority of patients treated with cryotherapy. Cryotherapy was also associated with a resolution of cystoid macular edema, suggesting a role for cryotherapy in treating structural complications and vision loss caused by pars planitis.

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Commercial Relationships: Michael Karampelas, None; Rene Moya, None; Dawn A. Sim, None; Javier Zarranz-Ventura, None; David G. Charteris, None; Richard W. Lee, Genentech (C); Carlos E. Pavesio, None

525 Clinical and Translational Studies in Ocular Inflammation Thursday, May 09, 2013 10:30 AM-12:15 PM
606/607 Paper Session
Program #/Board # Range: 5928-5934
Organizing Section: Immunology/Microbiology

Program Number: 5928
Presentation Time: 10:30 AM - 10:45 AM
Targeted B-cell Therapy with Rituximab for the Treatment of Autoimmune Retinopathy: Results of a Pilot Clinical Trial
H Nida Sen1, Monica D. Dalal1, Landon Grange1, Yujuan Wang1, John R. Heckenlively2, Patti R. Sherry2, Chi-Chao Chan2, Robert B. Nussenblatt1. 1National Eye Institute, National Institutes of Health, Bethesda, MD; 2Kellogg Eye Center, University of Michigan, Ann Arbor, MI.
Purpose: Nonparaneoplastic Autoimmune retinopathy (AIR) is an immune mediated disorder characterized by antiretinal autoantibodies and progressive vision loss in the absence of malignancy. Rituximab is a chimeric monoclonal antibody which binds specifically to CD20 antigen on B lymphocytes. The objective of this study is to investigate the safety and potential efficacy of rituximab treatment for AIR.
Methods: Five participants with AIR with positive serum antiretinal antibodies, visual field (VF) and electoretinography (ERG) abnormalities enrolled in this prospective, open-label pilot clinical trial. Rituximab is administered as a cycle consisting of two separate rituximab infusions of 1,000 mg each, two weeks apart. Participants with partial or complete treatment success at 6 months were eligible for a second cycle. Treatment success was defined as 25% improvement in ERG or improvement in the VFs according to predefined criteria. Study visits were performed every three months for the study duration of 18 months. The primary outcome was the number of participants who met the definition of treatment success within 6 months. Safety outcomes included the number and severity of adverse events.
Results: Four of the 5 patients were female, all were Caucasian with a median age of 52 years (range: 41-65). Three of the 5 (60%) patients had associated autoimmune systemic disease. Median visual acuity at enrollment was 84 ETDRS letters in the better eyes and 80 letters in the worse eyes; at 6 months, median vision was 82 and 79 ETDRS letters, respectively. All patients met the primary outcome of partial or complete response to treatment, most achieving stability. However, in most participants, improvement in visual function measures was not clinically significant. All patients tolerated Rituximab infusions well with no serious adverse events attributable to the study drug.
Conclusions: Autoimmune retinopathy continues to be a poorly defined challenging disease to manage. Rituximab infusions appear safe and are well tolerated among AIR patients, although with modest effect on the visual function. Randomized, masked clinical trials are needed to distinguish the treatment effect from natural history.
Commercial Relationships: H Nida Sen, None; Monica D. Dalal, None; Landon Grange, None; Yujuan Wang, None; John R. Heckenlively, None; Patti R. Sherry, None; Chi-Chao Chan, None; Robert B. Nussenblatt, None
Support: NEI Intramural Research Program
Clinical Trial: NCT01086631

IV Secukinumab Is An Effective Treatment In Patients With Noninfectious Uveitis Requiring Steroid Sparing Immunosuppressive Therapy
Erik Letko1, Steven Yeh2, Cynthia L. Grosskreutz3, Uwe Pleyer4, C. Stephen Foster5, Mitchell G. Briggel1, 1Corneal Consultants of Colorado, Denver, CO; 2Ophthalmology Eye Center, Atlanta, GA; 3Global Translational Medicine Head for Ophthalmology Novartis Institutes for Biomedical Research, Cambridge, MA; 4Ophthalmology University Charite Berlin, Berlin, Germany; 5Ophthalmology Ocular Immunol & Uveitis Foundation, Cambridge, MA; 6Novartis Institutes for BioMedical Research, Inc., Cambridge, MA.
Purpose: Secukinumab is a fully human monoclonal antibody that binds to IL-17A. In a proof of concept study 13/16 patients with active noninfectious uveitis were able to taper corticosteroids over 6 weeks with reduction of vitreous haze (VH) by at least one grade, following 1 or 2 intravenous (IV) infusions of Secukinumab 10 mg/kg (Sci Transl Med 2010;2:52RA72). Subsequent randomized controlled studies (RCTs) using subcutaneous (sc) Secukinumab at doses of 150 - 300 mg in patients with Behcet’s uveitis or quiescent noninfectious uveitis failed to show efficacy (Ophthalmol, in press).
In this study we examined the efficacy of IV vs. sc Secukinumab in patients with active non-infectious intermediate, posterior, or panuveitis.
Methods: 37 patients requiring steroid sparing immunomodulatory therapy, were randomized to receive either Secukinumab 300 mg sc every 2 wks, 10 mg/kg IV every 2 wks or 30 mg/kg IV every 4 wks. Patients were required to have a VH of ≥ 1+ treated with topical or oral corticosteroids. Corticosteroids were tapered over the course of the trial. Responders were patients whose VH improved by 2 grades or to grade 0 or trace at week 8. A patient was in remission if the vitreous and anterior chamber showed no sign of inflammation. Superiority was defined as a ≥ 50% probability of one treatment having 30% more responders or remissions. Similarity was defined as a ≥ 50% probability that the true difference between treatment response or remission rates was ± 20%.
Results: The percent of responders to Secukinumab was similar between the two IV groups (62% 10 mg/kg; 73% 30 mg/kg) and both were superior to the 300 mg sc group (33%). A similar trend was seen for remissions with 27%, 37% and 17% of patients in remission at week 8 in the 30 mg/kg IV, 10 mg/kg IV, and 300 mg sc groups, respectively. The drug was well tolerated with no trend to increasing adverse events with increasing dose.
Conclusions: IV Secukinumab is an efficacious and safe steroid sparing treatment for patients with active non-infectious uveitis. The modest efficacy of 300 mg sc Secukinumab and its failure in prior...
uveitis RTCs could be explained by inadequate Cmax, since this dose has been shown to be sufficient to neutralize peripheral IL-17A. Our results suggest that high Cmax achieved by IV dosing is needed to have an effect on active non-infectious uveitis.

**Commercial Relationships:** Erik Letko, None; Steven Yeh, Bausch and Lomb (C); Cynthia L. Grosskreutz, Novartis Institutes for BioMedical Research (E); Uwe Pleyer, None; C. Stephen Foster, Abbott Medical Optics (C), Abbott Medical Optics (F), Alcon Laboratories, Inc. (C), Alcon Laboratories, Inc. (F), Allergan, Inc. (C), Allergan, Inc. (F), Eyegate Pharmaceuticals, Inc. (I), Eyegate Pharmaceuticals, Inc. (F), IOP Ophthalmics (C), Ista Pharmaceuticals (C), Lux Biosciences, Inc. (C), Lux Biosciences, Inc. (F), Novartis Pharmaceuticals Corporation (C), Novartis Pharmaceuticals Corporation (F), XOMA Ltd (C); Mitchell G. Brigell, Novartis (E)

**Clinical Trial:** NCT00685399

**Program Number:** 5930

**Presentation Time:** 11:00 AM - 11:15 AM

**Clinical Characteristics and Treatment Outcomes of Juvenile Idiopathic Arthritis Associated Uveitis**

Nisha Acharya1,2, Sarju Patel1, Gelareh Homayounfar1, Wayne Enanoria1,2, Akbar Shakoor1, Anindita Chakrabarti2, Debra A. Goldstein1,4, F.I. Proctor Foundation, University of California, San Francisco, San Francisco, CA; 1Department of Ophthalmology, University of California, San Francisco, San Francisco, CA; 2Department of Ophthalmology, University of Illinois College of Medicine at Chicago, Chicago, Chicago, IL; 3Department of Ophthalmology, Northwestern Memorial Feinberg School of Medicine, Chicago, IL.

**Purpose:** To assess treatment outcomes in juvenile idiopathic arthritis (JIA) associated uveitis, including corticosteroid-sparing control of inflammation with immunomodulatory therapy (IMT) and relapse rates upon discontinuation of therapy.

**Methods:** We conducted a retrospective cohort study of patients with JIA-associated uveitis seen at the University of Illinois at Chicago and F.I. Proctor Foundation uveitis clinics between 1988 and 2011. Information collected included control of ocular inflammation and relapse rates upon discontinuation of treatment. Steroid-sparing control of inflammation was defined as ≤0.5+ anterior chamber cells, no active retinal/choroidal lesions, prednisone dose ≤10 mg and prednisolone acetate dose ≤3 times/day.

**Results:** Of 66 patients with JIA-associated uveitis, 89% were female. Median follow-up was 854 days (interquartile range (IQR) 189-2280 days). Median age at onset of JIA was 2 years, and that of uveitis was 4 years. The most common type of JIA was oligoarticular (67%). Eighty-five percent had anterior uveitis, 11% had anterior and intermediate uveitis, and 96% had a chronic course of disease. Fifty-one patients (77%) received corticosteroid-sparing IMT either as sole or combination therapy, including methotrexate (67%), adalimumab (21%), and infliximab (17%). Steroid-sparing control of inflammation was achieved in 15/21 (71.4%) of patients on methotrexate as sole therapy, 10/11 (91%) patients on infliximab, and 6/13 (46.2%) on adalimumab. Attempts were made to discontinue treatment in 14/51 (27.5%) patients on IMT. Median duration of quiescence on IMT prior to attempting to taper or stop was 413 days (IQR 88-595). Ten (66.7%) of these patients experienced relapse, with a median time to relapse of 329 days (IQR 213-344). There was no difference in the duration of controlled inflammation on IMT in patients who relapsed vs. those that did not (p=0.89).

**Conclusions:** Steroid-sparing control of inflammation was achieved in the majority of patients. However, attempts to stop IMT were often unsuccessful. This highlights the need for continued follow-up of patients after discontinuation of therapy for presumed remission, and for further research into predictors for successful discontinuation of systemic IMT in patients with JIA-associated uveitis.

**Commercial Relationships:** Nisha Acharya, None; Sarju Patel, None; Gelareh Homayounfar, None; Wayne Enanoria, None; Akbar Shakoor, None; Anindita Chakrabarti, None; Debra A. Goldstein, Bausch and Lomb (C), Bausch and Lomb (R).

**Support:** Dr. Acharya is supported by NEI grant K23EY017897 and a Research to Prevent Blindness (RPB) Career Development Award. Ms. Homayounfar is supported by the Doris Duke Clinical Research Fellowship. This research was also supported by That Man May See Foundation. The UCSF Department of Ophthalmology is supported by NEI grant EY06190 and an unrestricted RPB grant. Dr. Goldstein is also supported by an unrestricted RPB grant. The sponsors or funding organizations had no role in the design or conduct of this research.

**Program Number:** 5931

**Presentation Time:** 11:15 AM - 11:30 AM

**12-Month Results of the SAVE Study - Sirolimus as Therapeutic Approach to UVEIts: A Randomized Study to Assess the Safety and Bioactivity of Intravitreal and Subconjunctival Injections of Sirolimus in Patients with Non-infectious Uveitis**

Quan Dong Nguyen1,2, Mohamed A. Ibrahim3, Anthony L. Watters2, Yasir J. Sepah2, Millena G. Bittencourt1, Jithin Yohannan1, Joel Naor2, Naveed K. Shams2, Diana V. Do1,3, Diseases of the Retina & Uveitis, Johns Hopkins Univ, Wilmer Eye Inst, Baltimore, MD; 2Retinal Imaging Research and Reading Center, Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; 3Santen, Inc, Emeryville, CA.

**Purpose:** Purpose: To present the one-year safety and bioactivity outcomes of sirolimus as an immunomodulatory therapeutic (IMT) agent, delivered subconjunctivally (SCJ) or intravitreally (IVT), in patients with non-infectious posterior, intermediate, or panuveitis.

**Methods:** Methods: 30 subjects were stratified at baseline (BL) into 3 categories: (1) active disease receiving no treatment; (2) active disease receiving prednisone ≥10 mg/day and/or at least one other IMT; (3) inactive disease receiving prednisone <10 mg/day and/or at least one other IMT. Patients in each category were randomized into SCJ or IVT arm (1:1). Study eyes received SCJ (1320 µg) or IVT (352 µg) injections at days 0, 60, and 120, with primary endpoint at day 180. Study subjects may be followed and treated until M12. Beyond safety, study parameters include change in visual acuity (VA), vitreous cells/haze, and reduction in dosage of systemic corticosteroid (CS) compared to BL.

**Results:** Analyses at the 12-month endpoint were performed using data from 28 subjects; two subjects discontinued before M6. Both SCJ and IVT sirolimus injections were well tolerated. None of the adverse events (AE) were deemed to be related to sirolimus. At M12, VA improved ≥ 5 letters in 21% and 50%, ITV and SCJ respectively and stabilized in 43% of all patients. Vitreous haze improved in 70% of patients with active uveitis (SCJ=71%; IVT=71%). 20 patients were on systemic CS therapy at BL; all had their doses of CS reduced at M12. The mean dose among subjects who were on CS decreased from 20.5 mg/day at BL to 3.5 mg/day at M12.

**Conclusions:** Locally administered (subconjunctival or intravitreal) sirolimus appears safe in patients with noninfectious uveitis. Sirolimus has exerted its bioactivity in reducing vitreous haze and cells, improving VA, and in decreasing need for systemic CS over a 12-month period. Larger controlled trials are indicated to confirm the role of locally-delivered sirolimus and to determine its appropriate dosage and frequency of administration.

**Commercial Relationships:** Quan Dong Nguyen, Genentech (F), Regeneron (F), Lux Biosciences (F), Abbott (F), GSK (F), Santen (F).
Visual outcomes in uveitis with angiographic macular leakage treated with the fluocinolone acetonide implant or standard treatment

Thomas A. Albini1, Debra A. Goldstein2, David Callanan1, Quan Dong Nguyen3, Sunit K. Srivastava4. Ophthalmology, Bascom Palmer Eye Institute, Miami, FL; 2Ophthalmology, Northwestern University, Chicago, IL; 3Ophthalmology, Texas Retina Associates, Dallas, TX; 4Ophthalmology, Johns Hopkins University, Baltimore, MD; 5Ophthalmology, Cleveland Clinic, Cleveland, OH.

Purpose: Macular leakage associated with cystoid macular edema is a common cause of severe vision loss in patients with uveitis. This study examines the visual acuity outcomes of eyes treated with implant as compared to fellow eyes or those treated with standard of care.

Methods: Patients with macular leakage at baseline were identified from three prospective, randomized clinical trials examining the use of fluocinolone acetonide in the treatment of non-infectious posterior uveitis. Fluorescein angiograms were performed on each patient at regular time points. A centralized reading center analyzed and graded each angiogram. Visual outcomes were examined and analyzed at baseline and the two year time point. Three specific treatment groups were identified and analyzed - those eyes treated with the fluocinolone acetonide implant (IMP), fellow eyes (FEL) of patients with implants and eyes treated with standard of care systemic (SOC) medications.

Results: A total of 250 eyes treated with the IMP device had macular leakage identified at baseline. In comparison, 163 FEL eyes and 59 SOC eyes had baseline macular leakage. The mean baseline logMAR visual acuity was significantly worse in IMP and FEL eyes (.56 in both) vs SOC eyes (.39), (p<.01). IMP eyes had a significantly greater improvement of vision over 24 months (.56 to .41) in comparison to both SOC eyes (.39 to .40) and FEL eyes (.56 to .64), (p<.01). In eyes without evidence of macular leakage at baseline, all groups had some improvement of vision over 24 months: IMP (.41 to .33), FEL (.28 to .24) and SOC (.12 to .09). The amount of improvement was not statistically different between these groups.

Conclusions: In eyes with fluorescein evidence of macular leakage, fluocinolone acetonide device resulted in a statistically significant greater improvement in visual acuity over 24 months vs FEL eyes and SOC eyes. In eyes with macular leakage, the fluocinolone acetonide device may offer superior improvement of visual acuity over 2 years in comparison to eyes treated with standard of care therapy.

Commercial Relationships: Thomas A. Albini, Bausch and Lomb (C), Allergan (C), Genentech (F), Eleven Biotherapeutics (C); Debra A. Goldstein, Bausch and Lomb (C), Bausch and Lomb (R); David Callanan, Alcon (C), Alcon (R), Allergan (C), Allergan (R), Bausch & Lomb (C), Bausch & Lomb (R), Thrombogenics (R), Santen (C), Forsight (I), Regeneron (F); Quan Dong Nguyen, Genentech (F), Regeneron (F), Lux Biosciences (F), Abbott (F), GSK (F), Santen (F), Santen (C), Bausch and Lomb (C), Optos (F), Heidelberg Engineering (F); Sunil K. Srivastava, Bausch and Lomb (F), Bausch and Lomb (C), Novartis (F), Allergan (F)

Support: Bausch and Lomb

Clinical Trial: NCT00407082
Purpose: Cost-effectiveness is usually evaluated by comparing treatment cost with a corresponding gain in a patient-centered outcome like health utility. We estimated change in a measure of health utility (EQ-5D) in participants randomized to receive treatment with flucinolone acetonide implant versus systemic therapy for active or recently active non-infectious intermediate, posterior, or panuveitis as a first step.

Methods: Data from the first 3 years following randomization for individuals enrolled in the Multicenter Uveitis Steroid Treatment (MUST) Trial were evaluated. The follow-up time period was based on the expected lifetime of the implant. The EuroQol EQ-5D questionnaire was scored at 6-month intervals. Linear regression models were fit using generalized estimating equations to account for repeated measurements while adjusting for disease stratum (intermediate v. posterior/panuveitis). Contrasts were used to construct the quality adjusted life years (QALYs), a weighted sum of the EQ-5D at all of the visits. The weighting discounted years 2 and 3 by dividing by 1.03 and 1.03², respectively. Separate analyses were performed for participants with unilateral and bilateral disease due to the large expected discrepancy in upfront cost.

Results: 255 individuals were randomized (31 unilateral and 224 bilateral). The differences in QALYs between the treatment groups were 0.057 (95% CI: 0.033 to 0.148, p = 0.12) for those with bilateral disease and 0.128 (95% CI: 0.033 to 0.290, p = 0.22) for those with unilateral disease, favoring the implant. For unilateral uveitis, there was a slight increase in EQ-5D from baseline (Range: 0.060 to 0.096) among implant patients at each visit that was significant for all visits except the 3 year visit. For bilateral uveitis, there was a slight but not statistically significant decline in EQ-5D from baseline (Range: -0.200 to -0.055) among systemic patients at each visit. There were no detectable trends in the change from baseline among bilateral implant or unilateral systemic participants.

Conclusions: The differences in QALYs by treatment assignment were not statistically significant for either disease cohort. However, the magnitude of the observed values is large enough that the potential for the implant treatment to be cost-effective remains, depending upon the total cost, despite its larger upfront cost.

Commercial Relationships: Elizabeth A. Sugar, None; Alyce E. Burke, None; Lea T. Drye, None; Janet Holbrook, None; John H. Kempen, Alcon (C), Allergan (C), Clearside (C), Can-Fite (C), Lux Biosciences (C), Xoma (C), NEI/NIH (F), FDA (F), Research to Prevent Blindness (F), Mackall Foundation (F), EyeGate Pharma (F), University of Pennsylvania (E); Jennifer E. Thorne, Allergan (C), XOMA (C), Santen (C); Kevin D. Frick, Center for Applied Value Analysis (C), National Association for Eye and Vision Research (C)

Support: EYU10EY014660
Clinical Trial: NCT00132691

534 Corneal Infection/Inflammation II
Thursday, May 09, 2013 10:30 AM-12:15 PM
Exhibit Hall Poster Session
Program #/Board # Range: 5988-5998/A0032-A0042

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ARVO 2013 Annual Meeting Abstracts by Scientific Section/Group – Immunology/Microbiology

global public health problem. Cytokines have profound influence on the activation, development and expansion of T cells that confer protection against HSV-1 and other infectious agents. The JAK/STAT pathway regulates the intensity and duration of cytokine signals and is in turn under feedback regulation by suppressors of cytokine signaling (SOCS) proteins. Recent reports indicate that STAT3 inhibits T-helper cell proliferation and IL-2 production while SOCS3 inhibits Treg proliferation and suppressive functions. In this study we investigated whether STAT3 and SOCS3 contribute to the regulation of cell-mediated immunity against HSV-1 infection.

Methods: We have generated mice with conditional deletion of STAT3 (STAT3KO) or SOCS3 (SOCS3KO) in CD4 and CD8 T cells and infected them by intraperitoneal injection with the HSV-1 strain REpICP0-EGFP (Journal of Virology, 79:10339-10347, 2005). Viremia was measured at different time points post immunization (p.i.) by plaque assay. Splenocytes and lymph node cells were analyzed for the expansion of HSV-1-specific CD8 cells using an anti HSV-1 gB Tetramer-PE antibody and [3H] Thymidine incorporation assay. T cell immunophenotype was characterized by FACSM analysis and cytokine secretion was analyzed by the intracellular cytokine assay.

Results: In comparison to WT mice, HSV-1-specific CD8 T cells were significantly elevated in STAT3KO mice at the peak of the infection while loss of SOCS3 in T cells correlated with substantial decrease in HSV-1-specific CD8 T cell numbers in the SOCS3KO mice.

Conclusions: Our data indicate that STAT3 and SOCS3 may have diametrically opposite effects on cell-mediated immune response to HSV-1 infection, with STAT3 inhibiting the expansion of HSV-1-specific CD8 T cells. As SOCS3 antagonizes Treg expansion and suppressive activities, our data suggests that it may promote the expansion of HSV-1-specific CD8 cells by restraining the inhibitory effects of Tregs. Taken together, targeting STAT3/SOCS3 axis might be a beneficial therapeutic strategy to modulate the CD8-mediated host immunity against HSV-1.

Commercial Relationships: Fatemeh Navid, None; Chenguong Yu, None; Ivy M. Dambuza, None; Gregory M. Frank, None; Charles E. Egwuagu, None

Program Number: 5990 Poster Board Number: A0034
Presentation Time: 10:30 AM - 12:15 PM

Targeting host kinases as potential therapeutic targets for the treatment of herpes keratitis
Oleg Alekseev, Jane Azizkhan-Clifford. Biochemistry & Molecular Biology, Drexel Univ College of Medicine, Philadelphia, PA.

Purpose: Herpes keratitis (HK) is the most common cause of both cornea-derived and infection-associated blindness in the developed world. Despite the availability of potent antivirals, such as acyclovir and famciclovir, a large number of cases are refractory to these agents and develop into permanent corneal damage that necessitates surgical intervention. Importantly, the emergence of acyclovir-resistant strains of herpes simplex virus (HSV) emphasizes the need for the development of novel approaches to combat HK. The purpose of this study is to identify new therapeutic targets against HSV and to assess their antiviral potential in tissue culture experiments, as well as in more sophisticated models of HK. Specifically, we focused on the involvement of ataxia telangiectasia mutated (ATM) and its downstream target Chk2 in facilitating productive HSV infection in corneal epithelium.

Methods: We used two human corneal epithelial cell lines - hTCEpi and HCE - as in vitro models of HK. To generate more physiologically-relevant conclusions, we developed an ex vivo model of HK, where we infected intact human and rabbit corneoscleral buttons that were maintained in organ culture. Using corneas obtained from human donors allowed us to extrapolate our tissue culture findings into a highly relevant experimental system. In addition, we are currently utilizing an in vivo mouse model of ocular herpes to further validate our findings. Small molecule inhibitors of ATM (KU-55933, wortmannin, caffeine) and Chk2 (Chk2 inhibitor II), as well as RNAi against ATM and Chk2, were used to inhibit these two kinases. We used plaque assays and quantitative PCR assays to assess the infectious particle production, genome replication, and transcriptional activity of HSV in corneal epithelium.

Results: Small molecule or RNAi-mediated inhibition of ATM or Chk2 greatly suppressed the replication and transcription of the viral genome, as well as its overall infectious particle production. This was observed in the tissue culture models and, importantly, in organotypically explanted human and rabbit corneas. Ongoing experiments address the molecular mechanisms of ATM activation by the virus, as well as the downstream significance of ATM activation event.

Conclusions: This study identifies two host kinases - ATM and Chk2 - as potential novel therapeutic targets against herpes keratitis.

Commercial Relationships: Oleg Alekseev, None; Jane Azizkhan-Clifford, None

Program Number: 5991 Poster Board Number: A0035
Presentation Time: 10:30 AM - 12:15 PM

Efficacy of HSV1-specific meganucleases in a mouse model of relapsing herpetic keratitis
Marc Labetoulle1,2, Eric E. Gabison3, Antoine Rousseau4, Nicolas Huot2, David Pasdeloup2, Sebastien Barradeau3, Charlotte Mahier4, Benoit Chapelier4, Magali Breckler5. Ophthalmology, Hôpital Bicêtre, South Paris University, Le Kremlin Bicêtre, France; 2Laboratoire de Virologie Moléculaire et Structurale, CNRS UPR 3296, Gif sur Yvette, France; 3Institut de la Vision, Paris, France; 4Genomic Vision, Bagneux, France.

Purpose: While conventional drugs used in the treatment of infections by Herpes simplex virus type 1 (HSV1) does not reduce the burden of latent virus and therefore the risk of viral reactivation, specific endonucleases (such as meganucleases) could be an issue to reduce the relapsing herpetic keratitis in previously treated tissues.

Methods: Three weeks after subconjunctival inoculation of rAAV (recombinant adenovirus-associated) encoding a meganuclease specific to HSV1 (or the Green Fluorescent Protein, GFP), mice were infected in the lip with a wild type strain of HSV1 (SC16) to induce a latent infection in the trigeminal ganglia (TGs) TG. After 28 days, mice were subjected to a reactivation stimulus (heatshock) and then sacrificed. Corneas and TGs were analyzed for the presence of HSV-1 genome and several viral transcripts (LAT, TK and UL18).

Results: In the corneas, as in TG, mice treated with rAAV encoding meganuclease had more copies of viral genomes (viral load) and LAT, TK and UL18 transcripts (signs of viral replication) that control mice, ie treated with rAAV encoding GFP (p = 0.002 to p = 0.0008), suggesting a significant reduction in the activity of viral replication after herpes reactivation stimulus.

Conclusions: The subconjunctival inoculation of rAAV encoding a specific HSV1 meganuclease in ocular tissues seems to reduce the importance of HSV1 reactivation in TGs (the site of herpetic latency) and corneas. These results suggest that specific HSV1 meganuclease could be tested in therapeutic protocols with the aim of reducing the risk of herpes reactivation in the cornea.

Commercial Relationships: Marc Labetoulle, None; Eric E. Gabison, None; Antoine Rousseau, None; Nicolas Huot, None; David Pasdeloup, None; Sebastien Barradeau, Genomic Vision

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**Methods:** Fusarium infection was initiated in Sprague-Dawley rats (n=24) by corneal epithelial abrasion and fitting CL soaked in Fusarium solani 105 CFU/ml for 4 hours. A saline soaked CL was used in control animals (n=6). Rats were treated daily with topical 0.15% Amphotericin B at days 1-7, 10, and 13 post-infection (n=6). 2mg/kg Rapamycin only (n=6), or in combination with Amphotericin B (n=6) was administered intraperitoneally in the same time regimen. The infection was monitored periodically by slit lamp (SL), and SD-OCT imaging correlated well with representative figures of each g.

**Results:** SD-OCT imaging correlated well with SL and revealed thickening of the cornea, endothelial plaques, and inflammatory cells infiltrates. In infected animals the average CT was highly increased, CCT=245.5±69.9; PCT=229.6±62.27, when compared with control, CCT=155.7±11.2; PCT=151.5±10.46. Amphotericin treatment alone remarkably reduced CT to CCT=176.2±31.5; PCT=174.9±29.4, and rapamycin further potentiated the effect of amphotericin to CCT=171.76±46.66; PCT=162.02±36.7. CT reached a peak of CCT=343.1±71.9; PCT=322.41±38.85 at 3 days post-infection. This peak was decreased to CCT=265.20±8.02; PCT=228.9±12.5 by amphotericin, and to CCT=201.7±5.37; PCT=201.64±5.13 by combination amphotericin and rapamycin treatment. Combination therapy resulted also in faster recovery, CT reaching normal values at day 7, while amphotericin only treatment at day 9 post-infection.

**Conclusions:** We were able to quantitatively evaluate by SD-OCT the disease progression and treatment in the CL Fusarium keratitis model. Combination therapy with amphotericin B, and rapamycin efficiently reduced the severity of the infection and the time of recovery.

**Commercial Relationships:** M Livia Bajenaru, None; Andrea Rachelle C. Santos, None; Mitchell J. Falter, None; Marco Ruggeri, Biopigen Inc. (F); Jamie Pomnattam, None; Eleut P. Hernandez, None; Mohamed Abou Shousha, None; Victor L. Perez, Alcon (C), Bausch & Lomb (C), Genentech (C), Cleveland Clinic Foundation (P), Alcon (F), Alcon (R); Darlene Miller, None; Eduardo C. Alfonso, Bio Tissue (C)

**Support:** University of Miami Scientific Awards Committee Pilot Grant (MLB). The Bascom Palmer Eye Institute is supported by a National Institutes of Health Core Grant NIH301430 and an unrestricted grant from Research to Prevent Blindness.
University of Oklahoma Health Sciences Center, Oklahoma City, OK.

**Purpose:** The cationic antimicrobial protein CAP37 mediates proliferation, migration, and adhesion of cultured human corneal epithelial cells (HCEC) and promotes corneal wound healing in the mouse. The purpose of this study was to investigate using multiplex analysis which cytokines were affected during wounding of the cornea, and to identify those cytokines modulated by treatment with CAP37 to determine the mechanism whereby CAP37 contributes to the recruitment of inflammatory cells and healing of the cornea.

**Methods:** In a mouse model of corneal wound healing, a 2 mm diameter abrasion of the corneal epithelium was created utilizing the Algerbrush® II. Wounds were treated at 0 and 16 h with the full length human recombinant CAP37 (250 and 500 ng/ml), or the vehicle. Wounds were visualized with fluorescein staining at 0, 16 and 24 h, and measured using ImageJ software. Re-epithelialization was assessed by histology, infiltration of inflammatory cells was analyzed by immunohistochemistry, and the cytokine profile was investigated by multiplex analysis.

**Results:** An accelerated wound closure was found in corneas treated with rCAP37. Re-epithelialization of the corneal epithelium in rCAP37 treated wounds was complete by 24h. Immunohistochemistry revealed greater neutrophil infiltration in treated than untreated wounded corneas. Monocyte chemoattractant protein-1 (MCP-1), granulocyte colony-stimulating factor (GCSF), keratinocyte-derived cytokine (KC), interferon gamma-induced protein 10 (IP-10), leukemia inhibitory factor (LIF), and interleukins 5 (IL-5) and 9 (IL-9) were found to be induced by wounding and were modulated by CAP37 treatment. In general, CAP37 appeared to differentially modulate the chemokines involved in monocyte and neutrophil migration.

**Conclusions:** These data demonstrate that CAP37 facilitates accelerated corneal wound healing in vivo and modulates the release of cytokines in the cornea. The recruitment of inflammatory cells in corneal wound healing remains controversial. These findings suggest that recruitment of neutrophils may be critical for corneal reepithelialization during the acute phase, implying that CAP37 has the potential for use as a therapeutic for corneal injuries.

**Commercial Relationships:** Anne Kasus-Jacobi, None; Gina L. Griffith, None; Megan R. Lerner, None; H A. Pereira, None

**Support:** NIH grant EY015534 and NIH grant ST32AI007633

**Program Number:** 5995 Postter Board Number: A0039
**Presentation Time:** 10:30 AM - 12:15 PM

**Expression of PAR1 and 2 on Human Corneal Epithelial Cells and Induction of Pro-inflammatory Cytokine Secretion by Acanthamoeba MIP-133 and aPA Hassan Alizadeh 1, 2, Mahshid Abdi 1, 2, 1Cell Biology and Anatomy, UNTHSC Fort Worth TX, Fort Worth, TX; 2North Texas Eye Research Institute, UNTHSC, Fort Worth, TX.

**Purpose:** Protease activated receptors (PARs) are G-protein coupled receptors, which initiate inflammatory responses when activated by serine proteinase. We hypothesized that MIP-133 (mannose induced protein) and aPA (Acanthamoeba plasminogen activator) activate PARs on the corneal epithelial cells, resulting in signal transduction and production of pro-inflammatory cytokines that modulate corneal inflammation in Acanthamoeba keratitis.

**Methods:** Human corneal epithelial cells were incubated with or without purified 15 ug aPA or 7.5 ug MIP-133 for 24 h. As a positive control the cells were incubated with a 10 uM-human thrombin, PAR1 agonist. Corneal epithelial cells were also stimulated with a PAR2 agonist such as 10nM bovine trypsin, and 100 uM peptide corresponding to the receptor-activating tethered domains of PAR2 (SLIGRL-NH2). The expression of PAR1 and PAR 2 mRNA in human corneal epithelial cells was examined by RT-PCR. IL-6 and IL-8 gene expression was determined by RT-PCR and the levels of secreted IL-8 were determined by ELISA.

**Results:** Stimulation of corneal epithelial cells with a PAR1 agonist (thrombin) and aPA or PAR2 agonist (SLIGRL-NH2), trypsin and aPA resulted in upregulation of PAR1 and PAR2 mRNA and a significant IL-8 (P<0.05) protein production (10-13 fold).

Thrombin failed to upregulate IL-6 and IL-8 genes expression in HEC cells, however, MIP-133 induced upregulation of IL-8 mRNA expression. Thrombin and MIP-133 were able to induce IL-8 protein production by corneal epithelial cells (2 fold).

**Conclusions:** MIP-133 and aPA interact with PAR1 and PAR2 on HCE cells and activate pro-inflammatory cytokines/chemokines from HCE cells. Disruption of PAR1 and PAR2 activity might have a major impact on preventing inflammatory responses in Acanthamoeba keratitis and bacterial infection.

**Commercial Relationships:** Hassan Alizadeh, None; Mahshid Abdi, None

**Support:** NIH Grant EY09756

**Program Number:** 5996 Poster Board Number: A0040
**Presentation Time:** 10:30 AM - 12:15 PM

**Role of ST2 Signaling in IL-33 Induced Inflammation in Human Corneal Epithelium Jing Lin, Guiqiu Zhao, Lili Zhang, Ophthalmology, the Affiliated Hospital of Medical College, Qingdao University, Qingdao, China.

**Purpose:** Interleukin (IL) 33, a member of IL-1 cytokine family, is well known to promote Th2 type immune responses by signaling through its receptor ST2. However, it is not clear whether ST2 is expressed by mucosal epithelium, and how it responds to IL-33 to induce inflammatory mediator. This study was to explore the expression and functional role of ST2 signaling in IL-33 stimulated production of proinflammatory cytokines by human corneal epithelium.

**Methods:** Human corneal tissues and cultured primary human corneal epithelial cells (HCECs) were treated with IL-33 in different concentrations without or with different inhibitors to evaluate the expression, location and signaling pathways of ST2 in regulating production of inflammatory cytokines and chemokine. The mRN consequence expression was determined by reverse transcription and real time PCR, and protein production was measured by ELISA, immunohistochemical and immunofluorescent staining.

**Results:** In ex vivo donor corneal epithelium, ST2 protein was detected to be located in superficial layers, and its immunoreactivity was enhanced by multiple layers of corneal epithelium exposed to IL-33. Primary HCECs also expressed ST2 at both mRNA and protein levels, which were stimulated in a dose-dependent manner when the cells exposed to IL-33. IL-33 significantly stimulated production of inflammatory cytokines (TNF-α, IL-1β and IL-6) and chemokine IL-8 by HCECs at both mRNA and protein levels. The stimulated production of these inflammatory mediators by IL-33 was blocked by ST2 antibody or soluble ST2 protein. Interestingly, IkB-α inhibitor BAY11-7082 or NF-κB activation inhibitor quinazoline blocked the nuclear translocation of NF-κB p65 protein, and further suppressed the production of these inflammatory cytokines and chemokine induced by IL-33.

**Conclusions:** These findings demonstrate for the first time that ST2 is present in human corneal epithelial cells, and ST2 signaling plays an important role in regulating IL-33 induced inflammatory responses, suggesting that IL-33 and ST2 could become novel molecular targets for the intervention of inflammatory diseases in ocular surface.

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Commercial Relationships: Jing Lin, None; Guiqiu Zhao, None; Lili Zhang, None
Support: National Natural Science Foundation of China (81170825)

Program Number: 5997 Poster Board Number: A0041
Presentation Time: 10:30 AM - 12:15 PM

Antimicrobial Peptides in the In Vitro Corneal Response to Fungal Pathogens
Satya Sree N. Kolar, Hasna Baidouri, Alison McDermott, Optometry and Vision Sciences, University of Houston, College of Optometry, Houston, TX.

Purpose: Antimicrobial peptides (AMPs), including defensins and cathelicidins form an integral part of the initial mucosal defense but little is known about their actions against fungal pathogens. In an effort to understand their role during fungal keratitis, we investigated AMP efficacy against Candida albicans (CA) and Fusarium solani (FS) and human corneal epithelial cell (HCEC) AMP expression following fungal challenge in-vitro.

Methods: Antifungal activity was tested using broth micro-dilution assays. Fungal cells were incubated with 0-50 µg/ml of cathelicidin LL-37 or defensin hBD-2 for 24h at 30°C then reaction mixtures plated to determine fungal growth. Sytox green uptake was used to determine if AMP antifungal activity occurred via membrane disruption. For the Sytox assay, fungal cells were pre-incubated with 1 µM sytox green, 25 µM LL-37 added and fluorescence intensity recorded over time. To test AMP expression in response to fungal or TLR2 treatments, HCEC (primary cultures and cell lines) were exposed to heat-inactivated CA or FS or 10 µg/ml TLR2 agonist zymosan for 24h. RT-PCR and immunoblotting were performed to determine the expression of hBD-2 and LL-37. Expression of Dectin-1, a fungal pattern recognition receptor, was also investigated by RT-PCR.

Results: Antifungal assays showed that 25 µg/ml LL-37 and 10 µg/ml hBD-2 resulted in >3 log units of killing of CA and FS (n=2). LL-37 promoted uptake of Sytox green indicating an antifungal mechanism involving membrane disruption. Exposure of immortalized HCECs to heat-inactivated FS and CA upregulated the expression of LL-37 by 2.78 and 1.86 log2 fold and of hBD-2 by 3.78 and 2.13 log2 fold compared to controls (n=3). Additionally, immunoblotting showed markedly increased LL-37 and hBD-2 protein secretion in supernatants of fungal treated cells compared to control. Primary cultures showed comparable data. RT-PCR showed robust HCEC expression of the functional isoform of dectin-1. Zymosan upregulated HCEC expression of hBD-2 and LL-37 by 1.78 and 2.15 log2 fold compared to untreated cells suggesting a role for TLR2 in AMP modulation.

Conclusions: LL-37 and hBD-2 have potent antifungal activity and were upregulated in HCEC in response to fungal challenge, likely via activation of dectin-1 and TLR2. These in vitro studies suggest that AMPs are an important defence against fungal keratitis in vivo.

Commercial Relationships: Satya Sree N. Kolar, None; Hasna Baidouri, None; Alison McDermott, None
Support: NIH grants EY13175 (AMM), UHCO Vision Grant to Advance Research (AMM), EY07551 (UHCO CORE grant)

Program Number: 5998 Poster Board Number: A0042
Presentation Time: 10:30 AM - 12:15 PM

Corneal epithelial-cell necrosis and apoptosis directly induced by fungal isolates from patients with keratomycosis
Pablo L. Goldschmidt, Radhia Zemihi, Djida Ghoubay-Benallaoud, Cyril Temstet, Laurence Batellier, Vincent Borderie, Laurent Laroche, Christine Chaumeil, 1Laboratoire, Centre Hospitalier National d’Ophtalmologie des Quinze-Vingts, Paris, France; 2Service 5, Centre Hospitalier National d’Ophtalmologie des Quis, Paris, France.

Purpose: Keratomycosis (KM) refers to an infective process associated to outdoor occupations and contact lenses wear. The main agents triggering KM (Fusarium, Aspergillus and Candida) can be found in the air, soil, dust and plants. Without treatment, the inflammation may lead to corneal scarring, anterior chamber infection, perforation, and vision loss. The goal of this work was to assess if Fusarium solani, Aspergillus fumigatus and Candida albicans isolated from patients with KM are able per-se to induce corneal epithelial cell necrosis, cytotoxicity or to trigger programmed corneal cell death (apoptosis).

Methods: Fungal suspensions were introduced in microwells containing phenotypically-characterized human corneal epithelial cells. Non-viable Fungi served as negative controls. Viability was measured using the AFC substrate, which penetrates in cells with intact membranes and active metabolism. Fungal cytotoxicity was assessed following the incorporation by dead cells of the ApoTix-Glo TM luminescent reactant. Necrosis was confirmed with bis-AAF-R110, which enters through non-viable cell-pores and releases a fluorophore. Apoptosis was studied by activation of the cascade Caspase Glo 3/7 and cleavage of the luciferine labelled Z-DEVD substrate.

Results: Fusarium solani directly reduced corneal cell viability after 1 h of contact with human cells. After 2 h, viability reduction induced by Fusarium solani was 52%; Candida albicans 55% and Aspergillus fumigatus 43%. Significant fungal induced corneal cell-necrosis rates were confirmed after 2h (Candida albicans: 20%; Aspergillus fumigatus: 40% and Fusarium solani: 60%). Aspergillus fumigatus, Candida albicans and Fusarium solani induced detectable levels of corneal-cell apoptosis after 1 h. Apoptotic rates were significantly higher for all the species while comparing 1 h with 2 h of fungal-cell contact.

Conclusions: The results of this study show for the first time that viable Fungi isolated from patients with KM induce human corneal epithelial-cell necrosis and apoptosis. In addition to the classic anti-fungal and inflammatory agents, it appears necessary that the cytotoxic effects directly induced by Fungi should be integrated in the future development of therapeutic strategies to improve visual prognosis of people with KM.

Commercial Relationships: Pablo L. Goldschmidt, None; Radhia Zemihi, None; Djida Ghoubay-Benallaoud, None; Cyril Temstet, None; Laurence Batellier, None; Vincent Borderie, None; Laurent Laroche, None; Christine Chaumeil, None

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