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Research Article

Topical Corticosteroids Decrease IL-25 Expression by Immunohistochemistry

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Allergic rhinitis · Interleukin · Eosinophilia · Sinusitis

Abstract
Background: Interleukin-25 (IL-25) is an important contributing factor in the pathogenesis of allergic rhinitis. It leads to increasing peripheral and infiltrating eosinophilia as well as serum IgE, IgG, and Th2 cytokines (IL-4, IL-5, IL-13), which are responsible for the allergic symptoms. Intranasal steroids (INS) are effective in treating allergic rhinitis, but their effect on IL-25 release has not been studied. We aimed to study the link between IL-25 and the pathophysiology of allergic rhinitis as well as the effect of INS on its release. ***Methodology:*** This was a cohort, prospective, nonrandomized study that included 60 patients, 35 allergic rhinitis patients and 25 controls. We studied the effect of INS on IL-25 release. ***Results:*** Of allergic rhinitis patients 68.6% had strong cytoplasmic stain of IL-25 in the epithelial layer, while 25.7% had intermediate stain. INS caused significantly reduced IL-25 stain as only 14.3% of patients had intermediate stain and 85.7% had weak stain. Moreover, a correlation was found between nasal smear eosinophilia and the degree of IL-25 staining in the epithelial layer. ***Conclusion:*** Intranasal corticosteroids appear to be effective in the downregulation of IL-25, which may explain some of the utility of intranasal corticosteroid treatment in improving allergic rhinitis symptoms.

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Introduction

The allergic response has early and late phases. The early response occurs in sensitized individuals due to the response of mast cells to allergens. In the late response, the inflammatory cells such as eosinophils, basophils, mast cells, and T-lymphocytes migrate to the nasal mucosa [1, 2]. A variety of cytokines are released by these inflammatory cells including leukotrienes, kinins, histamine and interleukins, which results in facilitating the infiltration of eosinophils, T-lymphocytes, and basophils into the nasal mucosa and continuation of the symptoms [3]. Interleukin-25 (IL-25) was presented in 2001 by Lee et al. [4] as a cytokine that shares sequence similarity with the IL-17 family. Many studies have clarified its role in allergy. Yao et al. [5] have pointed that it can be produced by human eosinophils and basophils in allergic patients. In addition, its receptor (IL-17RB) was found to be expressed on Th2 cells and help in its differentiation and on the peripheral basophils in allergic rhinitis (AR) patients [6]. Binding of IL-25 to its receptor leads to further production of specific Th2 cytokines IL-4, IL-5, and IL-13, increased serum IgE and blood eosinophilia [7, 8]. Besides, an elevated level of IL-25 mRNA was found in the nasal epithelium of AR patients. These findings suggest that IL-25 has an important role in the pathogenesis of AR [9, 10].

On the other hand, intranasal corticosteroids (INS) inhibit both early and late responses. They also decrease IgE production and eosinophilia by inhibiting the secretion of IL-4, IL-5, and IL-13. Some studies also demonstrated their effect on lowering IL-17 [11, 12]. Our aim was to study the effect of INS on the release of IL-25 and consequently its role in the control of AR symptoms.

Patients and Methods

This was a cohort, prospective, nonrandomized study. Data were collected from patients attending Otorhinolaryngology outpatient clinic of Fayoum University Hospital, during the period from July 2015 to July 2017. Patients were divided into two groups.

Group A consisted of 35 patients presenting with AR symptoms who did not use topical corticosteroids at least 4 weeks before the first visit. They were asked to use INS in the form of mometasone furoate nasal spray for 3 months. Group B, the control group, consisted of 25 candidates for septoplasty surgery, without any symptoms suggestive of AR. Exclusion criteria included cases with allergic fungal sinusitis, aspirin sensitivity, and those that underwent endoscopic sinus surgery earlier. All patients were subjected to detailed history taking, endoscopic examination, CBC, nasal smear eosinophilia (NSE), skin prick test, CT scan, and nasal biopsy. The severity of nasal symptoms (i.e., nasal obstruction, rhinorrhea, and sneezing) was graded on a 4-point scale as follows; 0 = no symptoms, 1 = mild, 2 = moderate, 3 = severe. Nasal smear was performed to count eosinophils. The count was positive if ≥10 eosinophil cells were detected by high-power field (HPF). Biopsies (4 × 3 mm) were harvested from the nasal mucosa of the anterior end of the inferior turbinate under local anesthesia at the outpatient clinic twice, one at the first visit and the other after 3 months of INS. Biopsies from the control cases were taken under general anesthesia during septoplasty surgery. The samples of nasal biopsies were sent for immunohistochemical study using primary antibody anti-IL-25 (IgG1 rabbit monoclonal, 1:50 to 1:200 dilutions, US Biological 221212, clone C19orf10; US Biologic Life Sciences, USA). Slides were stained with avidin-biotin complex-alkaline phosphatase. The release of IL-25 was graded subjectively according to the degree of positivity and intensity of the stain all over the slide: 0 = negative; up to 30% = weak stain; 30–60% = intermediate stain; and 60–100% = strong stain.

Data were collected, tabulated, and statistically analyzed using SPSS software statistical computer package version 18. An independent t test was used to compare both groups. A dependent t test was performed to test changes in IL-25 before and after treatment, while the χ^2 test was used as a test of significance. $p \leq 0.05$ was considered significant.

Table 1. The severity of nasal symptoms in both groups

Grade	Group A (allergic rhinitis)		Group B (control)
	before treatment	after treatment	
Normal, *n* (%)	0 (0)	8 (22.86)	25 (100)
Mild, *n* (%)	2 (5.71)	23 (65.71)	0
Moderate, *n* (%)	12 (34.29)	3 (8.57)	0
Severe, *n* (%)	21 (60)	1 (2.86)	0
Total	35	35	25

Table 2. Results of NSE

	Group A (allergic rhinitis)		Group B (control)
	before treatment	after treatment	
≥10/hpf, *n* (%)	30 (85.71)	5 (14.29)	0 (0.0)
<10/hpf, *n* (%)	5 (14.29)	30 (85.71)	25 (100)
Total	35	35	25

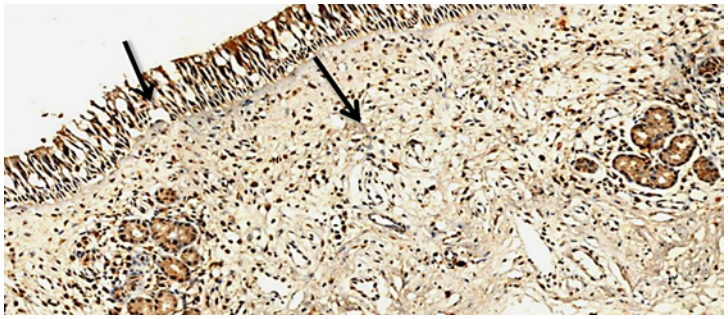

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Fig. 1. Nasal mucosa section showed strong positivity for IL-25 antibody (black arrows) in both epithelium and subepithelium. ×100.

Results

This study was performed on 60 patients. The first groups were 22 males and 13 females with an average age of 25.2 years, while the control group was 14 males and 11 females with an average age of 25.4 years. The severity of nasal symptoms is summarized in Table 1. Sixty percent of AR patients (n = 21) had severe symptoms that affected their daily life, while only 5.71% of patients (n = 2) had mild symptoms. After 3 months of INS, 22.86% of patients (n = 8) had complete relief of symptoms, 65.71% had markedly improved (n = 23), while only 8.57% of patients (n = 3) had moderate symptoms, and 2.86% had persistent severe symptoms (n = 1). The NSE showed that 85.71% of AR patients (n = 30) had positive results (<10 eosinophils/HPF) but only 14.29% had negative results (<10 eosinophils/HPF) (n = 5). This result denotes the correlation between the allergic process and the eosinophilic count. After 3 months of INS, there was marked reduction in the eosinophilic count as only 14.29% of patients (n = 5) had persistent positive results. The entire control group had negative results (Table 2). There was statistically significant difference ($p < 0.0001$) in NSE before and after treatment in group A. The mean count of NSE was 12.74/HPF and became 6/HPF after nasal steroids. This result also reflects the effect of INS on eosinophilia count and the allergic process in general.

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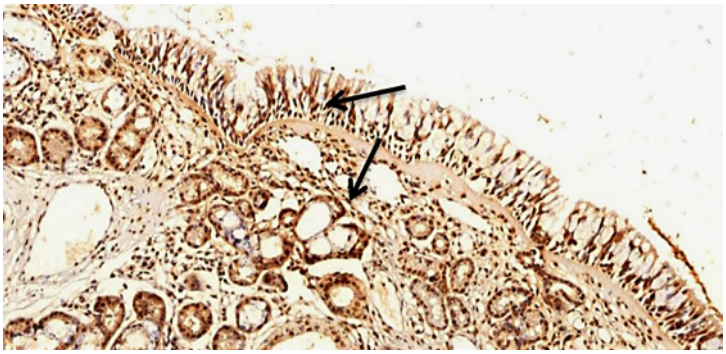

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Fig. 2. Another nasal mucosa section showed strong positivity for IL-25 antibody (black arrows) in both epithelium and subepithelium (higher magnification). ×200.

Table 3. Results of the release of IL-25 in the epithelial layer

Grade	Group A (allergic rhinitis)		Group B (control)
	before treatment	after treatment	
Negative (0%), *n* (%)	0 (0.0)	0 (0.0)	23 (92)
Weak (up to 30%), *n* (%)	2 (5.71)	30 (85.7)	2 (8)
Intermediate (30–60%), *n* (%)	9 (25.71)	5 (14.3)	0 (0)
Strong (60–100%), *n* (%)	24 (68.58)	0 (0.0)	0 (0)
Total	35	35	25

Table 4. Results of the release of IL-25 in the subepithelial layer

Grade	Group A (allergic rhinitis)		Group B (control)
	before treatment	after treatment	
Negative (0%), *n* (%)	0 (0.0)	0 (0.0)	23 (92)
Weak (up to 30%), *n* (%)	5 (14.3)	28 (80)	2 (8)
Intermediate (30–60%), *n* (%)	10 (28.6)	7 (20)	0 (0)
Strong (60–100%), *n* (%)	20 (57.1)	0 (0.0)	0 (0)
Total	35	35	25

Immunohistochemistry Examination

In AR patients, 68.58% (*n* = 24) had strong cytoplasmic stain of IL-25 in the epithelial layer, while 25.71% (*n* = 9) of patients had intermediate stain, and limited cases had weak stain (5.71%, *n* = 2) (Fig. 1). On the other hand, after administration of INS for 6 months, an obvious drop of the cytoplasmic stain occurred as only 14.3% (*n* = 5) of patients had intermediate stain and 85.7% (*n* = 30) had weak stain. Strong stain was no longer noticed. Most of the control cases (92%, *n* = 23) had negative stain (Table 3) (Fig. 2). The mean intensity of IL-25 stain before treatment was 70% (range from 40–80%), while after INS, it became 29.9% (range 10–50%). That drop was statistically significant ($p < 0.0001$). The results of the cytoplasmic stain of IL-25 in the subepithelial layer were nearly the same as epithelial layer, as shown in Table 4. Before treatment, 57.1% (*n* = 20) of patients had strong stain and 28.6% (*n* = 10) had intermediate stain, and only 14.3% (*n* = 5) had weak stain. While after treatment,

Table 5. Correlation between nasal smear eosinophilia (NSE) and the degree of IL-25 staining in the epithelial layer before and after medical treatment

IL-25 intensity in epithelial layer (IHC)	NSE			
	before treatment		after treatment	
	positive >10 (*n* = 30)	negative <10 (*n* = 5)	positive >10 (*n* = 5)	negative <10 (*n* = 30)
Negative, *n* (%)	0 (0.0)	2 (40)	2 (40)	28 (93.3)
Intermediate, *n* (%)	7 (23.3)	2 (40)	3 (60)	2 (6.7)
Strong, *n* (%)	23 (76.7)	1 (20)	0 (0.0)	0 (0.0)

80% (*n* = 28) of patients had weak and 20% (*n* = 7) had intermediate stain. Most of the control group (92%, *n* = 23) showed negative stain (Fig. 1, 2). Also, the mean intensity of IL-25 stain before treatment was 61.43% (range 35–65%), while after INS it was reduced to 31.43% (range 10–60%). That drop was statistically significant ($p < 0.0001$). A marked correlation was found between NSE and the degree of IL-25 staining in the epithelial layer. Before medical treatment, 30 patients had positive NSE, 76.7% of them (*n* = 23) had strong stain and increase in the expression of IL-25 in the epithelial layer, and 23.3% (*n* = 7) had intermediate intensity. Five patients with negative nasal smear showed less expression of IL-25 (Table 5). After INS administration, 30 patients had negative NSE, 93.3% of them (*n* = 28) had weak IL-25 stain, while only 6.7% (*n* = 2) had intermediate stain. On the other hand, 5 patients with persistent positive nasal smear had weak and intermediate staining. This correlation reflects the relation between the improvement in the AR symptoms and the reduction of IL-25 by local steroid treatment.

Discussion

This study is tried to emphasize the effect of INS on the release of IL-25 and consequently its effect on controlling AR symptoms. Many studies have pointed to the role of IL-25 in AR. It acts as a link between the innate and adaptive airways [9]. In their experimental study, Fort et al. [13] proved that IL-25 production leads to increasing peripheral and infiltrating eosinophilia as well as serum IgE, IgG, and Th2 cytokines (IL-4, IL-5, IL-13). These cytokines are responsible for exacerbation of allergic symptoms, mucous production, and airway hyperresponsiveness. Our results support that theory. We have found that nearly 70% of AR patients had strong cytoplasmic stain of IL-25, and 26% had intermediate stain, while 92% of the control had negative stain. Consistent with our findings, Kim et al. [9] and Shin et al. [7] found significant elevation of IL-25 expression and the levels of mRNAs in the nasal epithelium of AR patients in relation to controls. Interestingly, Kim et al. [9] have also found a relationship between IgE and IL-25-immunoreactive cells in allergic patients. There is no doubt that INS are now a powerful treatment option for AR [14]. They reduce all AR symptoms, and are thus used as a first-line treatment in many cases [15]. They have multiple modes of action [1, 15]. They can reduce IgE production and eosinophilia by inhibiting the secretion of IL-4, IL-5, and IL-13 and lower IL-17 [12, 15]. To our knowledge, our study is the first to investigate their effect on IL-25.

We observed that INS significantly reduced the IL-25 expression in the epithelium and subepithelium of AR patients ($p < 0.0001$) after 6 months of treatment. Reduction of IL-25 was associated with improvement of all allergic symptoms. These finding reflect the effect of

INS on IL-25. INS may reduce its release from the nasal epithelium or the Th2 cells, thus downregulating the production of cytokines (IL-4, IL-5, IL13) and IgE. In addition, INS may block the action of IL-25 on the target cells, thus reducing the allergic symptoms. It should be noted that, blocking the action of IL-25 reduces the allergic reaction as previously reported [7, 16, 17]. We also noticed that eosinophils in nasal smear were higher in AR patients than the controls. These results were consistent with other reports [18, 19]. INS were efficient in significantly reducing NSE ($p < 0.0001$). We should mention that Patel and Nagpal [19] have also found a good correlation between severity of allergic symptoms and NSE. We conclude that NSE are a good diagnostic test for AR as well as a good means of monitoring the severity of AR symptoms. Interestingly, in this study a new correlation was found between NSE and the release of IL-25 in AR patients. NSE-positive patients showed strong and intermediate IL-25 stain. On the other hand, INS reduced NSE and IL-25 release as well reduced AR symptoms. Lam et al. [10] also found a correlation between NSE and the degree of IL-25 staining, but they did not study the effect of local steroids on eosinophils or IL-25. The previous discussion confirmed the important link between IL-25 and AR. It also clarified the role of INS in the release of IL-25. Unfortunately, this paper did not discuss the mechanism of action of INS on IL-25. We opened a door for future studies to clarify this mechanism.

Conclusion

Intranasal corticosteroids appear to be effective in downregulating IL-25, which may explain some of the utility of intranasal corticosteroid treatment in improving AR symptoms.

Statement of Ethics

This study was approved by the local ethical committee. Written consent was obtained from all patients.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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