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Mesenchymal stem cell transfusion for desensitization of positive lymphocyte cross-match before kidney transplantation: outcome of 3 cases

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Abstract

Objectives: Donor specific antibodies (DSA) and a positive cross-match are contraindications for kidney transplantation. Trials of allograft transplantation across the HLA barrier have employed desensitization strategies, including the use of plasmapheresis, intravenous immunoglobulins, anti-B-cell monoclonal antibodies and splenectomy, associated with high-intensity immunosuppressive regimens. Our case 1 report suffered from repeatedly positive lymphocyte cross match after 1st renal transplantation. Graft nephrectomy could not correct the state of sensitization. Splenectomy was done in a trial to get rid of the antibody producing clone. Furthermore plasmapheresis with low dose IVIG could not as well revert the state of sensitization for the patient.

Material and methods: About 50 millions donor specific MSCs were injected to the patient.

Results: MSCs transfusion proved to be the only procedure which could achieve successful desensitization before performing the second transplantation owing to their immunosuppressive properties.

Conclusion: This case indicates that DS-MSCs is a potential option for anti-HLA desensitization. In cases 2 and 3 IV DS-MSCs transfusion was selected from the start as a successful line of treatment for pre renal transplantation desensitization to save other unnecessary lines of treatment that were tried in case 1.

Introduction

Sensitization to human leucocyte antigens (HLA) may be induced by previously administered blood products, pregnancy or prior transplantation; it represents an important barrier to renal transplantation. Procedures adopted to achieve desensitization include single high-dose intravenous immunoglobulin (IVIG) (1) or low-dose IVIG in combination with antibody reduction *via* plasmapheresis, with or without splenectomy (2–4). Mesenchymal stem cells (MSCs), amongst all their properties, have an important immunomodulatory effect and have been shown to inhibit anti-HLA allo-antibody production *in vitro* (5).

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Case reports

Case 1

A male patient, 34 years of age, was highly sensitized to donor-specific antibodies (DSA) with positive cross-match and panel reactive antibodies (PRA), with blood group B +ve, HLA A -,-; B 18, 35 and DR 4, -. His first donor HLA typing was HLA A -,-; B 35,12 and DR 4,-, HLA matching 2 of 6 and mismatching 1 of 6. Second donor HLA typing was HLA A -,-; B 35,14 and DR 4,-; HLA matching was 2 of 6 (2/6) and mismatching 1 of 6 (1/6). The first renal transplant was performed in July 2005 when he suffered chronic allograft dysfunction (CAD) and by December 2005 he had returned to regular dialysis therapy (RDT). The patient's dithiothreitol (DTT) cross-match 2006 to May 2007 was 20% positive for allogenic B lymphocytes at room temperature and at 37 °C; however, cross-match for T cells was negative. PRA was 33% against class I HLA and zero% against class II; graft nephrectomy was performed in May 2007. The patient's cross-match was persistently positive even after graft nephrectomy, and splenectomy was performed in September 2007 – but positive cross-match persisted. The patient underwent desensitization with 7 sessions of plasma exchange with low-dose intravenous immunoglobulin (IVIG; 100 mg/kg after each plasmapheresis session) in December 2007, but the positive cross-match still persisted. Thus, intravenous

donor-specific mesenchymal stem-cell (IV DS-MSCs) transfusion was performed, in 2 sessions 1 week apart, in February 2008. Two months later, cross-match was repeatedly negative (3 times). Anti-thymocyte globulin (ATG) 6 mg/kg body weight was received by the patient as an induction immunosuppressant. A second renal transplant was performed in July 2008 and triple post-transplant maintenance immunosuppression with cyclosporine (CsA), mycophenolate mofetil (MMF) and prednisolone (PRD) was administered. Follow-up of the patient by clinical assessment and laboratory investigation, including serum creatinine, creatinine clearance, cross-match and PRA testing was carried out. Serum creatinine improved to levels of around 0.8 mg/dl and creatinine clearance reached 85 ml/min; these values persisted until the time of reporting; cross-match and PRA were repeatedly reported to be negative.

Case 2

A male patient, 62 years of age, was diagnosed to have end-stage renal disease (ESRD) of unknown aetiology. He received RDT for 4 years and his blood group was A1. He suffered from ischaemic heart disease, and coronary artery bypass grafting was performed in July 2009. His donor was an unrelated female, 22 years of age, her blood group also being A1. The patient's HLA typing was HLA A 2, 19; B 21, 15 and DR 4, 13. HLA matching was 4 of 6 (4/6) and mismatching was 1 of 6 (1/6). His donor HLA typing was HLA A 19, -; B 21, 12 and DR 4, 13. HLA matching was 4 of 6 (4/6) and mismatching was 1 of 6 (1/6). DTT cross-match in October 2009 was 15% positive for allogenic B lymphocytes at 4 °C and at room temperature, and 10% positive for allogenic T lymphocytes at 4 °C; PRA were 23.7% against class I HLA and 7.8% against class II. Basiliximab (anti CD25) (20 mg) was administered on days zero 4 as induction therapy. Triple maintenance immunosuppression with CsA, MMF and PRD was received by the patient and slowly declining serum creatinine, reaching 1.98 mg% by day 9, was elicited. Graft biopsy was performed and revealed acute tubular injury. Trough cyclosporine level was adjusted between 200 and 250 ng/ml and serum creatinine level continued to decline to 1.07 mg% by January 2012. DTT cross-match and PRA reverted to negative values after renal transplantation continuing to the present. The patient had post-transplantation hyperglycaemia controlled by insulin (Table 1).

Case 3

A female child, 11 years of age, was diagnosed as ESRD, also being obese (BMI 43), of mental subnormality (IQ 70), with epilepsy and retinitis pigmentosa diagnosed as oculo-cerebro-renal syndrome; a younger brother suffered from a similar condition (CKD stage II). The patient had been on RDT since November 2009 and was scheduled for kidney transplantation from her mother. Her blood group was O positive; HLA typing A 1, 2; B 44, 47 and DR 4, 11 and HLA matching was 4 of 6 (4/6) and mismatching was 2 of 6 (2/6). Donor HLA typing was HLA A 1, 36; B 15, 47 and DR 4, 11 with HLA matching 4 of 6 (4/6) and mismatching 2 of 6 (2/6). Pre-transplant induction therapy with single-dose basiliximab (20 mg) was administered on day zero. The patient's 161 DTT cross-match elicited alloantibodies against B and T cells with 20% positivity at 4 °C and 15% against B cells at room temperature, in January 2010. PRA was 57.5% against class I HLA and 13%

Table 1. Cross-match results before and after MSC transfusion (case 2)

	4 °C		Room temperature		37 °C	
	T lymphocytes	B lymphocytes	T lymphocytes	B lymphocytes	T lymphocytes	B lymphocytes
20/10/2009	10%	15%	-ve	15%	-ve	-ve
15/11/2009	10%	15%	-ve	20%	-ve	-ve
07/12/2009	10%	15%	-ve	20%	-ve	-ve
07/12/2010	PRA 23.7% against Class I HLA, 7.8% against Class II					
04/01/2010	Donor specific MSC					
18/01/2010	10%	10%	-ve	10%	-ve	-ve
31/01/2010	5%	5%	5%	5%	-ve	-ve
07/02/2010	-ve	-ve	-ve	-ve	-ve	-ve
09/03/2010	-ve	-ve	-ve	-ve	-ve	-ve
17/03/2010	-ve	-ve	-ve	-ve	-ve	-ve
27/03/2010	-ve	-ve	-ve	-ve	-ve	-ve
29/03/2010	PRA zero% against Class I HLA, zero% against Class II					
01/04/2010	Transplantation					

Table 2. Cross-match results before and after MSC transfusion (case 3)

	4 °C		Room temperature		37 °C	
	T lymphocytes	B lymphocytes	T lymphocytes	B lymphocytes	T lymphocytes	B lymphocytes
9/1/2010	20%	20%	-ve	15%	-ve	-ve
24/1/2010	PRA 57.5% against Class I HLA, 13% against Class II					
1/3/2010	20%	30%	-ve	15%	-ve	-ve
7/4/2010	Donor specific MSC					
20/4/2010	-ve	-ve	-ve	-ve	-ve	-ve
26/4/2010	PRA 7.5% against Class I HLA, zero% against Class II					
7/5/2010	-ve	-ve	-ve	-ve	-ve	-ve
15/5/2010	Transplantation					

against class II. DS-MSCs were transfused in April 2010. Triple maintenance immunosuppression with CsA, MMF and PRD was received by the patient. Two months later, cross-matching was found to be negative and DSA reduced to 7.5% for class I HLA and had reached zero for class II (Table 2).

Declaration of ethics

This study was approved by the review board of Kasr Al Aini hospital and written informed consent was obtained from all patients according to the Helsinki guidelines of research ethics.

Materials and methods

Cross-matches

All patients had lymphocyte cross-match by complement-dependent micro-lymphocytotoxicity (CDC) according to the method described by (6).

Panel reactive antibodies

Panels made of fresh lymphocytes were used to screen a serum sample; panels consisted of cells from around 30 donors. One microlitre serum was added per well of Terasaki plates, and was incubated with cells for 60 min. Incubation for a further 30 min with complement, followed by staining, and reading was performed.

Mesenchymal stem cell transfusion

Fifty millilitre bone marrow (BM) was aspirated from donor-specific iliac bone under local anaesthesia and placed in sterile tubes with preservative-free heparin. Bone marrow aspirate (BMA) was diluted with PBS containing 2 mM EDTA (PBS/EDTA buffer). Mononuclear cells (MNCs) were separated by density-gradient centrifugation at 300 g for 20 min. MNCs were seeded at 2×10^5, and plated in Dulbecco`s modified Eagle's medium (DMEM) 1,000 cells/cm^2, and supplemented with 10% heat-inactivated foetal calf serum (FCS), penicillin (100 U/ml), streptomycin (10 mg/ml), 100 µl amphotericin B and 20 ng/ml basic fibroblast growth factor (b-FGF; R&D System, Minneapolis, MN, USA); they were then incubated at 37 °C in 5% CO_2. One day later, non-adherent cells were removed and adherent cells were cultured in the presence of mesenchymal medium for 3 weeks, during this period the media was changed every 3–4 days. (Human MesenCult™ Proliferation Kit is a standardized, serum-containing medium for culture and expansion of human MSCs) (Cambrex BioScience, Nottingham, UK). After 80% confluence was reached, MSCs were harvested by incubation with trypsin/EDTA and were counted using a haemocytometer. Surface expression of MSCs using anti-CD271, -CD29 and -CD34 monoclonal antibodies was analyzed by flow cytometry (Figs 1 and 2). Thirty to fifty million MSCs (0.4–0.7 million MSCs/kg body weight) were infused intravenously in 2 divided doses 1 week apart (Fig. 3).

Results and discussion

Removing anti-HLA allo-antibodies responsible for pre-transplantation donor-specific sensitization is not an easy task and is not always successful; having avoided blood transfusion generally reduces incidence of pre-transplant sensitization. Terminating afferent antigen stimulus by graft nephrectomy may occasionally terminate allo-sensitization. Suppression or elimination of any allo-antibody-producing clone, by immunosuppressive agents and/or by splenectomy has been attempted to terminate anti-HLA sensitization, and transplantation against ABO incompatibility (2,7).

Complementary removal of these antibodies by plasmapheresis with simultaneous suppression of antibody production by immunosuppressive agents or IVIG, has

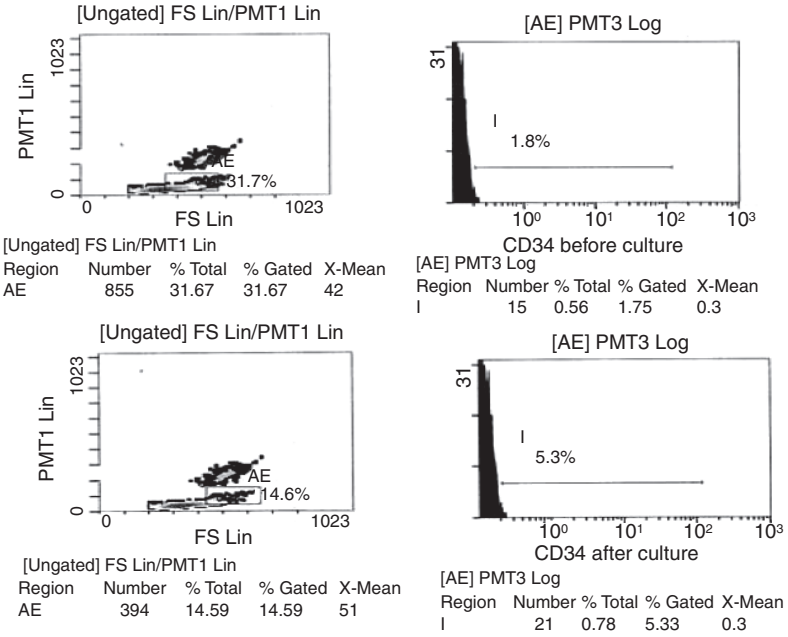

Figure 1. CD34 expression by flow cytometry, before and after culture, revealing no expression after culture.

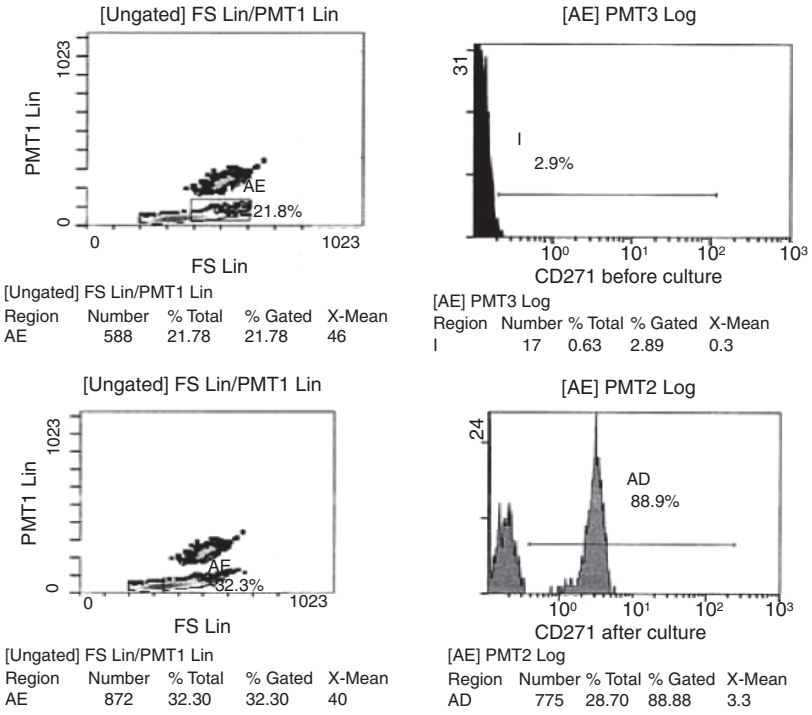

Figure 2. CD271 expression by flow cytometry, before and after culture, revealing positive expression after culture.

resulted in various degrees of success (3,4), but resistance to desensitization remains a challenging problem in successful transplantation, in some cases requiring novel immunomodulatory approaches.

Stem cells, in addition to their regenerative capabilities, have other important effects related to their paracrine functions and cell fusion properties (8,9) and MSCs have an important immunomodulatory function (10–12). MSCs avoid allogenic recognition (13,14) and these cells from autologous, allogenic haploidentical or third-party sources may be safely used to achieve the required therapeutic potential (15–17). Indications for stem-cell therapy are indicated to benefit from such of their properties.

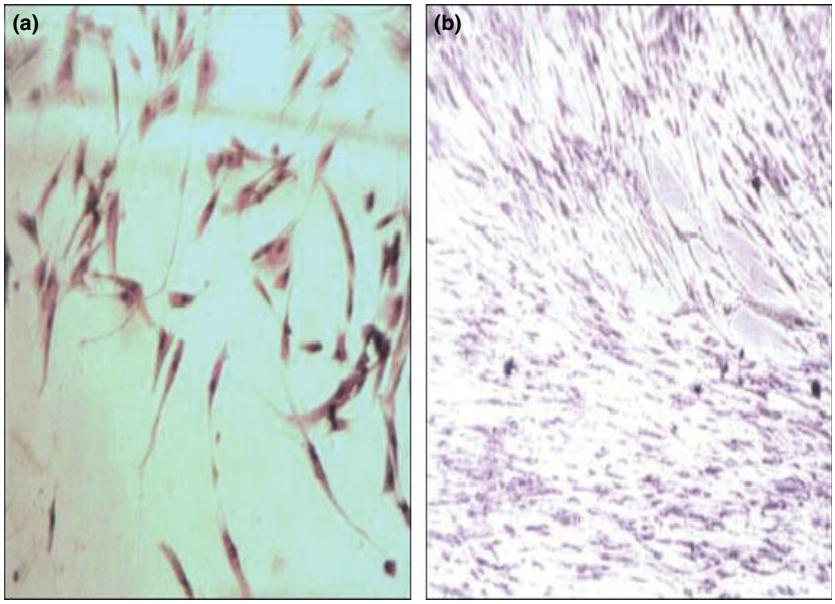

Figure 3. MSCs in culture; (a) MSCs 20% confluence, (b) MSCs 90% confluence.

Effects of MSCs on inhibition of antibody production by B-lymphocytes on exposure to allo-antigens is consistent in animal models (18), yet, there are contradictory reports concerning effects of human MSCs on antibody production by B-lymphocytes *in vitro*, on exposure to allogenic cells (19).Of important interest in clinical application of MSCs is establishment of optimum dosing that would achieve best results with least or absent side effects. In our study, around 0.4–0.7 $\times$ 10^6 MSCs/kg body weight were used and *in vitro* studies have shown that immunosuppressive effects occurred at (MSC:PBMC) ratios of 1:10 or higher. Such high concentrations of MSCs may not be achievable in clinical applications, as the majority of MSCs may disappear, as a result of distribution to other organs, and of cell loss caused by immunological or mechanical stress after infusion. A more recent multicentre trial has shown that doses of 0.5 $\times$ 10^6–9 $\times$ 10^6 cells per kg body weight did not lead to adverse side effects.

In case 1, graft nephrectomy did not correct sensitization to the scheduled new living donor. The latter was intended to avoid HLA mismatches of the first donor. Splenectomy; in a trial to ablate any antibody producing clone, similar to the procedure adopted for desensitization in both ABO incompatibility and HLA allo-sensitization, still could not revert donor-specific sensitization. Furthermore, plasmapheresis with low-dose IVIG, considered for desensitization, also could not revert the state of sensitization of the patient.

After approval of both the donor and the recipient, about 50 million donor-specific MSCs were injected into the patient in a trial, to benefit from their immunosuppressive and immuno-modulatory properties. This proved to be the only procedure that achieved successful desensitization, which had been persistent for the three months prior to transplantation. Being a second transplant and due to the history of donor-specific sensitization, the patient was treated with induction by ATG, which is known to upregulate T-regulatory cells, apart from its lymphocyte-depleting properties.

Patients 2 and 3 suffered pre-renal transplant sensitization due to previous frequent blood transfusions. In these patients, IV DS-MSCs transfusion was selected from the outset to be a successful line of treatment for pre-renal transplantation desensitization, in order to obviate other lines of treatment attempted in case 1.

The effect of MSCs to induce upregulation of T-regulatory cells and microchimaerism, and to promote a state of immunotolerance or immunosuppression, awaits to be proved. Here we have described a case that indicates that DS-MSCs were a potential option for anti-HLA desensitization. Cases 2 and 3 upheld the hypothesis derived after Case 1 treatment.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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