CASE REPORT

Stable graft function on low-dose steroid monotherapy in spite of donor-specific antibodies in renal transplantation combined with stem cell infusion

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SUMMARY

Transplantation using immunosuppression/induction therapies has controlled acute rejections; however, there is no answer for chronic graft attrition. Donor-specific antibodies (DSA) are believed to cause antibody mediated rejections eventually causing chronic graft loss. Regulatory T cells (T-regs) are believed to protect the graft from immune injury. We report a 53-year-old woman transplanted with her son's kidney using donor-specific transfusion and stem cells (SC) under non-myeloablative conditioning of cyclophosphamide, anti-T and anti-B-cell antibodies and Bortezomib. The patient was on low-dose steroid monotherapy under immune monitoring of DSA and serum creatine. Graft biopsy at 1 and 3.5 years post-transplant was unremarkable in spite of the presence of DSA. Peripheral T-regs (pTregs) at 3.5 years post-transplant were 3.54%. This case shows that DSA are not necessarily detrimental to the renal allograft. We further hypothesise that pTregs were induced from SC and sustained to protect this graft from cytotoxic T cells and DSA.

BACKGROUND

Transplantation using induction therapies and standard triple drug immunosuppression has become an acceptable therapy for end stage renal disease (ESRD). Acute rejections have been controlled; however, chronic rejection still remains an unanswered enigma. Cell therapy is becoming an attractive option to improve graft survival and minimise immunosuppression requirement thereby preventing morbidity and mortality associated with the use of immunosuppression.¹ We have been using donor stem cell infusion in renal transplant (RTx) recipients to achieve minimisation of immunosuppression with variable non-myeloablative conditioning regimens with approval of the Institutional Review Board.²

CASE PRESENTATION

A 53-year-old woman with ESRD due to chronic glomerulonephritis presented for RTx with stem cell infusion. The patient had four children, was on maintenance haemodialysis for 6 months and had received 26 units of blood transfusion. The patient had been treated for cervical tuberculous lymphadenopathy 1 year before admission. The patient was 163 cm tall, weighing 49 kg with pulse rate 90/min and unremarkable general and systemic

examination except blood pressure of 140/90 mm Hg controlled with Captopril, 3.125 mg twice a day.

INVESTIGATIONS

Her serum creatine (SCr) was 4.9 mg/dl, haemoglobin, 8 g/dl; total white cell count, $11.5 \times 10^4/\mu\text{l}$; platelet count, $1.8 \times 10^5/\mu\text{l}$; non-reactive for hepatitis B/C and HIV infections and blood group was 'O' Rh positive. Her antinuclear antibodies, anticytoplasmic antibodies and anticardiolipin antibodies were negative with normal serum C3 and C4 levels. Liver functions, electrolytes, blood sugar, lipid profile and uric acid were within normal limits. Urine routine examination revealed +2 albumin, microscopy showed 30–35 pus cells and 5–6 RBCs/high power field and was sterile on culture sensitivity testing. Ultrasonography revealed bilateral shrunken kidneys measuring 8.2×3.1 and 8.2×2.7 cm. Echocardiography showed global left ventricular hypokinesia with ejection fraction of 40%. Fundus examination showed grade 1 hypertensive retinopathy. Her lymphocyte cross-match (LCM) using standard CDC technique was negative, single antigen assay (SA) using Luminex platform showed absence of antibodies and flow-cross-match (FCM) with T and B cells (cut-off 50 median channel shift (MCS) for T cells and 150 MCS for B-cells) were negative.

TREATMENT

The patient was subjected to RTx with her 27-year-old son's kidney with human leucocyte antigen (HLA) 3/6 match, blood group 'o' positive, in November 2008 along with combined donor-specific transfusion (DST), 60 ml with nucleated cell count, $11.2 \times 10^4/\mu\text{l}$; cultured bone marrow (CBM), 100 ml with nucleated cell count, $3.36 \times 10^4/\mu\text{l}$; peripheral blood stem cells (PBSC), 135 ml; nucleated cell count, $1.33 \times 10^5/\mu\text{l}$ with total CD34+, $1.3 \times 10^6/\text{kg}$ BW under non-myeloablative conditioning of Cyclophosphamide, 20 mg/kg BW; rabbit-antithymocyte globulin, 1.5 mg/kg BW; Rituximab, 375 mg/m² and Bortezomib, 1.3 mg/m² in four divided doses before and after RTx with no immunosuppression other than Prednisone, 10 mg/day (figure 1). PBSC and CBM were injected into bone marrow of posterior superior iliac crest of recipient and DST was administered intravenously. Peritransplant induction

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Figure 1 Tolerance induction protocol.

included methylprednisone, 500 mg×3 days and intravenous immunoglobulin, 5 g×5 days. Immune monitoring consisted of LCM, SA and FCM at weekly intervals. Her complete blood counts including platelet counts, SCr and urine routine examination were monitored twice weekly for first month, weekly for next 2 months, fortnightly for next 3 months and monthly thereafter. The patient was also monitored for cytomegalovirus and hepatitis B/C infections at 3 monthly intervals. Prednisone was discontinued at 10 months post-transplant.

OUTCOME AND FOLLOW-UP

Stem cell infusion, conditioning and RTx were uneventful. At 1 year post-transplant her graft biopsy was unremarkable; however, the patient developed rise in donor-specific antibodies (DSA) (DQA1) with mean fluorescence intensity (MFI) of 11 000 units which continued to rise to around 14 000 MFI by 15 months. Prednisone, 10 mg/day was started with rise in DSA

and continued thereafter. The patient was subjected to one cycle of Bortezomib with methylprednisone, 125 mg followed by two plasmapheresis sessions and then Bendamustine (alkylating agent used in treatment of lymphoma/leukaemia), 55 mg/m² body surface twice at an interval of 15 days. However, DSA did not decrease significantly. Repeat biopsy at 3.5 years post-transplant was also unremarkable with absence of C4d deposits on peritubular capillary membranes (figure 2). Interestingly, her SCr remained around 0.9–1.1 mg/dl throughout the post-transplant course (figure 2). Her peripheral regulatory T cells (pTregs) (CD127^{low}CD25^{high}CD4⁺) were 3.54% at 3.5 years which have been sustained (5.2%) at 4.5 years.

DISCUSSION

Transplantation tolerance can be viewed as two interlinked phases—tolerance induction and its maintenance. It has now been proved that T-regs play a key role in tolerance induction and maintenance.^{3–6} This could be an organ-specific phenomenon. There are several phenotypes of regulatory cells like Tr1, Th3 and B-regs, all of them work complementary to each other through the phenomenon of linked suppression and infectious tolerance. While looking for tolerance signature in a cohort of our patients on minimum/no immunosuppression, we found the presence of >2.5% pTregs as a common denominator in all these patients (unpublished data) similar to the present case.

SH, 53/F (Donor: Son) TX: 26 Nov '08

28 Dec '11 POD: 1127

Figure 2 Graph showing complete status of the patient; on top is her age, initials, mentioning her son as donor and transplant date. On upper hand corner is the date when this graph was prepared. Human leucocyte antigen match status of patient (PT) and donor (DN) are mentioned. On the X-axis is the follow-up in days since the patient was inducted in the protocol, on Y-axis, left is antibody measurement by luminex assay (measured in mean fluorescent intensity) and on right side is serum creatine (mg/dl). The different coloured spikes are for antibodies and black line is for serum creatine. On the X-axis below the line are arrow-heads showing different treatment strategies given to the patient. This figure shows that serum creatine has remained fairly stable in spite of rise in donor specific antibodies. On right corner is photomicrograph showing renal allograft biopsy performed at 3.5 years post-transplant showing normal glomeruli with surrounding tubules and one artery, H&E stain, ×100.

This case suggests that DSA are not necessarily detrimental to the renal allograft in spite of maintenance on low-dose steroid monotherapy. We hypothesise that Tregs were generated from stem cells and protect the graft from immune injuries caused by not only cytotoxic T cells but also antibodies including DSA.

Learning points

- ▶ Renal transplantation is a well known therapy for end organ failure.
- ▶ Presence of donor-specific antibodies (DSA) are believed to be detrimental to graft function by causing antibody rejection.
- ▶ However, this case shows that graft injury caused by DSA can be prevented by stem cell therapy.
- ▶ Regulatory T cells generated from stem cell therapy can protect the graft from immune injury in addition to the benefit of absence of using conventional immunosuppression which may be called as induction and sustenance of clinical transplant tolerance.

Contributors HLT designed research work, wrote manuscript, carried out literature survey and patient care; AVV carried out laboratory research work, and wrote the manuscript; SD carried out lab work.

Competing interests None.

Patient consent Obtained.

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