

*Case Report***Alloimmune haemolytic anaemia resulting from anti-A and anti-B antibody induced by group O graft in renal transplant recipients**

R. Peces, R. A. Navascués, A. S. Laurés, J. Baltar, J. García-Gala, C. Sanzo, P. Rodríguez, F. Ortega and J. Alvarez-Grande

Services of Nephrology and Haematology, Instituto Reina Sofía de Investigación Nefrológica, Hospital Central de Asturias, Oviedo, Spain

**Key words:** alloantibodies; cyclosporin; immunosuppression; haemolytic anaemia; passenger lymphocytes; renal transplantation; tacrolimus

**Introduction**

Several cases of autoimmune haemolytic anaemia have been reported after transplantation of ABO-minor-incompatible organs [1–19]. Antibodies to autologous red blood cells (RBC) most frequently appeared within the first weeks after transplantation from a O-blood group donor to an A-, B-, or AB-blood group recipient and in the majority of the cases the immunosuppressive therapy included cyclosporin [5–19]. To our knowledge only one case of alloimmune haemolytic anaemia has been described after an organ-solid transplantation with ABO incompatibility and under tacrolimus therapy [20].

We present here three cases of alloimmune haemolytic anaemia due to minor ABO incompatibility after renal transplantation in two patients treated with tacrolimus and in one patient receiving cyclosporin. The pathogenetic mechanisms involved and strategy for treatment are discussed.

**Case reports***Case 1*

A 51-year-old man with end-stage renal disease secondary to nephrosclerosis began continuous ambulatory peritoneal dialysis (CAPD) in May 1994. On 31 December 1996 he received a cadaveric renal transplantation from a 64-year-old donor. HLA typing of recipient was A1, A2, B8, B35, DR16, DR17, and of

donor was A2, A11, B49, B51, DR15. Blood groups of the patient and his donor were B Rh(+) and O Rh(+) respectively. Initial immunosuppressive therapy consisted of prednisone (30 mg/day), tacrolimus (12 mg/day) and azathioprine (1.5 mg/kg/day). The graft functioned initially. In the immediate post-operative period the patient needed transfusion of 3 units of B group packed erythrocytes. Blood tacrolimus levels ranged between 8 and 11 ng/ml. On the 10th day after transplantation laboratory analysis revealed haematocrit 10.4%, haemoglobin 5.4 g/dl, WBC 35 400/mm<sup>3</sup>, platelets 232 000/mm<sup>3</sup>, reticulocytes 156 000/mm<sup>3</sup>, lactic dehydrogenase (LDH) 1906 U/l, serum haptoglobin 17 mg/dl (normal 40–200 mg/dl), serum indirect bilirubin 2.8 mg/dl, and direct bilirubin 1.3 mg/dl. Free plasma haemoglobin and haemoglobinuria were noted. Peripheral blood smear revealed erythrocyte morphology was normochromic and normocytic. All of these findings suggested the diagnosis of haemolytic anaemia and prompted tacrolimus and azathioprine discontinuation that were replaced by mycophenolate mofetil.

Direct antiglobulin tests (DAT) were positive with anti-IgG and anti-C3d. An eluate prepared from the patient's red cells showed anti-B antibodies. Moreover, anti-B antibodies were detected in the patient's serum. Studies with dithiothreitol demonstrated that the antibody consisted of IgG. A test to identify a tacrolimus-dependent antibody, which might be implicated in drug-induced hemolysis was negative. Four units of O group packed erythrocytes were transfused. Over the following days renal function worsened quickly and the patient need dialysis. The blood group of the patient changed to O group and the DAT became negative, but anti-B antibodies were still positive.

Immunosuppressive therapy was continued with mycophenolate mofetil (2 g/day), methylprednisolone (500 mg/day) for 5 days, followed by prednisone (30 mg/day), and OKT-3 (5 mg/day) for 14 days. During the time of OKT-3 administration, prophylactic treatment with ganciclovir (5 mg/kg/day) was given.

*Correspondence and offprint requests to:* Dr R. Peces, Servicio de Nefrología, Hospital Central de Asturias, Celestino Villamil s/n, E-33006 Oviedo, Spain.

Two days after the discontinuation of OKT-3 the patient presented leukopenia of 800 WBC/mm<sup>3</sup>. Mycophenolate mofetil was discontinued and changed to cyclosporin (7 mg/kg/day), and treatment with granulocyte-colony-stimulating factor (G-CSF) (300 µg/day) was initiated. Ten days later the WBC normalized and G-CSF could be stopped. Haemolysis persisted unmodified and anti-B antibodies remained positive 2 weeks later. The graft was non-functioning and the patient continued to need dialysis and blood transfusions. A biopsy taken at the 54th post-operative day showed evidence of acute tubular necrosis.

On day 62 the patient developed pulmonary aspergillosis and was treated with itraconazole 200 mg/day for 3 months. Allograft function was never recovered and immunosuppressive drugs were stopped and the patient regressed to CAPD. Subsequent serological evaluation revealed no evidence of haemolysis. He remains clinically well on CAPD over 6 months later.

#### Case 2

A 47-year-old man with end-stage renal disease secondary to interstitial nephropathy initiated haemodialysis in January 1993. On 13 April 1997 he received a cadaveric renal transplantation from a 64-year-old woman. HLA typing of recipient was A2, A28, B44, B14, DR1, DR15, and of donor was A2, A9, B7, DR4, DR15. Blood groups of the patient and his donor were B Rh(+) and O Rh(+), respectively. Immunosuppressive therapy consisted of prednisone (30 mg/day), cyclosporin (7 mg/kg/day) and azathioprine (1.5 mg/kg/day). He received transfusion of 1 unit of B group packed erythrocytes. Graft function was immediate and the post-operative period was without complications. In the 10th day the patient was discharged with haematocrit 28%, haemoglobin 9.4 g/dl, serum creatinine 2 mg/dl, and creatinine clearance 36 ml/min. Blood cyclosporin levels ranged between 241 and 274 ng/ml (target range 150–200 ng/ml).

On the 22nd day after transplantation laboratory analysis revealed haematocrit 17.7%, haemoglobin 6.4 g/dl, WBC 14 000/mm<sup>3</sup>, platelets 187 000/mm<sup>3</sup>, reticulocytes 300 000/mm<sup>3</sup>, LDH 616 U/l, serum haptoglobin 20 mg/dl, serum indirect bilirubin 1.4 mg/dl, and direct bilirubin 1.3 mg/dl. Peripheral blood smear revealed normal erythrocyte morphology. The patient was admitted to the hospital and DAT was positive with anti-IgG. An eluate prepared from the patient's red cells showed anti-B antibodies. Moreover, anti-B antibodies were detected in the patient's serum. The prednisone dose was increased to 60 mg/day during 3 days and the patient received transfusion of 2 units of O group packed erythrocytes. Prednisone 20 mg/day was continued, and cyclosporin was progressively reduced from the initial dose of 450 mg/day to 250 mg/day (3.8 mg/kg/day) while blood trough levels decreased to 158 ng/ml. Five days after admission the patient was discharged with serum creatinine 1.5 mg/dl and creatinine clearance 57 ml/min. The signs of

haemolysis gradually disappeared and 2 weeks later, a new control showed haematocrit 31%, haemoglobin 10.1 g/dl, WBC 6000/mm<sup>3</sup>, platelet 146 000/mm<sup>3</sup>, total bilirubin 1.2 mg/dl, serum creatinine 1.1 mg/dl, and creatinine clearance 88 ml/min.

#### Case 3

A 59-year-old man with end-stage renal disease secondary to interstitial nephropathy initiated CAPD in April 1995. In May 16, 1997 he received a cadaveric renal transplantation from a 42-year-old donor. HLA typing of the recipient was A3, A28, B8, B14, DR17, DR17, and of the donor was A1, A2, B8, DR3, DR6. Blood groups of the patient and the donor were A Rh(+) and O Rh(+) respectively. Initial immunosuppression consisted of prednisone (30 mg/day) and tacrolimus (16 mg/day). Graft function was immediate and the post-operative period was without complications. On the 10th post-operative day the patient was discharged with haematocrit 30%, haemoglobin 10.3 g/dl, serum creatinine 1.7 mg/dl, and creatinine clearance 68 ml/min. Blood tacrolimus levels ranged between 12.3 and 14.1 ng/ml. On the 23rd day after transplantation laboratory analysis revealed haematocrit 22%, haemoglobin 7.3 g/dl, WBC 11 200/mm<sup>3</sup>, platelets 220 000/mm<sup>3</sup>, reticulocytes 100 000/mm<sup>3</sup>, LDH 363 U/l, serum haptoglobin <5 mg/dl, serum indirect bilirubin 1.08 mg/dl, and direct bilirubin 0.26 mg/dl. Peripheral blood smear revealed normal erythrocyte morphology. The serum creatinine was 1.9 mg/dl and the creatinine clearance 60 ml/min.

At admission to the hospital DAT were positive with anti-IgG and anti-C3d. An eluate prepared from the patient's red cells showed anti-A antibodies. Moreover, anti-A antibodies were detected in the patient's serum. The patient received methylprednisolone (500 mg/day) by 3 days, the prednisone dose was continued to 15 mg/day and 1 unit of O group packed erythrocytes was transfused. The tacrolimus dose was reduced to 14 mg/day while blood trough levels were maintained between 7 to 10 ng/ml. The signs of haemolysis gradually improved and 5 days after admission he was discharged with serum creatinine 1.9 mg/dl. The serum haptoglobin levels progressively increased to 92 mg/dl in 4 weeks and haemoglobin gradually normalized.

#### Discussion

Haemolysis has already been reported after transplantation. Some cases are clearly related to haemolytic-uraemic syndrome (HUS) and usually attributed to cyclosporin treatment [21]. HUS has, however, also been described in a patient receiving tacrolimus [22]. Acute haemolysis has also been related to anti-erythrocyte alloantigens and autoantibodies in some kidney transplant recipients [1–3,5–10,23–25]. In most cases patients from blood group A, B, or AB had received an ABO-compatible although ABO-non-identical organ, mostly from group O. Haemolysis

usually appeared within the first 2 weeks after transplantation and lasted from 2 weeks to several months. In these cases, the haemolysis has been related to alloantibodies derived from passenger B lymphocytes transplanted together with the organ [6,10,12,21,25–28]. Several such cases have been reported after renal transplantation [10,12,24], although it is more common after liver transplantation [20,26,27]. The occurrence of this complication, a form of graft-versus-host disease (GVHD), no doubt reflects the better immunosuppression achieved with cyclosporin.

Acute haemolytic anaemia has also been reported in patients treated with tacrolimus [20,29]. In these cases, three mechanisms of haemolysis have been described: drug-induced haemolysis, autoimmune haemolysis, and haemolysis secondary to alloantibodies derived from passenger lymphocytes contained within the graft [20,29].

The clinical picture of the patients presented here was completely different from HUS. The existence of severe anaemia without schistocytosis, indirect hyperbilirubinaemia, increased serum LDH levels and decreased serum haptoglobin levels in the presence of good graft function suggested in all cases a haemolytic anaemia. A striking feature of these patients was the onset of alloimmune haemolysis in the first weeks after transplantation covered by heavy immunosuppressive therapy including tacrolimus or cyclosporin. Two patients were group B positive and one patient group A positive, and all received a group O graft. The DAT showed that antierythrocyte antibodies responsible of haemolysis were of the IgG isotype. Furthermore, the alloantibodies eluted and the antibodies identified in the serum of the three patients had the anti-B or the anti-A specificity, as would be expected in the passenger lymphocyte syndrome (Table 1). Diagnostic certainly is achieved by the detection of donor HLA antigens in the host bone marrow or peripheral blood (chimerism), or by DNA studies. However, circulating passenger lymphocytes have been shown after transplantation in a significant percentage of recipients, but their presence would not necessarily indicate that they are producing autoantibodies [30,31]. Therefore, the immunohaematological evaluation supports alloimmune haemolytic anaemia resulting from passenger lymphocyte syndrome in these cases. Moreover, although the severity of haemolysis in the three cases varied widely, the clinical picture resolved within a few weeks.

Immunosuppressive drugs used to prevent rejection, cyclosporin and tacrolimus, are more effective and selective, but still induce a marked immunological deficit. Such deficit can be accentuated when additional factors are present, e.g. cytomegalovirus infection, administration of OKT-3, or drug-induced neutropenia (ganciclovir, azathioprine, or mycophenolate mofetil). They predispose to the development of GVHD when the patient receives immune competent lymphocytes present in the graft. Since haemolysis has been most frequently related to cyclosporin therapy, some authors have suggested that B lymphocytes were mainly allowed to proliferate and to produce antibodies because it inhibits T-cell function and relatively spares B-cell activity [32,33]. It is hypothesized that cyclosporin prevented the recipient's immune system from damaging the donor's B lymphocytes and that a subtype of these B-cells, which were also resistant to cyclosporin, produced anti-A and anti-B antibodies [34,35]. This could be the mechanism of haemolysis in case 2. Similarly, in cases 1 and 3 the alloimmune haemolytic anaemia may reflect an analogous phenomenon in relation to tacrolimus therapy. Therefore, a deep T cell suppression may have played an indirect role in producing the alloimmune haemolytic anaemia by permitting the clonal development of donor B lymphocytes in the recipients. The long duration of haemolysis together with the persistence of anti-B antibodies in case 1 could be favoured by concomitant OKT-3 and ganciclovir therapy.

No treatment has been proved effective in transplant patients who develop haemolytic anaemia. Although some cases have responded to high-dose corticosteroids, this therapy has not been uniformly successful. Other immunosuppressor drugs have been used with little success, perhaps because these agents further compromise the immune system. On the other hand, although in some cases the improvement was related to the decrease in immunosuppression, it is also certain that spontaneous remission within 2 months is the usual outcome.

In conclusion, optimal management is unknown. Prevention is therefore very important to avoid this complication. It could be achieved by avoiding profound immunosuppression in the early post-transplant period in patients with higher risk. In situations such as that presented here slight adjustment of cyclosporin or tacrolimus dose may be an adequate treatment. Since cyclosporin and tacrolimus are T specific, more

**Table 1.** Data for case histories 1–3

Case	Age	Blood group		Antibody		Onset (days)	Duration (days)	Immuno-suppression	Treatment
		Donor	Recipient	Specificity	Isotype				
1	51	O	B	Anti-B	IgG	10	90	Tacro + Aza + P	Discontinuation
2	47	O	B	Anti-B	IgG	22	15	CsA + Aza + P	CsA reduction
3	59	O	A	Anti-A	IgG	23	21	Tacro + Aza + P	Tacro reduction

Tacro, tacrolimus; CsA, cyclosporin; Aza, azathioprine; P, prednisone.

potent B-cell suppression such as the addition of mycophenolate mofetil to baseline immunosuppression may be an alternative.

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Received for publication: 27.10.97

Accepted in revised form: 23.2.98