Haemolysis after kidney transplantation: beware of the graft

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Introduction

Haemolysis may occur after organ transplantation. Well-known causes of haemolysis are medication, infection, and microangiopathy. It is important to include graft-versus-host antibody formation in the differential diagnosis. We present two cases illustrating the role of the graft in antibody formation, and briefly discuss some aspects important to the clinician involved in renal transplantation.

Case reports

Case 1

A 53-year-old man with end-stage renal disease due to adult polycystic kidney disease received a 2-antigen matched cadaveric kidney transplant. The blood group of the patient was B, rhesus positive, and the blood group of the donor was O, rhesus positive. The post-operative course was complicated by a haemorrhage and a temporary decline in renal function due to cyclosporin toxicity. No transfusions were given. The patient was discharged on day 18 with a good graft function (creatinine 155 μmol/l). His medication consisted of cyclosporin 100 mg b.i.d., prednisone 10 mg b.i.d., mycophenolate mofetil 500 mg b.i.d., omeprazole 20 mg, and co-trimoxazole 480 mg. On day 27 the haemoglobin dropped from 5.7 to 4.5 mmol/l. A slight rise in unconjugated bilirubin (24 μmol/l), LDH (450 U/l), and a reticulocytosis (108%) were observed; a diagnosis of haemolytic anaemia was made.

There was no evidence of microangiopathy, infection, or a metabolic disturbance. Drug-induced haemolysis was considered, but in view of the blood group mismatch we first looked for the presence of erythrocyte antibodies. Indeed, the direct antiglobulin test was positive with polyspecific and anti-IgG antiglobulin sera (anti-IgG titre 32). The serum and an eluate of the patient’s erythrocytes contained anti-B antibodies. A screening panel for irregular antibodies remained negative. These results confirmed the diagnosis of a haemolytic anaemia caused by antibodies produced by donor lymphocytes against the erythrocytes of the recipient. After the initial drop the haemoglobin level stabilized and the reticulocytosis gradually subsided. The medication was left unchanged and transfusions were not necessary. From day 66 the haemoglobin started to rise and the LDH fell to normal values. The direct antiglobulin test was negative on day 145. The patient has remained in good health until now, 2 years after the transplantation.

Case 2

A 62-year-old man with end-stage renal failure of unknown cause received a 3-antigen matched cadaveric kidney transplant. The patient’s blood group was A, rhesus positive (ccDEE) and the donors blood group was A, rhesus negative (ccdee). The postoperative course was uneventful and the patient was discharged on the eighth day. His medication consisted of cyclosporin 100 mg b.i.d., prednisone 10 mg b.i.d., mycophenolate mofetil 2000 mg b.i.d., famotidine 20 mg, isoniazide 300 mg, pyridoxine 20 mg and co-trimoxazole 480 mg. On day 18 we received a message from Eurotransplant that haemolysis had occurred in the recipients transplanted with the liver and the lungs of the same donor. In both patients anti-D antibodies had been found. Although there were no clinical signs of haemolysis we tested our patient for antibodies. On day 20 the direct antiglobulin test was positive with polyspecific and anti-IgG antisera (titre 128). The serum and an eluate of the patient’s erythrocytes contained anti-D antibodies. The direct antiglobulin test remained positive until day 84; the indirect antiglobulin test was negative from day 51. In contrast to the other recipients no signs of haemolysis developed in our patient. Retrospectively anti-D antibodies were also found in the donor’s serum.

Discussion

Haemolysis after kidney transplantation is a challenging problem and several possible causes must be considered (see Table 1). In most cases the haemolysis is drug-related. Cyclosporin may cause a microangiopathic haemolytic anaemia [1]. Co-trimoxazole
Table 1. Causes of haemolytic anaemia after kidney transplantation [2,4]

<table>
<thead>
<tr>
<th>Category</th>
<th>Cause</th>
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<tbody>
<tr>
<td>Antibody-related</td>
<td>graft-versus-host, anti-lymphocyte globulin, post-transfusion</td>
</tr>
<tr>
<td>Medication</td>
<td>co-trimoxazole, dapsone, cyclosporin</td>
</tr>
<tr>
<td>Microangiopathy</td>
<td>cyclosporin, rejection, haemolytic—uraemic syndrome</td>
</tr>
<tr>
<td>Infection</td>
<td>mycoplasma, clostridium, gram-negative sepsis</td>
</tr>
<tr>
<td>Metabolic</td>
<td>hypophosphataemia</td>
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and dapsone, used as prophylactic agents against Pneumocystis carinii infections, cause haemolysis via oxidative denaturation of haemoglobin [2]. Preparations of antithymocyte globulin, used as an antirejection treatment, sometimes contain anti-erythrocyte antibodies [3]. Other known causes of haemolysis are severe hypophosphataemia, which is often present in the first weeks after kidney transplantation, and infections such as Mycoplasma pneumoniae [2].

An intriguing source of haemolysis after transplantation is the graft. Two separate mechanisms can be distinguished: erythrocytes can be damaged mechanically by microangiopathy in the graft, resulting from rejection, the haemolytic–uraemic syndrome or the use of cyclosporin [1]. Alternatively, haemolysis may be the result of a graft-versus-host disease. In these cases, donor lymphocytes transplanted with the graft produce antibodies against ‘minor mismatched’ erythrocyte antigens of the recipient [4–10]. ‘Minor mismatched’ or ‘non-identical’ transplantation refers to transplantation of a blood group O organ into a non-O recipient, transplantation of a non-AB organ into an AB recipient, or a rhesus non-identical transplantation [4,5]. By allotyping of the antibodies it has been demonstrated unequivocally that the antibodies are of donor origin [11–15]. Also, irradiation of the graft largely prevented the formation of these antibodies [14]. In one recently described case of a heart–lung transplantation, leukocytes of donor origin have been identified in the recipient’s lungs and peripheral blood using a PCR for human leukocyte antigen class II genotype [16].

The formation of antibodies against erythrocytes by graft-derived lymphocytes has been described in over 100 case reports. Ramsey analysed 106 cases published by 1991, mostly involving recipients of a kidney (n = 46) or liver (n = 45) transplant [5]. All cases dealt with the formation of ABO or rhesus antibodies. Although conceivable, the formation of antibodies against other erythrocyte antigens, leading to haemolysis after kidney transplantation, has never been described.

ABO-antibodies, mainly of the IgG class, appeared within a few days after transplantation. Haemolysis developed in about half of these patients at a median of 10 days after transplantation. The antibodies generally disappeared within 2 months but sometimes were present much longer. These findings are consistent with a secondary immune response by previously primed B-lymphocytes [5,17]. As cyclosporin is a more selective inhibitor of T-cell function and has only little effect on the B-cell-mediated secondary immune response, antibody production could be more frequent in cyclosporin-treated patients [5,14,17]. However, Ramsey found no difference in the incidence of antibody formation between groups of patients treated with azathioprine or cyclosporin, although more cases of overt haemolysis were found in the cyclosporin-treated group [5]. No data are available regarding possible effects of mycophenolate mofetil. The incidence of antibody formation appears to depend on the transplanted organ mass: it was found more often after heart/lung and liver transplantation than after kidney transplantation. No influence was found for sex, pregnancies, race, ABH secretor status, or cadaveric vs living donor organs. In most cases haemolysis was mild, but severe cases requiring massive blood transfusions or leading to acute renal failure have been described [5].

Although prospective studies regarding the incidence of rhesus antibody formation are lacking, this seems to be a rare event. Only nine cases had been described, of which seven had haemolysis. Antibodies were directed against several rhesus antigens, mainly C and D. In most of these cases the donor had been sensitized before transplantation [5,18,19]. This finding, together with the discrepancy between the proportion of transplantation of Rh(D)-negative organs into Rh(D)-positive recipients (about 15%) and the apparently much lower incidence of formation of Rhesus antibodies, strongly supports the assumption that anti-erythrocyte antibodies are produced only via a secondary immune response.

Data on a possible relationship between antibody formation and kidney graft survival have been contradictory [20,21]. Ramsey found that if haemolysis occurred, graft survival was decreased after liver transplantation but not after kidney transplantation [5]. The largest and most recent report comes from the UNOS registry. The incidence of early graft rejection in six HLA-antigen matched, ABO-non-identical grafts was higher than in the ABO-identical grafts, but one and three year graft survival were identical [22].

Several measures have been proposed to prevent antibody-induced haemolysis or its complications after solid-organ transplantation: avoidance of transplantation of lymphocytes as much as possible by flushing the graft thoroughly and by removing lymph nodes from the perirenal fat [23], use of the monoclonal antibody OKT3 instead of the polyclonal antithymocyte globulin [23], and performing serial direct antiglobulin testing and addition of cyclophosphamide to the immunosuppressive therapy if antibody production is detected [11].

The value of these prophylactic measures is not proven. In our opinion routine application of these measures is not required since haemolysis most often is mild and resolves spontaneously [5]. If haemolysis is severe (decline in graft function, severe hyperbilirubinaemia) several approaches are possible: a course of plasmapheresis could be given [11,23], cyclosporin could be replaced by another immunosuppressive agent [10], or high-dose steroids could be added [17]. It is
important to note that transfusions, when necessary, should be of donor type to avoid further haemolysis [5].

In conclusion, in identifying the cause of haemolysis after kidney transplantation it is important to evaluate blood group differences critically. In cases of a ‘non-identical’ transplantation the possibility of a graft-versus-host reaction must be considered. Initial laboratory evaluation should therefore include direct and indirect antiglobulin testing [with routine and specific sera and cells] and the analysis of eluted antibodies. If indeed the haemolysis is due to antibody formation by donor lymphocytes, in most cases no specific measures are necessary.

References