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CASE REPORT

Heart transplantation across the antibodies against HLA and ABO

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Summary

We have intentionally performed heart transplantation in a 5-year-old child, despite the most unfavourable risk factors for patient survival; the presence of high level of antibodies against donor's human leucocyte antigen (HLA) class I/II and blood group antigens. Pretransplant treatment by mycophenolate mofetil, prednisolone, tacrolimus, intravenous immunoglobulin, rituximab, protein-A immunoadsorption (IA) and plasma exchange reduced antibody titres against the donor's lymphocytes from 128 to 16 and against the donor's blood group antigen from 256 to 0. The patient was urgently transplanted with a heart from an ABO incompatible donor (A_1 to O). A standard triple-drug immunosuppressive protocol was used. No hyperacute rejection was seen. Antibodies against the donor's HLA antigens remained at a low level despite three acute rejections. Rising anti-A_1 blood group antibodies preceded the second rejection and were reduced by two blood group-specific IAs and remained at a low level. The patient is doing well despite the persistence of donor-reactive antibodies.

Introduction

The presence of donor-specific antibodies against human leucocyte antigen (HLA) [1–8] and ABO [9] antigens are still major obstacles for heart and other organ transplantation because of an enhanced risk of graft failure because of hyperacute, acute or chronic rejections. The hyperacute rejection of an organ transplant within minutes or hours after transplantation is rare and generally found in patients having high levels of antibodies against donor's HLA and ABO antigens [1–4]. Improved results in renal transplantation have been obtained in recent years in patients with preformed donor-reactive antibodies (DRA) using pretransplant reduction of antibodies to a low level by different preparative regimens [plasma exchange (PE)/splenectomi, PE and/or intravenous immunoglobulin G (IVIG) and PE or immunoadsorption (IA)/IVIG/anti-CD20] and immunosuppressive protocols, graft survival rate was comparable with that of antibody-negative patients [7–15]. However, except for very high titres of donor-specific antibodies, no other predictive factors or specific treatments were finally defined.

Graft failure in kidney transplantation leads to dialysis treatment. There is no such established treatment in heart

transplantation, the graft failure being the most common cause of patient death during the first year after heart transplantation. The experience of pretransplant treatment of DRA in heart transplantation is limited to a few cases [6,16].

This report concerns the first heart transplantation performed as a last resort in a highly immunized patient across the antibodies against the donor's HLA class I/II and blood group A_1 antigens.

Patient

A 5-year-old girl, blood group O, was considered for heart transplantation due to severe cardiac failure after complex surgery for congenital cardiac malformations. An aortic valvulotomy was performed in infancy due to aortic stenosis. Later on severe left ventricular (LV) outflow obstruction occurred, necessitating reoperation with a Ross-Konno procedure, including implantation of a homograft in the right ventricular outflow. Thereafter, progressive neoaortic incompetence developed in conjunction with LV dysfunction. In an effort to save the valve it was decided to perform a valve sparing procedure which acutely led to further LV dysfunction. Postoperatively, the sternum could not be closed even after 2 days of treatment with a left ventricle assist device (LVAD). In order to stabilize the sternum a vacuum-assisted closure system consisting of polyurethane foam dressing and a special pump unit [17] was used for almost a month.

Acute antibody screening was panel-reactive antibodies (PRA) 100% positive in both cytotoxic screen against test panel B/T cells and in flow cytometry using beads coated with purified HLA class I/II antigens (FlowPRA^{TM}; One Lambda, Canoga Park, CA, USA) class I and II screening tests (Fig. 1). Autologous B/T cells were negative. Cytotoxic antibody titres against one random test cell donor were 2048. Transplantation was the only treatment option, but was contraindicated without modulation of the antibody response. Treatment was started on day 21 pretransplant by i.v. mycophenolate mofetil (MMF) 40 mg/kg/day and IVIG 0.5 g/kg/day together with low-molecular weight heparin and five protein-A immunoadsorptions (PAIA). After 10 days of treatment cytotoxic antibody titres against cells from random test donors were sixfold reduced but increased threefold 3 days later. Therefore, tacrolimus 0.15 mg/kg/day and prednisolone 2 mg/kg/day were added to the protocol at day 7 pretransplant and one infusion of 375 mg/m^2 rituximab was given. On day 5 pretransplant ventricular fibrillation (VF) occurred shortly after the placement of a dialysis catheter (Vas-Cath) in the right femoral vein. The VF was resistant to defibrillations, bolus dose of amiodarone, lidocain, calcium cloride, magnesium and bicarbonate. A high blood level of potassium (9.5 mM) was found. The hyperkalemia was treated successfully, while performing external cardiac compressions for 45 min. When the potassium level was 5.5 mM the VF was easily defibrillated to sinus rhythm and spontaneous circulation regained. Fifteen minutes later a PAIA started and she moved spontaneously and normally. The reason for the hyperkalemia was unknown, but both the long-term treatment with glucose-insulin-potassium (GIK) and tacrolimus treatment may have contributed to the high level.

An urgent request for an ABO-compatible transplant was made at the beginning of the second series of four PAIA. Five days later at the end of the PAIA series followed by IVIG, an urgent call even for an ABO-incompatible heart transplant was sent. Five hours later a donor heart of blood group A_1 was offered and accepted.

The donor was blood group A_1, secretor. The HLA type of the donor (HLA- A*11,24;B*15,51;DRB1*04,14) was three HLA antigen matched with the patient (HLA-A*11,30;B*13,51;DRB1*07,14). IgM antibody titres (standard direct agglutination test) and IgG antibody titres (a standard indirect agglutination test for IgG antibodies and Bio-Rad AHG gel card test detecting IgG antibodies and C3d) (Bio-Rad Hercules, CA, USA) against donor erythrocytes were 32 and 2, respectively. A single PE (three times plasma volume) using the Sequestra 1000 (Medtronics, Parker, CO, USA) during cardiopulmonary bypass removed remaining antibodies against blood group A_1 but cytotoxic donor-specific crossmatch was still B cells-positive. Thus, the pretransplant treatment reduced antibody titres against the donor's lymphocytes from 128 to 16 and against the donor's blood group A_1 antigen from 256 to 0. Before reanastomosis, MMF, methylprednisolone 30 mg/kg, aprotinin l00 ml, 10 000 KIE/ml and IVIG were infused. The patient was uneventfully transplanted with a heart from a male donor who weighed almost triple her own weight. Ischaemic time was 60 min. She could be extubated on the third postoperative day. In spite of the long preoperative treatment with an open sternum there were no signs of infections.

Postoperative treatment consisted of tacrolimus (trough level of 20–25 ng/ml during the first month and 15–20 ng/ml during the second month and 8–12 ng/ml thereafter), MMF (30 mg/kg/day) prednisolone and IVIG (0.5 g/kg every third day for 3 months). Monitoring of antibodies against donor cells was performed using serial twofold dilutions of patient sera; daily in the first 6 weeks post-transplant, weekly in the third and fourth months and monthly thereafter. FlowPRA^{TM} class I/II screening tests were performed in parallel. Endomyocardial biopsies were performed twice a week for the first 5 weeks, weekly in the second and third months with the successive lengthening of the time interval; in all 25 times during

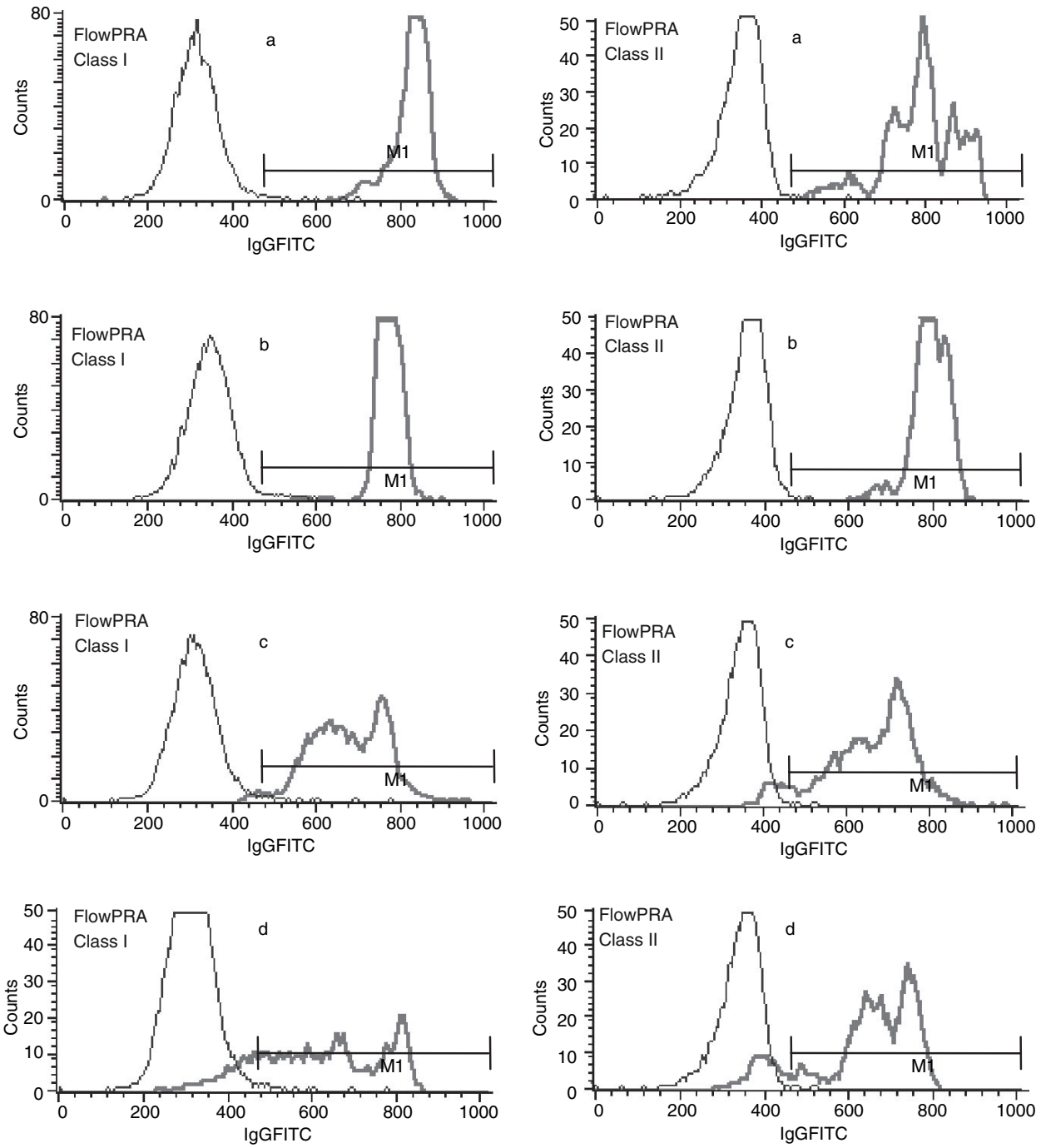

Figure 1 The follow-up results of FlowPRA^{TM} class I and II screening tests. (a) Positive and negative control sera. Negative control serum and patient's serum taken; (b) before conditioning treatment; (c) per-transplant and (d) 4 months post-transplant.

the first year. The light microscopy biopsies were assessed according to the International Society of Heart and Lung Transplantation [18] grading system. Indirect staining for complement split products or immunoglobulins was not carried out on cardiac biopsies, except for C4d immunohistochemistry, which was performed once in 10 months post-transplant and found positive for C4d staining on capillary endothelium.

No hyperacute rejection was seen. There were three acute rejection episodes under the follow-up period. The first rejection occurred on day 8 and biopsy was assessed as cellular rejections grade 3A [15]. It was reversed by

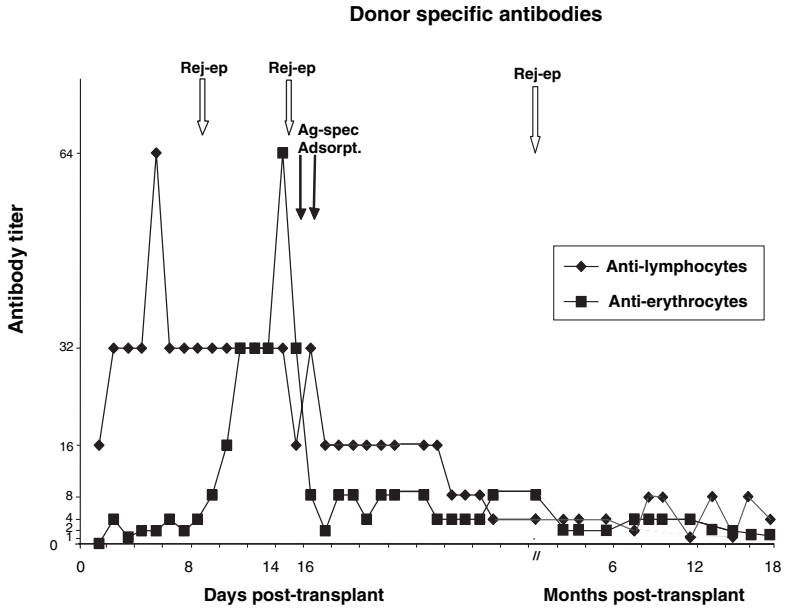

Figure 2 Antibody titer, highest serum dilution (reciprocal) of donor-specific antibodies giving positive reaction; ◆, complement-dependent cytotoxicity against lymphocytes; ■, against erythrocytes (Bio-Rad AHG gel card test); Rej-ep, acute rejection episode; Ag-spec adsorpt., blood group A antigen-specific adsorption (Glycosorb®-ABO).

15 mg/kg/day methylprednisolone for 3 days and three doses (1.5, 1.5 and 3.0 mg/kg) anti-thymocyte globulin (ATG). The second rejection occurred on day 14 and was preceded by rising anti-A blood group antibody. The biopsy was assessed as grade 4 with interstitial oedema and bleeding, cell necrosis, granulocyte and lymphocyte infiltration, indicating antibody-mediated vascular rejection. The rejection was treated by methylprednisolone (30, 7.5 and 7.5 mg/kg/day), and ATG (3.0, 1.5, 3.0 and 3.0 mg/kg). The rising anti-A1 antibody titres from 2 to 64 were reduced to 2 by two IAs using a low-molecular weight carbohydrate column with the blood group A antigen linked to a Sepharose matrix (Glycosorb®-ABO, Glycorex Transplantation, Lund, Sweden), on 2 consecutive days and thereafter remained at a low level (Fig. 2). The third rejection occurred at day 35 and biopsy was assessed as cellular rejections 3A and was promptly resolved by 7.5 mg/kg/day methylprednisolone treatment. All control biopsies after rejection episodes were negative.

A prophylactic photochemotherapy with 8-methoxypsoralen was started at 7 weeks post-transplant with totally 15 sessions for 3 months aiming to reduce the doses of tacrolimus and prednisolone because of drug-induced diabetes. During this period one septic episode (infected central venous catheter) and asymptomatic activation of cytomegalovirus (CMV) replication were successfully treated by antibiotics respectively i.v. ganciclovir.

Sixteen months after heart transplantation the DRAs are still present at low titres; against erythrocytes 1–4 and against lymphocytes 4–16. Cytotoxic PRA are positive, 72% for B cells and 50% for T cells whereas FlowPRA screening tests were positive, 85% for class I and 80% for class II. Except for a drug-induced diabetes, the patient is doing well on maintenance drug therapy by tacrolimus, MMF and prednisolone and without clinical or biopsy signs of rejection or vasculopathy.

Discussion

We have successfully performed a heart transplantation despite the most unfavourable risk factors; the presence of high levels of donor-specific antibodies against HLA class I, class II and blood group A_1 antigens in a blood group O patient. The patient had a classical predisposing history for high immunization; heart-lung-machine operations, multitransfusion, valve allograft and LVAD. The cryopreserved homograft may have played a major role in the development of anti-HLA class I antibodies as it is known to give a long-lasting immune response [19].

The procedure was based on recent findings in kidney transplantation showing that temporary reduction of preformed or *de novo* donor-specific antibodies to a low level might prevent and reverse antibody mediated rejection (AMR) and result in long-term allograft survival [6,14,15,20]. The encouraging results in heart transplantation, treated by plasmapheresis and IVIG once before the transplantation has been previously shown [16]. Moreover, we have positive experience of conditioning treatment using per-transplant IVIG without plasmapheresis in patients with preformed DRA in organ (heart, lung and kidney) transplantation (unpublished data).

We primarily tried to reduce antibody production to avoid hyperacute graft failure. Treatment was started by IVIG-based protocol [21] and MMF with a moderate

effect on antibody reduction. Thereafter, PAIA was added with a good but temporary effect on lowering of antibody titre. One week before the transplantation, anti-CD20, tacrolimus, PAIA, MMF and IVIG protocol [13] was implemented. We expected that successful pretransplant reduction of IgG and to lesser extent, IgM antibodies by PAIA and a modulation of antibody response might sufficiently reduce the anti-HLA antibodies as well as the anti-ABO antibodies. The present results confirmed our expectations. The timing of the transplantation at a low antibody level was considered much more important than the additional adverse effects of ABO-incompatibility. Thus, we were prepared to handle both crossmatch-positive and ABO-incompatible heart transplants.

Per-operative ATG induction therapy, which is included in a standard immunosuppressive protocol at our centre, was not used because the level of immunosuppression was sufficiently high at the time of transplantation and the patient had a low lymphocyte number in the peripheral blood. Furthermore, ATG was saved for eventual treatment of rejection episodes.

In the post-transplant period we wished by closely monitoring DRAs without prophylactic IA to promote a graft accommodation [22] and the modulation of the patient's immune response by allograft [23].

The present results illustrate the lack of clear indications for prophylactic post-transplant IA of DRA. The relatively high level of anti-lymphocyte DRA (titres 16–64) and the increase of antibody titre by two serum dilutions (considered as a nonsignificant increase) preceded the first rejection episode, which according to the biopsy was a pure cellular rejection without indirect signs of DRA involvement. Thereafter, the DRA level successively decreased despite two more acute rejection episodes and without postoperative PAIA. On the other hand, the significant increase in anti-A_1 titres (three or more serum dilutions) during the first few days should motivate IA before the clinical signs of rejection. However, we showed that blood group-specific IA is a very effective treatment for removal of anti-ABO antibody even during acute rejection.

No ABO-compatible heart was offered for 5 days. By requesting an ABO-incompatible transplant we were offered and accepted an A_1 donor heart after 5 h and within the next 5 h four more hearts, all ABO-incompatible, were offered. Consequently, by accepting an ABO-incompatible heart allograft we obtained an optimal timing with regard to the antibody reduction treatment, a short ischaemia time and a three HLA-A, -B, -DR antigen-matched transplant. Thus, accepting an ABO-incompatible transplant might enhance the chances for an urgent transplantation or to find a crossmatch-negative donor.

Our patient received heavy preparative and postoperative regimens with high doses of tacrolimus, MMF, prednisolone and rituximab but not antibody induction therapy. Furthermore, a whole arsenal of other immunomodulating procedures (IVIG, PAIA, plasmapheresis, Glycosorb and photopheresis) but without splenectomy or prophylactic IA was applied. The optimal preoperative and postoperative protocols are still unknown. There is a clear tendency towards the less aggressive conditioning regimens; from splenectomy [11], to anti-CD20 [13], to IVIG [24]; all included PE/IA. The role of immunosuppressive therapy by tacrolimus and MMF in this context might be underestimated.

We believe that successful organ transplantation across the DRAs should include a short but intensive preparative regime, close postoperative monitoring and a standard triple-drug and antibody induction protocol to create the conditions for graft accommodation and the modulation of immune response by allograft.

In conclusion, the patient is doing well on maintenance drug therapy despite the persistence of DRAs against lymphocytes, HLA and blood group antigens. Despite the presence of C4d deposition on capillary endothelium, there is, so far, no sign of graft vasculopathy, in contrast to previous finding [25]. The rise of DRAs in connection with graft rejection was against the blood group A_1 antigen but not, as we primarily expected, against the HLA antigen. The results of recent analysis of the post-transplant course of DRAs against HLA antigens indicate the existence of a specific and allograft-induced downregulative process on antibody production (personal communication).

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