Efficacy of Immunoabsorption To Reduce Donor-Specific Alloantibodies in Kidney-Transplant Candidates

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Abstract

Objectives: We implemented a desensitization program at our center to enable transplant in kidney-transplant candidates who have a living human-leukocyte antigen-incompatible (HLAi) donor. We report on the efficacy of semispecific immunoabsorption to allow HLAi kidney transplant in 6 highly sensitized patients.

Materials and Methods: We chose immunoabsorption as the apheresis technique coupled to hemodialysis as a means to decrease donor-specific alloantibodies in kidney transplant candidates submitted to a pretransplant desensitization program to remove detrimental antibodies.

Results: Six highly sensitized kidney-transplant patients (5 females), awaiting their first (n = 1) or second (n = 5) kidney transplant from a living donor, were enrolled in this desensitization program. They had 1 (n = 2), 2 (n = 1), 3 (n = 2), or 4 (n = 1) donor-specific alloantibodies; their mean fluorescent intensities at predesensitization ranged from 1200 to 19 000. Each patient underwent between 10 and 16 immunoabsorption sessions. At the time of transplant, donor-specific alloantibodies were undetectable in 2 patients (A24, DR3); donor-specific alloantibodies decreased by > 50% in 8 patients (A11, B44, DR3, DR11, DQ3 thrice, DQ5); donor-specific alloantibodies remained unchanged in 2 patients (B50, DR13); and mean fluorescent intensities were slightly increased in 2 patients (Cw6, DQ8). In the analysis of final outcomes, 2 patients experienced no rejection (1 experienced donor-specific alloantibody elimination, and 1 experienced a > 50% decrease in donor-specific alloantibodies). One patient presented with acute antibody-mediated rejection, which required immunoabsorption sessions and eculizumab therapy (donor-specific alloantibodies between 5000 and 19 000). Two patients presented with subacute antibody-mediated rejection; 1 was treated by plasmapheresis/rituximab therapy, and the other was treated with plasmapheresis/methylprednisolone pulses. Another patient presented with chronic antibody-mediated rejection, which was treated unsuccessfully with plasmapheresis/rituximab; a tentative of rescue therapy with eculizumab was attempted without success.

Conclusions: Desensitization of the human-leukocyte antigen using this immunoabsorption procedure effectively reduced or eliminated donor-specific alloantibodies in 71% of patients undergoing kidney transplant, at the time of transplant.

Key words: Eculizumab, Apheresis, Antibody-mediated, Rejection, HLA sensitized patients

Introduction

The number of end-stage renal disease patients is increasing rapidly in the Western world and is associated with major costs.1 Its treatment relies on dialysis and for some patient we can offer kidney transplantation. Although patient treatment in the first year following a kidney transplant is as expensive as hemodialysis treatment for a year, the cost and quality-of-life is much improved thereafter.2 Because of the shortage of organs from deceased...
donors, one means to cope with the huge numbers of patients with end-stage renal disease treated with dialysis and awaiting a kidney transplant is to implement living-donor kidney transplant. However, in some cases, the potential recipient has antibodies against the donor, which can render kidney transplant problematic. These antibodies can be directed against human-leukocyte antigens [donor-specific alloantibodies (DSAs)] and/or against ABO antigens [anti-A or /and anti-B isoagglutinins].

If kidney donation is to be undertaken in the setting of incompatibility to human-leukocyte antigens (HLAi) and/or ABO incompatibility (ABOi), medical personnel must implement pretransplant desensitization protocols. These rely on (1) immunomodulatory agents (eg, intravenous immunoglobulin [IV-Ig]) and/or immunosuppressants (eg, rituximab, tacrolimus, mycophenolate mofetil, steroids), and (2) apheresis. Apheresis is used to remove deleterious antibodies from the blood, which could trigger acute antibody-mediated rejection. Immunomodulatory and immunosuppressant agents are then given to prevent resynthesis of these deleterious antibodies. Apheresis techniques include plasmapheresis, double-filtration plasmapheresis, and immunoadsorption. We chose to use immunoadsorption because, relative to plasmapheresis or double-filtration plasmapheresis, it removes only immunoglobulins and has no effect on clotting factors or other components in the plasma. In the setting of HLAl kidney transplant, we use semispecific immunoadsorption using protein-A Immunosorba® absorber columns (Fresenius Medical Care, Bad Homburg, Germany), as previously described. The number of pretransplant immunoadsorption sessions needed is guided by the levels of DSAs expressed in mean intensity fluorescent (MFI) units. In cases where HLAl is associated with ABOi, semispecific immunoadsorption also can be used to decrease isoagglutinin titers.

Materials and Methods

We prospectively included 6 hemodialysis kidney-transplant candidates. There were 5 females; the median age was 52 years (range, 32-62 y). One patient was a liver-transplant recipient with calcineurin-inhibitor-induced end-stage renal disease; the 5 other patients have had a previous kidney transplant that had failed. Because they were highly sensitized to human-leukocyte antigens, they had been waiting for a second kidney transplant for many years. All patients had a potential living-related donor (see Table 1). However, every patient had at least 1 DSA (up to 4 in 1 patient). The study was approved by the Ethical Review Committee of our Institute. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all subjects.

To be included in our desensitization program, we required a negative T-cell crossmatch between the donor and recipient, as assessed by microlymphocytotoxicity. Two patients (Nos. 1 and 2) had positive B-cell crossmatches (as assessed by microlymphocytotoxicity). In the other 4 patients, both T- and B-cell crossmatches were negative (as assessed by microlymphocytotoxicity). We did not perform crossmatches by flow cytometry. All crossmatches were performed before any rituximab infusion to avoid false positive results on B cells.

With regards to the desensitization protocol, all patients followed the same protocol (as shown in Figure 1). At 40 days pretransplant and during an hemodialysis session, the patients were perfused with IV-Ig at 1 g/kg. At 30 and 15 days pretransplant, all patients received rituximab at 375 mg/m², paracetamol (1 g), plus methylprednisolone (1 mg/kg).

At 10 days pretransplant, immunosuppression was started with tacrolimus (0.1 mg/kg bid), mycophenolate mofetil (1 g bid), and prednisone (0.5 mg/kg/d). At the same time, we implemented prophylaxis against Pneumocystis jirovecii using sulfamethoxazole (400 mg)/ trimethoprim (80 mg) every other day for a period of 1 year.

Immunoadsorption sessions were started at 17 days pretransplant and were then scheduled on pretransplant days 16, 15, 12, 11, and 10. Subsequently, they were performed according to the DSA levels assessed at days 10 and 5 pretransplant, i.e IA sessions were done if DSA(s) were > 3000.

The immunoadsorption procedure was performed as previously described. In all cases, the immunoadsorption sessions were performed as a tandem procedure with hemodialysis.

Kidney transplant was performed on the day scheduled, regardless of the results of the DSA MFI at
5 days pretransplant. Induction therapy used antithymocyte globulin at 1 mg/kg, which was given IV just before transplant and then postoperatively on days 2 and 4 at the same dosage.

Donor specific alloantibodies (DSA) were detected by Luminex assays using the LABScreen Single antigen kit (One Lambda, Canoga Park, CA, USA) according to the manufacturer’s instructions. The presence and specificity of antibodies were then detected using a LABScan TM 100 (One Lambda) and the mean fluorescence (baseline value) for each sample in each bead was evaluated. The baseline value was calculated as follows: (raw sample MFI – raw negative MFI) – (negative-bead raw MFI with sample – negative-bead raw MFI with negative serum control). A baseline value of > 1000 was considered positive.

Results

Patients underwent from 10 to 16 sessions of immunoadsorption before receiving a kidney transplant (see Table 1). At the time of transplant, this resulted in (1) elimination of 2 DSAs; (2) the reduction of 8 DSAs by > 50%; (3) two stable DSAs; but (4), an increase in 2 DSAs (Cw6 and DQ8). Overall, DSAs were eliminated or reduced by more than 50% in 71% of patients by the time of transplant. At the last follow-up, DSAs remain negative in 5 settings; they remain reduced > 50% in 6 settings; they are stable in 1 setting, but have increased in 2 settings.

The median posttransplant follow-up was 11 months (range, 7-15 mo). Patient- and graft-survival rates were 100% and 84%. Three of the 6 patients had a (sub)acute humoral-rejection episode and 1 had a chronic antibody-mediated rejection. None of the patients presented with an acute cellular rejection (Table 2). Two of the patients (Nos. 4 and 5) did not have an acute-rejection episode, and both had significantly decreased DSA levels after the immunoadsorption sessions (Table 3).

Patient 1 presented with an acute antibody-mediated rejection episode on postoperative day 4. She recovered renal function by postoperative day 4, but then suddenly became oligo-anuric, was subfebrile (38°C), and had features of thrombotic microangiopathy, which included schistositic hemolytic anemia and thrombopenia. At this time, tacrolimus trough levels were 8 ng/mL. Because a diagnosis of acute antibody-mediated rejection was apparent, she was given pulses of methylprednisolone (10 mg/kg/d) and daily plasmapheresis with fresh frozen plasma as a replacement fluid. After 4 days of this therapy, there was no improvement. We then performed a kidney-allograft biopsy, which confirmed acute antibody-mediated...

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**Figure 1.** Desensitization Protocol Used at Toulouse University Hospital in the Setting of HLA-Incompatible Kidney-Transplant Recipients

**Table 1.** Demographic Data of Patients Within the Study Group

<table>
<thead>
<tr>
<th>Patients No.</th>
<th>Gender</th>
<th>Age at the Time of Desensitization (y)</th>
<th>Previous KTx Y/N (n)</th>
<th>Time on HD (mo)</th>
<th>IA (PP) Before KTx</th>
<th>Posttransplant Immunosuppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>62</td>
<td>Y (1)</td>
<td>45</td>
<td>16 (4)</td>
<td>Tac-MMF Cs</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>52</td>
<td>Y (1)</td>
<td>56</td>
<td>16 (3)</td>
<td>Tac-MPA Cs</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>55</td>
<td>Y (1)</td>
<td>40</td>
<td>16</td>
<td>Tac-MMF Cs</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>32</td>
<td>N</td>
<td>NA</td>
<td>10</td>
<td>Tac-MPA Cs</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>46</td>
<td>Y (1)</td>
<td>57</td>
<td>10</td>
<td>Tac-MPA Cs</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>39</td>
<td>Y (1)</td>
<td>48</td>
<td>16</td>
<td>Tac-MMF Cs</td>
</tr>
</tbody>
</table>

**Abbreviations:** Cs, corticosteroids; DSA, donor-specific alloantibodies; HD, hemodialysis; IA, immunoadsorption; KTx, kidney transplant; MMF, mycophenolate mofetil; MPA, mycophenolic acid; NA, not applicable; PP, plasmapheresis; Tac, tacrolimus
rejection with diffuse C4d deposits. Assessment of DSAs showed that there was rebound of anti-DQ5 (MFI = 18 000 vs 8000 at the time of transplant). Facing a severe episode of acute antibody-mediated rejection, we gave the patient eculizumab (1200 mg) on days 1 and 8, and then 900 mg every 2 weeks for 3 months. At 1 week after the first dose, urine output was present, renal function had recovered quite quickly, and the thrombotic microangiopathy had disappeared. By 2 weeks after starting eculizumab therapy, the patient was discharged; serum creatinine was 130 μmol/L. At 3 months after transplant, serum creatinine was 100 μmol/L. At this point, a surveillance kidney-transplant biopsy was performed: this was almost normal. Thus, we decided to stop eculizumab therapy. At 1 year after transplant, serum creatinine was 110 μmol/L.

Although DSA were nil, because there was an increase in isoagglutinine titers from 1/2 to 1/16, in this patient who was also an ABO incompatible kidney transplant patient we assumed that she had an acute antibody-mediated rejection. This was treated with 6 plasmapheresis sessions and a large increase in prednisone (1 mg/kg/d for 10 days). Serum-creatinine levels improved within 5 days and have returned to baseline values.

Patient 3 presented at 4 months posttransplant with a slight increase in serum creatinine, from 90 to 130 μmol/L. DSAs were stable. We performed a kidney biopsy, which showed capillaritis, glomerulitis, and diffuse C4d staining. We treated this patient with pulses of methylprednisolone (10 mg/kg/d, 3 days), 6 plasmapheresis sessions, and 2 doses of rituximab (375 mg/m² each). Thereafter, her renal function stabilized at ~100 μmol/L of serum creatinine. One month later, she developed severe hypertension, which was resistant to quadruple therapies. An echo-Doppler echogram of the kidney revealed severe stenosis of the renal-artery anastomosis. We performed a percutaneous angioplasty, which was successful but was complicated with a dissection of the renal artery. In the subsequent days, serum creatinine rose to 300 μmol/L, but then stabilized at ~250 μmol/L. Since then, her blood pressure has been controlled and, although renal function is impaired, it is stable.

Patient 2 had a serum creatinine level of 180 μmol/L at the time of discharge. At that time he had no proteinuria. At 5 months posttransplant, he developed overt proteinuria of ~2 g/d, slightly increased serum creatinine levels at (220 μmol/L), but his DSA levels were stable. Because he was receiving anticoagulant therapy for an aortic mechanical valve, we did not perform a kidney biopsy. With the assumption that he had a subacute antibody-mediated rejection, we performed 6 plasmapheresis sessions and gave 2 injections of rituximab (375 mg/m² each, at 1 week apart).
Subsequently, there was no change in his renal parameters. At 8 months posttransplant, because serum creatinine had risen to 300 μmol/L and because proteinuria was 6 g/d, we performed a percutaneous kidney biopsy despite the anticoagulant therapy. This primarily showed features of a chronic antibody-mediated rejection. Thus, we decided to implement eculizumab therapy (1200 mg/week, for 2 weeks, and then 900 mg every 2 weeks). The renal parameters stabilized for 2 months but subsequently deteriorated and he returned to hemodialysis by 11 months posttransplant.

Discussion

Herein, we report on the effect of pretransplant desensitization, using immunoadsorption with conventional immunosuppression, in the setting of incompatibility to human-leukocyte antigens with a living-kidney transplant. We demonstrate that this therapy is very effective at reducing pretransplant DSA levels; despite that, two-thirds of patients presented with antibody-mediated rejection posttransplant.

We included 6 patients (5 women and 1 man) who were highly sensitized to human-leukocyte antigens; of these, 5 patients had received a previous kidney transplant. All had been waiting for a deceased donor for > 40 months. When their serums were tested against a potential living donor we found that the recipients had at least 1 DSA against the donor; 12 of the 14 DSAs had MFI values > 3000 units. However, 2 of the patients had a positive crossmatch (assessed by microlymphocytotoxicity) with B lymphocytes; conversely, crossmatches on T lymphocytes were always negative in the 6 patients.

Recently, Montgomery and associates have shown that, in the setting of live-donor kidney transplant, desensitization using plasmapheresis and low doses of IV-Ig provides a significant survival benefit for patients with sensitization to human-leukocyte antigens when compared to those awaiting a compatible organ from a deceased donor. By 8 years, this survival advantage, compared with those that remained on dialysis and on the kidney-waiting list, had more than doubled.6

In previous studies, desensitization has been conducted without apheresis, or with plasmapheresis, or with plasmapheresis and specific immunoadsorption. To the best of our knowledge, our study is the first to report on the efficacy of semispecific immunoadsorption using Immunosorba® columns in the setting of incompatibility to human-leukocyte antigens in living-kidney transplants.

It was necessary to perform between 10 and 16 immunoadsorption sessions to decrease DSAs to a sufficient level. The 2 patients needing in addition plasmapheresis sessions pretransplant were those with a positive B-cell crossmatch.

Our procedure, which was combined with pretransplant immunosuppression, resulted in either the disappearance or a decrease of > 50% of DSAs (according to MFI) in 71% of the patients. These results were sustained: at the last follow-up, 5 DSAs were no longer detectable, and MFI values were < 50% of those at predesensitization for 2 DSAs.
Altogether, at last follow-up, as compared to predesensitization, 80% of DSAs had decreased. However, despite these good results, only 2 of the 6 patients did not experience an antibody-mediated rejection. For the 4 patients who experienced antibody-mediated rejection, this was very acute in 1 patient and required eculizumab therapy; rejection was subacute in the other 3 patients. One of these 3 patients subsequently developed a chronic antibody-mediated rejection, which required eculizumab. Other recent case studies also report that eculizumab, a humanized anti-C5a antibody, saved the kidneys of patients with acute humoral rejection and who were resistant to all other therapies.9,10

In the setting of living kidney donation with DSAs, the use of eculizumab can be valuable in prevention of acute antibody-mediated acute rejection. Hence, Stegall and associates have reported on the efficacy of terminal complement inhibition with the humanized anti-C5 antibody, eculizumab, in the prevention of antibody-mediated rejection (AMR) in renal transplant recipients with a positive crossmatch against their living donor. The incidence of biopsy-proven AMR in the first 3 months posttransplant in 26 highly sensitized recipients of living-donor renal transplants who received eculizumab posttransplant was compared to a historical control group of 51 sensitized patients treated with a similar plasma exchange (PE)-based protocol without eculizumab. The incidence of AMR was 7.7% (2/26) in the eculizumab group compared to 41.2% (21/51) in the control group (P = .0031). Eculizumab also decreased AMR in patients who developed high levels of DSAs early after transplant that resulted in proximal complement activation. With eculizumab, AMR episodes were easily treated with PE, reducing the need for splenectomy. On 1-year protocol biopsy, transplant glomerulopathy was found to be present in 6.7% (1/15) eculizumab-treated recipients and in 35.7% (15/42) of control patients (P = .044). These researchers concluded that inhibition of terminal complement activation with eculizumab decreases the incidence of early AMR in sensitized renal transplant recipients.11 Randomized clinical trials are currently underway evaluating the benefit of adding eculizumab to prevent acute AMR in de novo kidney transplant patients.

We conclude that semispecific immuno-adsorption can decrease DSAs at pretransplant in more than two-thirds of cases when it is combined with immunosuppression, thus allowing kidney transplant from a live donor. Despite the associated high rate of posttransplant antibody-mediated rejection, our findings indicate that such rejection may be treated by treatment with plasmapheresis, rituximab or eculizumab if needed.

References