Mycophenolate Mofetil, Together with Cyclosporin A, Prevents Anti-OKT3 Antibody Response in Kidney Transplant Recipients

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Abstract. OKT3 monoclonal antibody, a murine IgG2a monoclonal antibody targeting the T cell CD3 antigen, elicits a neutralizing humoral response in 20 to 50% of kidney transplant recipients when the concomitant immunosuppression consists of CsA-Sandimmun (SAND) and azathioprine (AZA). In the present study, we investigated the impact of the newer agents, CsA-Neoral (NEO) and mycophenolate mofetil (MMF) on OKT3 sensitization. Sixty-two consecutive kidney transplant recipients received prophylactic OKT3 (5 mg/d) from days 0 to 13, together with steroids. Concomitant immunosuppression consisted of either AZA + SAND (n = 20), AZA + NEO (n = 31), or MMF + NEO (n = 11). The following doses were used: AZA, 2 mg/kg per d from days 0 to 13, then 1 mg/kg per d; MMF, 2 g/d starting on day 1; and CsA, either SAND or NEO, 6 mg/kg per d from day 6. At least two serum samples per month were available during the initial 3 mo for each patient. IgG anti-OKT3 antibodies were first evaluated by enzyme-linked immunosorbent assay. Patients were considered sensitized if their serum scored positive at a dilution ≥1/1000. Peak titers of IgG anti-OKT3 antibodies and the incidence of patients harboring neutralizing anti-idiotypic antibodies were also determined. A first reduction in OKT3 sensitization was seen in patients receiving Neoral instead of Sandimmun (AZA + SAND: 10 of 20 [50%] patients sensitized versus 6 of 31 [19%] in the AZA + NEO group; P = 0.03). This was probably related to the achievement of higher mean CsA trough blood levels in the NEO group during the first month (253 ± 44 versus 186 ± 49 ng/ml in SAND patients). Peak antibody titers and the proportion of patients with anti-idiotypic antibodies were similar in the AZA + SAND and AZA + NEO groups. A further reduction in the sensitization rate was observed with the replacement of AZA by MMF (MMF + NEO: 0% sensitized patients; P = 0.0013). It is concluded that the combination of CsA-Neoral and MMF efficiently prevents sensitization against OKT3.

The prophylactic use of the murine anti-CD3 IgG2a monoclonal antibody (mAb) OKT3 in renal transplantation has improved graft survival in several categories of high immunologic risk patients compared with a regimen combining CsA-Sandimmun (SAND) and azathioprine (AZA) (1–3). With regard to rejection therapy, OKT3 has proved its value as a first-line therapy (4) and at reversing corticosteroid-resistant rejection episodes (5,6). One of the side effects of OKT3 has been the development of an anti-OKT3 antibody response. The specificity of anti-OKT3 antibodies may be either anti-idiotypic, directed at IgG2a murine determinants, or anti-idiotypic, targeting the OKT3 variable Fab fragments involved in CD3 binding (7,8). The anti-idiotypic sensitization may result in OKT3 neutralization and hence prevent OKT3 reuse if it consists of high titers of IgG antibodies (≥1/1000 of serum dilution) (9–13).

The incidence of OKT3 sensitization is affected by the immunosuppressive drugs used concomitantly. Anti-OKT3 antibodies were detected in 100% of the patients when OKT3 was used as the sole immunosuppressive agent (7), and this figure fell to approximately 40 and 25% with the concomitant use of AZA and SAND, respectively (14–17). Both drugs have the ability to inhibit primary antibody responses (18–21).

The recent availability of CsA-Neoral, the microemulsion formulation of cyclosporine, and mycophenolate mofetil (MMF) might further reduce the incidence of OKT3 sensitization. CsA-Neoral (NEO) has improved bioavailability compared with SAND (22), and MMF is a potent inhibitor of antibody synthesis (23,24). The aim of the present study was to evaluate the impact of both Neoral and MMF on anti-OKT3 antibody formation in kidney transplant recipients.

Materials and Methods

Patients and Immunosuppressive Protocols

We retrospectively evaluated 62 consecutive recipients of cadaveric kidney grafts who received induction therapy with OKT3. OKT3 was given from the day of transplantation (day 0) until day 13 at a dose of 5 mg/d, together with steroids. Concomitant immunosuppression in three consecutive groups of patients consisted of either AZA + SAND (n = 20); AZA + NEO (n = 31); or MMF + NEO (n = 11). Patient characteristics are shown in Table I. The dose of AZA was 2 mg/kg per d from days 0 to 13 and 1 mg/kg per d thereafter.

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CsA trough levels were between 150 and 507% of the value of the positive pool. Titers were calculated by interpolation and expressed as a percentage.

NEO, was initiated on day 6, at a dose of 6 mg/kg per d. Target blood CsA, either SAND or MMF was initiated on day 1 at a dose of 2 g/d. CsA trough levels were between 150 and 300 ng/ml during the first 3 posttransplant months.

Detection of Anti-OKT3 Antibodies by Enzyme-Linked Immunosorbent Assay

Serum samples were obtained at least twice monthly during the first trimester. The mean number of samples per patient was 14.4 (range, 11 to 20) in AZA + SAND patients, 14.6 (range, 7 to 24) in AZA + NEO patients, and 15.3 (range, 10 to 20) in MMF + NEO patients (P = NS). Anti-OKT3 IgG antibodies were detected by an enzyme-linked immunosorbent assay method as described previously (25). Briefly, OKT3 was coated on microtiter plates before the addition of several serum dilutions. The titration curves included a 1/100 and a 1/1000 dilution of serum. Bound anti-OKT3 antibodies were revealed by an Fc-specific antihuman IgG peroxidase-conjugated goat antibody (Sigma A0170). Because only one of the 62 patients from this series (AZA + SAND group) developed anti-OKT3 sensitization limited to low titers (1/100), further analysis was restricted to patients who were positive at the 1/1000 dilution in one or more serum samples. Positivity was defined as an optical density value equal or superior to twofold the optical density of the negative control pool at the 1/1000 dilution. Because the coating was performed with the intact antibody, the enzyme-linked immunosorbent assay is able to pick up both anti-idiotypic and anti-isotypic antibodies.

To evaluate more precisely the intensity of the anti-OKT3 antibody response, titration curves of sera from sensitized patients were compared with a serum pool from patients immunized against OKT3. Titers were calculated by interpolation and expressed as a percentage of the value of the positive pool.

Detection of Neutralizing Anti-Idiotypic Anti-OKT3 Antibodies

The principle of this assay lies in the ability of anti-idiotypic antibodies to bind to OKT3, therefore preventing its binding to CD3-positive cells (8). Briefly, FITC-conjugated OKT3 was incubated with serum samples from immunized patients before the addition of normal peripheral blood mononuclear cells. The number of peripheral blood mononuclear cells positive for OKT3 and the intensity of OKT3 staining were analyzed by fluorescence-activated cell sorter. A decrease in either parameter was indicative of the presence of anti-idiotypic anti-OKT3 antibodies.

Statistical Analyses

Differences between the three groups were assessed by ANOVA for continuous variables and by the χ² test for categorical variables.

Results

Impact of Neoral and MMF on OKT3 Sensitization

The consecutive replacement of Sandimmun by Neoral and of AZA by MMF was associated with a successive, significant decrease in the incidence of patients sensitized to OKT3 (Table 2). First, the substitution of CsA-Sandimmun by CsA-Neoral in patients on AZA was accompanied by a reduction from 50 to 19% in the incidence of patients sensitized to OKT3 (AZA + SAND versus AZA + NEO groups; P = 0.03). In addition, peak titers of anti-OKT3 antibodies among sensitized patients decreased approximately twofold in AZA + NEO versus AZA + SAND patients, but this difference did not reach statistical significance (Table 2). The proportion of sensitized patients

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AZA + SAND</th>
<th>AZA + NEO</th>
<th>MMF + NEO</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>31</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>38.4 ± 2.5</td>
<td>42.8 ± 1.9</td>
<td>46.6 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>14/6</td>
<td>21/10</td>
<td>4/7</td>
<td>NS</td>
</tr>
<tr>
<td>No. with PRA &gt;5%</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Cold ischemia time (hours)</td>
<td>21.8 ± 1.0</td>
<td>23.5 ± 1.2</td>
<td>22.1 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>HLA A + B + DR mismatches</td>
<td>1.5 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>1.8 ± 0.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

*All patients were recipients of their first allograft. AZA, azathioprine; SAND, cyclosporin A Sandimmun; NEO, cyclosporin A Neoral; MMF, mycophenolate mofetil; PRA, panel-reactive antibodies.

Table 2. Impact of Neoral and MMF on OKT3 sensitization

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AZA + SAND</th>
<th>AZA + NEO</th>
<th>MMF + NEO</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. sensitized (titers ≥1/1000)</td>
<td>10 of 20 (50%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6 of 31 (19%)</td>
<td>0 of 11 (0%)</td>
<td>0.0013</td>
</tr>
<tr>
<td>Anti-OKT3 Ab titers</td>
<td>426% (95 to 1816)</td>
<td>202% (71 to 542)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>(% of positive pool)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5/9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3/6</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Ab, antibody. Other abbreviations as in Table 1.
<sup>b</sup> P = 0.03 versus AZA + NEO and 0.005 versus MMF + NEO by Fisher exact test.
<sup>c</sup> Mean percentages, with ranges shown in parentheses. The nonparametric Wilcoxon test was used.
<sup>d</sup> Sera from one patient were not available for analysis.
that developed anti-idiotypic antibodies was not different between AZA + SAND and AZA + NEO groups (Table 2). Because improved bioavailability of CsA Neoral over CsA-Sandimmun might play a role in these results, we analyzed the CsA doses as well as CsA trough blood levels during the first posttransplant month. CsA doses did not differ between the three treatment groups, whereas patients on Neoral had significantly higher CsA levels than those on Sandimmun (AZA + SAND: 186 ± 49 ng/ml; AZA + NEO: 253 ± 44 ng/ml; and MMF + NEO: 272 ± 53 ng/ml; \( P < 0.0001 \)) (Figure 1).

Second, a further reduction in the sensitization rate was observed with the replacement of AZA by MMF (0% sensitized patients in the MMF + NEO group; \( P = 0.0013 \) by \( \chi^2 \) for trend).

Rejections and Adverse Events during the First Trimester

Because the intensity of immunosuppression obviously has been increased by the successive replacement of Sandimmun by Neoral and of AZA by MMF, it was important to compare the three groups for the main clinical events occurring after transplantation. As shown in Table 3, the number of patients who experienced rejection was not significantly different, although there was a trend toward fewer rejections in MMF + NEO patients. Importantly, the increased levels of CsA did not adversely affect plasma creatinine at either 1 or 3 mo. The incidence of urinary tract infections, cytomegalo virus diseases, lymphomas, and death was not different between the three treatment groups.

Discussion

The main finding of this study is that the combination of Neoral and MMF was remarkably effective in the prevention of the humoral response against the OKT3 mAb in kidney transplant recipients. Both drugs appear to have played a role in this process. Indeed, there was an initial drop from 50 to 19% in the incidence of OKT3 sensitization when Sandimmun was replaced by Neoral in patients receiving AZA. Higher trough CsA blood levels, an expected consequence from the improved bioavailability of Neoral, probably account for this observation. CsA has the ability to inhibit primary antibody responses mainly by preventing the T cell-derived cytokine production required for antibody production (26,27), but possibly also through direct effects on B cells (18–20).

A further drop in the OKT3 sensitization rate was seen when MMF was used instead of AZA. The direct comparison with the AZA + NEO group did not reach significance, probably because of the limited number of patients in the MMF + NEO group. However, the independent role of MMF is suggested by a significant, progressive reduction in the proportion of sensitized patients in the three consecutive AZA + Sandimmun, AZA + Neoral, and MMF + Neoral groups (\( P = 0.0013 \) by \( \chi^2 \) for trend). MMF inhibits the proliferation of T and B lymphocytes, and as a result, this drug has been shown to powerfully inhibit antibody production in vivo in rodents (28,29) and in vitro in humans (24). With regard to the impact of MMF on in vivo antibody generation in humans, recent work by Kimball et al. showed that MMF, given at a dose of 2 or 3 g/d, efficiently reduced both the incidence as well as the maximal antibody response against the antithymocyte gamma-globulin horse polyclonal preparation compared with AZA (30). Our observations suggest that the in vivo inhibition of humoral response in humans extends to murine monoclonal antibodies. This might prove beneficial not only by allowing the reuse of OKT3 if necessary, but also when therapy with chimeric or humanized mAb is considered. Indeed, the idiotypic portions of these antibodies may still remain immunogenic and induce a neutralizing anti-idiotypic response (31–33).
the concomitant use of Neoral and MMF might prevent the residual immunogenicity of these antibodies.

Finally, recent studies indicate that the generation after transplantation of alloantibodies reactive with MHC class I or class II antigen disparities might be detrimental to the graft and contribute to chronic rejection (34–37). Additional studies should elucidate whether the Neoral-MMF combination will prove effective in preventing this humoral allosensitization.

### Acknowledgments

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### References


### Table 3. Rejections and adverse events during the first trimestera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AZA + SAND</th>
<th>AZA + NEO</th>
<th>MMF + NEO</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>31</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>DGFb</td>
<td>3 of 20 (15%)</td>
<td>5 of 31 (16%)</td>
<td>2 of 11 (18%)</td>
<td>NS</td>
</tr>
<tr>
<td>No. with rejection</td>
<td>6 of 20 (30%)</td>
<td>9 of 31 (29%)</td>
<td>1 of 11 (9%)</td>
<td></td>
</tr>
<tr>
<td>Graft lossc</td>
<td>1 of 20 (5%)</td>
<td>2 of 31 (6%)</td>
<td>0 of 11 (0%)</td>
<td></td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)d</td>
<td>1.82 ± 0.17</td>
<td>1.66 ± 0.17</td>
<td>1.56 ± 0.15</td>
<td>NS</td>
</tr>
<tr>
<td>1 mo</td>
<td>1.78 ± 0.21</td>
<td>1.57 ± 0.22</td>
<td>1.59 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>3 mo</td>
<td>5 of 20 (25%)</td>
<td>8 of 31 (26%)</td>
<td>3 of 11 (27%)</td>
<td></td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>2 of 31 (10%)</td>
<td>1 of 31 (3%)</td>
<td>1 of 11 (9%)</td>
<td></td>
</tr>
<tr>
<td>CMV diseasec</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>PLTD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

a DGF, delayed graft function; CMV, cytomegalovirus; PTLD, posttransplant lymphoproliferative disease. Other abbreviations as in Table 1.

b Defined as the need for at least one dialysis during the first week.

c All losses were due to rejection.

d Mean ± SEM. Only patients with a functioning graft were considered.

e Tissue-invasive disease requiring therapy.