Frequency and Clinical Implications of Development of Donor-Specific and Non–Donor-Specific HLA Antibodies after Kidney Transplantation

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The involvement of immunologic and nonimmunologic events in long-term kidney allograft failure is difficult to assess. The development of HLA antibodies after transplantation is the witness of ongoing reactivity against the transplant, and several studies have suggested that the presence of HLA antibodies correlates with poor graft survival. However, they have not discriminated between donor-specific (DS) and non–specific (NDS) antibodies. A total of 1229 recipients of a kidney graft, transplanted between 1972 and 2002, who had over a 5-yr period a prospective annual screening for HLA antibodies with a combination of ELISA, complement-dependent cytotoxicity, and flow cytometry tests were investigated; in 543 of them, the screening was complete from transplantation to the fifth year postgrafting. Correlations were established between the presence and the specificity of the antibodies and clinical parameters. A total of 5.5% of the patients had DS, 11.3% had NDS, and 83% had no HLA antibodies after transplantation. NDS antibodies appeared earlier (1 to 5 yr posttransplantation) than DS antibodies (5 to 10 yr). In multivariate analysis, HLA-DR matching, pretransplantation immunization, and acute rejection were significantly associated with the development of both DS and NDS antibodies and also of DS versus NDS antibodies. The presence of either DS or NDS antibodies significantly correlated with lower graft survival, poor transplant function, and proteinuria. Screening of HLA antibodies posttransplantation could be a good tool for the follow-up of patients who receive a kidney transplant and allow immunosuppression to be tailored.


Although the humoral response in the pathogenesis of acute or hyperacute vascular rejection in patients who have pretransplantation HLA immunization has been studied extensively, less attention has been paid to the role of MHC antibodies in chronic rejection, although it long has been suspected that their early development after renal transplantation leads to graft loss (1). The role of HLA antibodies is presently revisited, following the publication of several studies in the past 10 yr, showing that the appearance of HLA antibodies after transplantation is associated, in kidney, pancreas, heart, and lung transplantation, with poor transplant outcome and the occurrence of acute but also chronic rejection (review in 2). In the few reports that have analyzed serially the humoral response after kidney transplantation, HLA antibodies were generally detected before chronic rejection or transplant failure (3,4), suggesting that they could be responsible for transplant dysfunction. Another study showed that the percentage of immunized patients increased with the number of acute rejections, but the presence of HLA antibodies was also found to be an independent factor for graft failure (5). The importance of humoral immunity in organ transplantation was summarized by Terasaki (6).

HLA antibodies are generally not detected on transplant biopsy. The studies from Feucht (7) highlighted the role of the complement product C4d as a marker of complement activation by antibody deposition in the graft capillaries and established the “missing link” between antibodies and transplant histology of acute or chronic rejection. Nickelheit et al. (8) and Regele et al. (9) also reported, in biopsied recipients of a kidney transplant, a strong association between C4d detection and transplant glomerulopathy. HLA antibodies were screened in the study of Nickelheit et al. (8) and found 43% of the patients positive for C4d versus 19% of those negative for C4d (P = 0.001).

Previous studies have found wide variations in the percentage of patients with HLA antibodies after kidney transplantation, ranging between 12 and 60% (2–5,10). However, the tech-
niques used for HLA antibody screening were different from one study to the other, and most of the cohorts had <100 patients. In addition, antibody specificity against the donor antigens was generally supposed but not identified, as the patients who were tested and found positive for the presence of HLA antibodies were not immunized before transplantation. A recent study, which included 4763 patients, reported that the frequency of HLA antibodies among kidney recipients was 20.9% (11). However, the reactivity of these antibodies against the donor was not analyzed.

The recent development of ELISA and flow cytometry tests for identification of HLA antibody specificity that are more sensitive than the routine complement-dependent cytotoxicity (CDC) led us to reassess in our patients the risk for and implications of posttransplantation donor specific (DS) and non-donor-specific (NDS) HLA immunization. Our study is a systemic annual analysis over a 5-yr period of the presence and the specificity identification of posttransplantation HLA antibodies in a large cohort of 1229 kidney transplant recipients who had a functioning graft at 1-yr posttransplantation. For this purpose, they had an annual screening of their HLA antibodies, detected and identified by CDC, ELISA, and/or flow cytometry (Luminex). The aims of the study were to evaluate the percentage of patients with DS and NDS HLA antibodies, to determine the pre- and posttransplantation parameters that predict HLA immunization posttransplantation, and to correlate this immunization with transplant outcome.

Materials and Methods

Patients

All recipients of a kidney or combined kidney and pancreas transplantation who were followed at our institution between January 1998 and March 2003 were analyzed for the development of HLA antibodies. Among the patients who received a transplant within this period, only those who had a functional transplant for at least 1 yr were included in the study. Screening of HLA antibodies was part of an annual check-up. Two to five sera were available per patient.

The patients had received a transplant between 1972 and 2002, and according to the year of transplantation, different initial and maintenance immunosuppressive protocols were administered. From 1972 to 1982, the maintenance immunosuppressive treatment was based on the combination of steroids and azathioprine and after 1982 consisted of one calcineurin-inhibitor (CNI), mainly cyclosporine (CsA) associated with steroids and azathioprine or mycophenolate mofetil (MMF) after 1996. In the patients who received CNI, steroids were generally stopped in the first 3 mo posttransplantation. From 1981 to 1992, all patients received induction treatment with polyclonal globulins (lympho- or thymoglobulin; Sangstat-Imtix, Lyon, France). After 1992, these were restricted to patients who received a second transplant and patients who were immunized (panel reactive antibodies [PRA] \( \geq 15\% \)) and those with long cold ischemia time (\( \geq 36 \) h). However, after 1998, recipients of a first transplant who had initial CNI triple therapy were also given induction with an anti-IL-2 receptor mAb, basiliximab (Novartis Pharma, Basel, Switzerland) and NDS antibodies were included in experimental protocols administering other mAb or related molecules (anti-LFA1, anti-CD4, or CTLA4-Ig), sirolimus, everolimus, or FTY720.

In all cases, acute rejection episodes were treated with steroid boluses as first-line therapy and with anti-lymphocyte globulins in case of corticoresistance. The immunosuppressive treatment could be modified according to clinical events but not on the knowledge of the presence of HLA antibodies after transplantation alone.

HLA Antibody Studies

One serum sample was harvested for each patient at the date of the transplantation anniversary as part of a systematic check-up. HLA antibodies were detected by an ELISA screening test for class I and class II (LAT-M; One Lambda, Canoga Park, CA). HLA class I and class II antibodies were identified by a combination of CDC and the flow cytometry Luminex (LABScreen; One Lambda) and in some cases by ELISA (LAT ID-1288; One Lambda). An additional identification of DS and NDS antibodies was performed in patients with multiple HLA antibodies using a high-definition, single-antigen ELISA (LS1A01; One Lambda). Patients were said to have DS or NDS antibodies whenever their antibodies were directed against donor HLA class I or class II, identified by at least one test. It was presumed that those who were positive for HLA antibodies in ELISA/Luminex and negative in CDC had complement nonfixing antibodies, but they were equally considered as having DS or NDS antibodies. The patients who had both DS and NDS antibodies were included in the DS group.

CDC was performed on a panel of 36 selected HLA-typed and separated T and B lymphocytes on platelet-absorbed and -unabsorbed sera. T lymphocytes were purified using mAb (LymphoKwick T; One Lambda), and B lymphocytes were isolated using magnetic beads (Dynabeads M-450 pan HLA class II; Dynal, Oslo, Norway). The ELISA (12) and Luminex (13) were performed as recommended by the manufacturer.

HLA Typing and Pretransplantation Cross-Match

For the great majority of the patients, donor and recipient HLA A, B, DR, and DQ typing were performed using routine lymphocytotoxicity, completed after 1993, for the recipient, by genomic DNA typing, using PCR–sequence specific primer (SSP) for the determination of HLA class I (Kit Olerup-SSP; Genovision, Oslo, Norway), or HLA-DR and DQ subtypes (MicroSSP class II; One Lambda). In addition, as DQ typing was not routinely available before 1993, the donor specificity of the DQ antibody was in some cases extrapolated from the donor DR antigens, according to the disequilibrium links between DR and DQ.

Pretransplantation cross-match was performed by CDC on separated T and B lymphocytes, according to National Institutes of Health recommendations (14). A positive T cell cross-match was a contraindication to transplantation.

Statistical Analyses

The statistical analysis compared clinical and immunologic parameters in three groups of patients: Those without HLA antibodies, those with DS antibodies, and those with NDS antibodies. The SAS System Version 8.2 was used to process the data and perform the statistical analysis. We first performed a descriptive analysis to study the association of pre- and posttransplantation parameters with the development of HLA antibodies. Global hypothesis tests were used to analyze either the global difference among the three groups of patients (ANOVA global F) or the global dependency between one parameter and the absence of HLA antibodies or presence of HLA DS or NDS antibodies (Pearson \( \chi^2 \) and/or Cochran-Mantel-Haenszel row mean score). A multivariate analysis was then performed with the Cox model. Risk factors for antibody development were selected from the results of the descriptive analysis. Pairwise comparisons were done among the three groups of patients (HLA antibodies: negative versus NDS; negative versus DS; DS versus NDS). For some variables, an ANOVA model was used and
the pairwise comparison was assessed with a linear contrast corresponding to each comparison. The null hypothesis of equality was tested with an F test. For the other binary variables, a logistic model and a linear contrast corresponding to each comparison were performed. The null hypothesis of group equality was tested by a \( H_9273 \) test. A confidence interval of 95% was required for significance in all tests.

**Results**

**Description of Population and Percentage of Patients with and without HLA Antibodies after Transplantation**

A total of 1229 patients who received a transplant between 1972 and 2002 were included in the study. Ninety-three percent had received a cadaveric transplant, and 10% had a combined kidney and pancreas transplantation. According to the HLA antibody screening, 1021 (83%) had no HLA antibodies, 68 (5.5%) had DS antibodies, and 140 (11.3%) had NDS HLA antibodies after transplantation. Once the antibodies were detected, they did not disappear during the follow-up. Pretransplantation characteristics of the three groups are given in Table 1, and posttransplant data are given in Table 2. A significant difference was found in the interval between transplantation and the date of first antibody screening, 77 ± 59 mo for patients without HLA antibodies, 83 ± 59 and 63 ± 60 for those with DS and NDS antibodies, respectively (\( P = 0.014 \)), and a significantly higher number of patients with DS antibodies were between 5 and 10 yr posttransplantation (39.7 versus 26.7% of the patients without antibodies and 23.6% of those with NDS antibodies; \( P < 0.021 \)). A total of 543 patients (44% of the population), who received a transplant during the study period or 1 yr before, had a complete screening with a serum tested every year. It is interesting that in this subgroup that was submitted to a prospective follow-up, 442 (81.4%) had no HLA antibodies, 26 (4.8%) had DS antibodies, and 75 (13.8%) had NDS antibodies. Their demographic characteristics were very similar to those of the overall population.

**Pre- and Posttransplantation Parameters Associated with Development of HLA Antibodies after Transplantation**

Among the parameters studied, the descriptive statistical analysis evidenced both in the overall population and in the subgroup of patients with a complete annual screening a significant influence of retransplantation; the duration of the pretransplantation dialysis; the presence of immunization against T or B lymphocytes before transplantation; overall HLA-A, -B, -DR matching; and HLA-DR matching alone in the development of HLA antibodies posttransplantation (Table 1). Among posttransplantation parameters, the administration of antithymocyte globulin (ATG) as induction treatment was also associated with the presence of posttransplantation HLA antibodies, independent of their specificity, whereas acute rejection, cytomegalovirus infection, and delayed graft function (DGF) did not (Table 2).

The parameters associated with the development of HLA antibodies and their DS or NDS character then were studied in a pairwise comparison and in a Cox model estimation. In pairwise comparison, HLA-DR matching, >10% PRA against T or B lymphocytes before transplantation, overall HLA-A, -B, and -DR matching; and HLA-DR matching alone in the development of HLA antibodies posttransplantation (Table 1). Among posttransplantation parameters, the administration of antithymocyte globulin (ATG) as induction treatment was also associated with the presence of posttransplantation HLA antibodies, independent of their specificity, whereas acute rejection, cytomegalovirus infection, and delayed graft function (DGF) did not (Table 2).

### Table 1. Pretransplantation characteristics of patients with DS, with NDS, and without HLA Ab posttransplantation

<table>
<thead>
<tr>
<th></th>
<th>Ab Negative (n = 1021)</th>
<th>DS Ab (n = 68)</th>
<th>NDS Ab (n = 140)</th>
<th>( P^{b} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>43 ± 14</td>
<td>38 ± 13</td>
<td>43 ± 14</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Gender (% female/% male)</strong></td>
<td>42/58</td>
<td>48/52</td>
<td>49/51</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Retransplantation (%)</strong></td>
<td>13</td>
<td>27</td>
<td>54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Duration of dialysis (mo)</strong></td>
<td>29 ± 34</td>
<td>42 ± 51</td>
<td>32 ± 38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Immunization: % of patients with</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anti-T Ab ≥10%</td>
<td>18</td>
<td>34</td>
<td>55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>anti-B Ab ≥10%</td>
<td>10</td>
<td>19</td>
<td>48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HLA A-B-DR mismatches (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.5</td>
<td>0</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.1</td>
<td>9.5</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18.3</td>
<td>12.6</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>23.6</td>
<td>29.8</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>24.8</td>
<td>27</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>16.3</td>
<td>16.6</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3.4</td>
<td>4.5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>HLA-DR mismatches (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>6</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>1 to 2</td>
<td>72</td>
<td>94</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

*aDS, donor-specific; NDS, non–donor-specific; Ab, antibodies.*

*b\( P \): F test, \( \chi^2 \), or Cochran-Mantel-Haenszel row mean score.*
antibodies versus patients without HLA antibodies, in both the overall population and the subgroup with a complete annual screening. The age of the recipient also had a significant influence on the presence of DS HLA antibodies (Table 3). In addition, the analysis reported the interval between transplantation and the date of antibody detection as a significant factor in the development of HLA antibodies, with NDS antibodies appearing in the first 5 yr posttransplantation (Table 4) and DS antibodies in the 5 to 10 yr posttransplantation (Table 3). This finding was difficult to interpret as 56% of the patients had no longitudinal screening from the day of transplantation. However, when the multivariate analysis was performed in the subpopulation that had a complete annual screening, this parameter was also present (Tables 3 and 4). The Cox model estimation confirmed the significant influence of DR matching and/or pretransplantation immunization in the development of DS and NDS antibodies, but, surprisingly, it also evidenced a role for MMF administration in the overall population but not in the patients with a complete annual screening (Table 3). This finding was difficult to interpret as 56% of the patients had no longitudinal screening from the day of transplantation. However, when the multivariate analysis was performed in the subpopulation that had a complete annual screening, this parameter was also present (Tables 3 and 4). The Cox model estimation confirmed the significant influence of DR matching and/or pretransplantation immunization in the development of DS and NDS antibodies, but, surprisingly, it also evidenced a role for MMF administration in the overall population but not in the patients with a complete annual screening (Table 3). The pairwise comparison evidenced again recipient’s age, DR matching, one or more acute rejection episodes, and >10% PRA against T or B lymphocytes before transplantation as significant and independent factors associated with the development of DS versus NDS antibodies (Table 6).

**Description of Group of Patients with DS Antibodies**

The 68 recipients with DS HLA antibodies were significantly younger (37.5 yr; range 13 to 69; Tables 3 and 6). Despite the presence of DS antibodies, none presented acute vascular rejection. All but one developed DS HLA class II antibodies: DR in 43%, DQ in 42%, and a mixture of DR and DQ antibodies in 13%. Twenty-five (37%) of these 68 recipients had a transplant biopsy. Transplant glomerulopathy was found in 18, chronic allograft nephropathy was found in three, recurrence of the initial glomerulopathy was found in two, and acute rejection was found in two. Among the 21 biopsies examined for the presence of C4d, 14 were found positive. The great majority of them were administered ATG as induction treatment, but a significantly higher number of them received azathioprine rather than MMF as initial treatment (P < 0.05) or at the time of antibody detection (P < 0.02; Table 2), when they were compared with patients without HLA antibodies or with NDS antibodies.

**Implications of Development of HLA Antibodies Posttransplantation for Transplant Outcome**

During the follow-up period, 101 patients lost their transplant or died: 10 (15%) in the DS group, 25 (18%) in the NDS group, and 66 (6.5%) in the group without HLA antibodies. The correlations between the development of the antibodies and transplant function as well as proteinuria were also investigated. In addition to the expression of these variables as mean values, subgroups were done according to the level of creatinine clearance (≥50, 30 to 50, ≤30 ml/min) or proteinuria (<1, 1 to 3, ≥3 g/d). The statistical analysis (χ² and Cochran-Mantel-Haenszel row mean score) evidenced that the presence of either type of antibodies correlated significantly with poor transplant function (P < 0.001) and the onset of proteinuria (P < 0.001). The differences in serum creatinine, creatinine clearance, and proteinuria between patients with and without HLA antibodies (Table 7) were already significant at the beginning of the study and remained so until the last screening point. In the patients who had a complete annual screening, serum creatinine significantly increased during the study in patients with DS immu-

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**Table 2. Posttransplantation clinical parameters and immunosuppressive treatment in patients with DS, with NDS, and without HLA Ab posttransplantation (Ab negative)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ab Negative (n = 1021)</th>
<th>DS Ab (n = 68)</th>
<th>NDS Ab (n = 140)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Ab detection (mo)</td>
<td>77 ± 59</td>
<td>83 ± 59</td>
<td>63 ± 60</td>
<td>0.014</td>
</tr>
<tr>
<td>Acute rejection (% patients)</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>none</td>
<td>79.5</td>
<td>72</td>
<td>79.3</td>
<td></td>
</tr>
<tr>
<td>1 episode</td>
<td>16.2</td>
<td>22.1</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>≥2 episodes</td>
<td>4.3</td>
<td>6</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Dialysis (% patients)</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>0 to 1 session</td>
<td>74</td>
<td>73</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>≥2 sessions</td>
<td>26</td>
<td>27</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Transplant day with CrCl ≥10 ml/min</td>
<td>6 ± 7</td>
<td>6 ± 8</td>
<td>6 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>CMV infection (%)</td>
<td>16</td>
<td>23</td>
<td>19</td>
<td>NS</td>
</tr>
<tr>
<td>Antithymocyte globulin induction (%)</td>
<td>58</td>
<td>72</td>
<td>78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment at time of Ab detection (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNI</td>
<td>95</td>
<td>93</td>
<td>94</td>
<td>NS</td>
</tr>
<tr>
<td>MMF</td>
<td>45</td>
<td>31</td>
<td>53</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>azathioprine</td>
<td>31</td>
<td>43</td>
<td>23</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>steroids</td>
<td>22</td>
<td>40</td>
<td>43</td>
<td>NS</td>
</tr>
</tbody>
</table>

*CrCl, creatinine clearance; CMV, cytomegalovirus; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil.

P: F test, χ², or Cochran-Mantel-Haenszel row mean score.
nization, whereas proteinuria was already significantly higher at 1 yr posttransplantation.

**Discussion**

Our study was a systematic analysis, conducted over a 5-yr period, of posttransplantation HLA immunization in recipients of a kidney transplant, whatever the date of transplantation between 1972 and 2002. The data of a screening performed within a period of time in patients who are at different intervals after transplantation could be difficult to interpret. However, 44% of our patients, who also had received the most recent transplant, had a complete annual screening from the day of transplantation to the end of the study period. That the results obtained in this subpopulation were for each parameter comparable with those of the overall population gives credit to the general conclusions. Besides the size of the cohort of patients, the sensitivity of the assays used for HLA antibody screening that allowed us to discriminate between DS and NDS antibodies was another important point. That distinction was lacking in the previous publications, with the exception of the study of Christiaans et al. (10), who included 143 patients, 17 of whom had DS antibodies.

HLA antibody screening used a combination of CDC and the most recent ELISA and flow cytometry tests for their detection and identification. The higher sensitivity of these two tests over CDC has been shown (15). According to that screening, 16.8% of the patients had HLA antibodies after transplantation, a percentage in agreement with the lower range of those reported in the literature (2–5,10). A recent study by Terasaki (11), using the same screening tests in a cohort of 4763 kidney recipients during the first year posttransplantation, found a frequency of 20.9%. Only 5.5% in our total population had DS antibodies, and all but one were class II. In 11.3%, HLA immunization was NDS and directed against HLA class I and/or class II. In the subgroup of patients who had a complete annual screening, the percentage of those with DS and NDS antibodies was similar (4.8 and 13.8%, respectively).

The statistical analysis evidenced predictive factors for the presence of HLA antibodies posttransplantation, and, not surprising, significant differences between patients with DS, with NDS, and without antibodies were seen for immunologic parameters: Percentage of retransplantations; percentage of patients with pretransplantation immunization; HLA matching, especially DR; and occurrence of acute rejection. A profile of patients who were prone to develop DS or NDS antibodies after transplantation could be drawn. Patients with NDS HLA anti-

**Table 3. Pairwise comparison of patients with DS Ab versus no HLA Ab**

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total population</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recipient age (yr)</td>
<td>5.48</td>
<td>2.06</td>
<td>8.904</td>
<td>0.0017</td>
</tr>
<tr>
<td>date of Ab detection (mo)</td>
<td>−5.40</td>
<td>−20.00</td>
<td>9.207</td>
<td>0.4686</td>
</tr>
<tr>
<td>1 rejection</td>
<td>2.47</td>
<td>1.40</td>
<td>4.360</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2 rejections</td>
<td>0.43</td>
<td>0.24</td>
<td>0.775</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3 rejections</td>
<td>0.36</td>
<td>0.08</td>
<td>1.655</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Ab detection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 5 yr postgraft</td>
<td>1.41</td>
<td>0.86</td>
<td>2.328</td>
<td>0.17</td>
</tr>
<tr>
<td>5 to 10 yr postgraft</td>
<td>0.55</td>
<td>0.33</td>
<td>0.918</td>
<td>0.02</td>
</tr>
<tr>
<td>&gt;10 yr postgraft</td>
<td>1.30</td>
<td>0.70</td>
<td>2.420</td>
<td>0.41</td>
</tr>
<tr>
<td>DR mismatches</td>
<td>0.16</td>
<td>0.06</td>
<td>0.454</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>peak anti-T ≥10%</td>
<td>0.42</td>
<td>0.25</td>
<td>0.719</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>peak anti-T ≥80%</td>
<td>0.23</td>
<td>0.10</td>
<td>0.533</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>peak anti-B ≥10%</td>
<td>0.50</td>
<td>0.26</td>
<td>0.936</td>
<td>0.03</td>
</tr>
<tr>
<td>peak anti-B ≥80%</td>
<td>0.10</td>
<td>0.03</td>
<td>0.313</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Subpopulation with a complete annual screening</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recipient age (yr)</td>
<td>6.51</td>
<td>1.18</td>
<td>11.843</td>
<td>0.0168</td>
</tr>
<tr>
<td>date of Ab detection (mo)</td>
<td>−4.34</td>
<td>−9.63</td>
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<td>0.1082</td>
</tr>
<tr>
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<td>2.83</td>
<td>1.18</td>
<td>6.796</td>
<td>0.02</td>
</tr>
<tr>
<td>2 rejections</td>
<td>0.45</td>
<td>0.17</td>
<td>1.182</td>
<td>0.11</td>
</tr>
<tr>
<td>3 rejections</td>
<td>0.19</td>
<td>0.04</td>
<td>0.980</td>
<td>0.05</td>
</tr>
<tr>
<td>DR mismatches</td>
<td>0.23</td>
<td>0.05</td>
<td>1.006</td>
<td>0.05</td>
</tr>
<tr>
<td>peak anti-T ≥10%</td>
<td>0.29</td>
<td>0.12</td>
<td>0.670</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>peak anti-T ≥80%</td>
<td>0.19</td>
<td>0.04</td>
<td>0.980</td>
<td>0.05</td>
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<td>0.17</td>
<td>0.913</td>
<td>0.03</td>
</tr>
<tr>
<td>peak anti-B ≥80%</td>
<td>0.07</td>
<td>0.02</td>
<td>0.231</td>
<td>&lt;0.01</td>
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*CI, confidence interval.*
Table 4. Pairwise comparison of patients with NDS Ab versus no HLA Ab

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<tr>
<th></th>
<th>Estimate</th>
<th>95% CI</th>
<th>P</th>
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<tbody>
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<td>Upper Limit</td>
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<td>Total population</td>
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<tr>
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<td>7.91</td>
<td>5.41</td>
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<td>0.13</td>
<td>0.275</td>
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<tr>
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<td>0.08</td>
<td>0.04</td>
<td>0.186</td>
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<tr>
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<td>0.66</td>
<td>0.46</td>
<td>0.946</td>
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<tr>
<td>1 to 5 yr postgraft</td>
<td>1.18</td>
<td>0.78</td>
<td>1.791</td>
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<tr>
<td>5 to 10 yr postgraft</td>
<td>1.56</td>
<td>0.98</td>
<td>2.501</td>
</tr>
<tr>
<td>&gt;10 yr postgraft</td>
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<td>1.99</td>
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</tr>
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<td>0.18</td>
<td>0.12</td>
<td>0.257</td>
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<td>peak anti-T ≥10%</td>
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<td>0.06</td>
<td>0.172</td>
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<td>0.09</td>
<td>0.188</td>
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<td>-2.46</td>
<td>4.135</td>
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<td>2.84</td>
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<td>7.33</td>
<td>22.112</td>
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<td>0.08</td>
<td>0.239</td>
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<tr>
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<td>0.08</td>
<td>0.03</td>
<td>0.223</td>
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<td>6.617</td>
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<td>0.07</td>
<td>0.192</td>
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<td>0.02</td>
<td>0.118</td>
</tr>
<tr>
<td>peak anti-B ≥10%</td>
<td>0.06</td>
<td>0.03</td>
<td>0.103</td>
</tr>
<tr>
<td>peak anti-B ≥80%</td>
<td>0.04</td>
<td>0.02</td>
<td>0.102</td>
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</table>

Table 5. Parameters associated with the development of DS and NDS Ab

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<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>$\chi^2$</th>
<th>P</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
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<td></td>
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<td>Lower Limit</td>
<td>Upper Limit</td>
<td></td>
<td></td>
</tr>
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<td>DS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DR mismatch &gt;0</td>
<td>2.27891</td>
<td>0.60681</td>
<td>14.1042</td>
<td>0.0002</td>
<td>9.766</td>
<td>2.973</td>
</tr>
<tr>
<td>azathioprine at Ab detection</td>
<td>0.24923</td>
<td>0.33453</td>
<td>0.5550</td>
<td>0.4563</td>
<td>1.283</td>
<td>0.666</td>
</tr>
<tr>
<td>MMF at Ab detection</td>
<td>1.09775</td>
<td>0.37878</td>
<td>8.3991</td>
<td>0.0038</td>
<td>2.997</td>
<td>1.427</td>
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<tr>
<td>peak anti-T ≥10%</td>
<td>-0.00277</td>
<td>0.34510</td>
<td>0.0001</td>
<td>0.9936</td>
<td>0.997</td>
<td>0.507</td>
</tr>
<tr>
<td>peak anti-T ≥80%</td>
<td>0.61998</td>
<td>0.47794</td>
<td>1.6827</td>
<td>0.1946</td>
<td>1.859</td>
<td>0.729</td>
</tr>
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<td>peak anti-B ≥10%</td>
<td>0.67755</td>
<td>0.42073</td>
<td>2.5935</td>
<td>0.1073</td>
<td>1.969</td>
<td>0.863</td>
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<tr>
<td>peak anti-B ≥80%</td>
<td>1.26900</td>
<td>0.59798</td>
<td>4.5036</td>
<td>0.0338</td>
<td>3.557</td>
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<tr>
<td>NDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-0.57007</td>
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<td>0.0018</td>
<td>0.565</td>
<td>0.396</td>
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<td>azathioprine at Ab detection</td>
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<td>1.5030</td>
<td>0.2202</td>
<td>0.729</td>
<td>0.440</td>
</tr>
<tr>
<td>MMF at Ab detection</td>
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<td>0.24137</td>
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<td>3.540</td>
<td>2.206</td>
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<tr>
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<td>0.43150</td>
<td>0.21219</td>
<td>4.1353</td>
<td>0.0420</td>
<td>1.540</td>
<td>1.016</td>
</tr>
<tr>
<td>peak anti-T ≥80%</td>
<td>0.45449</td>
<td>0.24946</td>
<td>3.3191</td>
<td>0.0685</td>
<td>1.575</td>
<td>0.966</td>
</tr>
<tr>
<td>peak anti-B ≥10%</td>
<td>1.49879</td>
<td>0.21635</td>
<td>47.9933</td>
<td>&lt;0.0001</td>
<td>4.476</td>
<td>2.929</td>
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<tr>
<td>peak anti-B ≥80%</td>
<td>0.54513</td>
<td>0.27218</td>
<td>4.0113</td>
<td>0.0452</td>
<td>1.725</td>
<td>1.012</td>
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</table>

*Cox model estimation.
bodies tended to be more immunized before transplantation and/or to receive retransplantation than the others. Because of their immunologic status, they received a well-matched kidney. The analysis of their individual chart revealed that the HLA specificities of the antibodies present after transplantation had always been identified at least once before transplantation. The resurgence of these pretransplantation antibodies generally occurred in the first posttransplantation years and could have been elicited by acute rejection episodes that were significantly more frequent than in patients without HLA antibodies. Patients with DS antibodies after transplantation were significantly younger than the others, a fact that has already been reported (3), and intermediate between patients with NDS antibodies and those without HLA antibodies, regarding immunization and percentage of retransplantations. Despite this immunologic risk factor, they were less matched with their donor than the patients with NDS antibodies and even patients without HLA antibodies. The difference with patients who had NDS antibodies was particularly striking for HLA-DR matching as 6% of them were 2 HLA-DR matched with their donor versus 56% of the patients with NDS antibodies. This could explain why they presented more acute rejection episodes. These antibodies appeared later, generally between 5 and 10 yr posttransplantation. Even when the analysis was restricted to the patients with a complete annual screening, who also had received the most recent transplant, this parameter of timing of antibody detection was found significant.

As DS antibodies were more frequently observed in patients between 5 and 10 yr posttransplantation, we hypothesized that it could be the result of the decrease of immunosuppression over time or the administration of less powerful immunosuppressive molecules. Indeed, an international cooperative study has already shown that patients who received CsA-MMF had significantly fewer HLA antibodies than patients who received CsA-azathioprine (11). Our results seemed to disagree with this conclusion, as MMF was positively associated with the development of DS and NDS antibodies in our series. However, we believe that we could explain this finding by the fact that we used to switch patients from azathioprine to MMF whenever they presented transplant dysfunction or onset of proteinuria, and patients with DS or NDS antibodies were more often in this category than patients without HLA antibodies posttransplantation.

The presence of DS antibodies was associated with lower graft survival, lower transplant function, and the presence of proteinuria. In most of the patients biopsied, transplant dysfunction was associated with transplant glomerulopathy and the presence of C4d. These histologic features are in agreement with the study of Regele et al. (9). That patients with NDS antibodies also had a significantly higher risk for transplant

<table>
<thead>
<tr>
<th>Table 6. Pairwise comparison of patients with DS versus NDS Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Estimate</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total population</td>
</tr>
<tr>
<td>recipient age (yr)</td>
</tr>
<tr>
<td>date of Ab detection (mo)</td>
</tr>
<tr>
<td>1 rejection</td>
</tr>
<tr>
<td>2 rejections</td>
</tr>
<tr>
<td>3 rejections</td>
</tr>
<tr>
<td>Ab detection</td>
</tr>
<tr>
<td>1 to 5 yr postgraft</td>
</tr>
<tr>
<td>5 to 10 yr postgraft</td>
</tr>
<tr>
<td>&gt;10 yr postgraft</td>
</tr>
<tr>
<td>DR mismatches</td>
</tr>
<tr>
<td>peak anti-T ≥10%</td>
</tr>
<tr>
<td>peak anti-T ≥80%</td>
</tr>
<tr>
<td>peak anti-B ≥10%</td>
</tr>
<tr>
<td>Subpopulation with a complete annual screening</td>
</tr>
<tr>
<td>recipient age (yr)</td>
</tr>
<tr>
<td>date of Ab detection (mo)</td>
</tr>
<tr>
<td>1 rejection</td>
</tr>
<tr>
<td>2 rejections</td>
</tr>
<tr>
<td>3 rejections</td>
</tr>
<tr>
<td>DR mismatches</td>
</tr>
<tr>
<td>peak anti-T ≥10%</td>
</tr>
<tr>
<td>peak anti-T ≥80%</td>
</tr>
<tr>
<td>peak anti-B ≥10%</td>
</tr>
<tr>
<td>peak anti-B ≥80%</td>
</tr>
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</table>
failure and decreased transplant function than patients without HLA antibodies was more striking. This suggested that although these patients received a transplant with good conditions of HLA matching, they remained high immunologic responders. Alternatively, as hypothesized by Terasaki, they might have developed low titers of DS antibodies that are trapped in the transplant and are not detectable.

These data suggest that the presence of HLA antibodies, DS but also NDS, is associated with transplant failure and that their screening is an important tool for the treatment of patients who receive a kidney transplant, as they could help in discriminating between chronic rejection and nonimmune allograft dysfunction. This discrimination could have therapeutic implications and could lead to specific treatment aimed at either inhibiting their production, such as MMF (16) or the CD20 mAb (17), or neutralizing their action, with plasma exchange and/or intravenous Ig (17–20), that are used with success in acute vascular rejection.

Acknowledgments
This study was presented at the American Transplant Congress, April 30 to May 4, 2003, in Washington, DC.

We are indebted to the staff of the Histocompatibility Laboratory for excellent technical work.

References
1. Soulillou JP, Peyrat MA, Guenel J: Association between treatment-resistant kidney allograft rejection and post-

Table 7. Correlations between presence of DS and NDS Ab and serum creatinine, CrCl, and proteinuria in patients with no HLA antibodies, with DS HLA Ab, and with NDS HLA Ab at entry and at end of study.

<table>
<thead>
<tr>
<th></th>
<th>Ab Negative</th>
<th>DS Ab</th>
<th>NDS Ab</th>
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<tr>
<td>Total population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>creatinine (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>entry</td>
<td>139 (66)</td>
<td>180 (108)</td>
<td>179 (163)</td>
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</tr>
<tr>
<td>end</td>
<td>149 (93)</td>
<td>197 (125)</td>
<td>195 (172)</td>
<td>&lt;0.001</td>
</tr>
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<td>CrCl (ml/min)</td>
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<td></td>
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<tr>
<td>entry</td>
<td>58 (27)</td>
<td>50 (26)</td>
<td>57 (27)</td>
<td>0.007</td>
</tr>
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<td>57 (27)</td>
<td>48 (27)</td>
<td>51 (28)</td>
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<td>proteinuria (g/d)</td>
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</tr>
<tr>
<td>entry</td>
<td>0.4 (0.9)</td>
<td>0.9 (2.6)</td>
<td>0.9 (2.3)</td>
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<td>1.0 (2.6)</td>
<td>1.0 (2.3)</td>
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<tr>
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<td>139 (66)</td>
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<tr>
<td>entry</td>
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<td>52 (24)</td>
<td>61 (26)</td>
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<tr>
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<td>1.5 (3.9)</td>
<td>0.5 (1.1)</td>
<td>&lt;0.001</td>
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</table>

aData are mean (SD).


Access to UpToDate on-line is available for additional clinical information at http://www.jasn.org/