Antibody Mediated Rejection: Update 2006

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It now is recognized that both acute and chronic allograft rejection may be mediated by alloantibodies. This constitutes a major shift in our thinking over the past 40 yr. We previously reviewed the articles that are relevant to the humoral theory: McKenna et al. (1), Terasaki (2), Cai and Terasaki (3), and Terasaki and Cai (4). Here we review articles that have appeared since our last review.

According to the humoral theory, antibody engagement with antigen is the original trigger; ultimate destruction of an organ requires a cascade of many events. Despite numerous publications, it still is not possible to convince everyone that antibodies are the cause of rejection, but the accumulated evidence, particularly in the past 5 yr, suggests that the theory is sufficiently valid to be useful in patient treatment.

The central diagnostic criteria today of antibody-mediated rejection is the demonstration of C4d in peritubular capillaries, inflammation, and/or tissue injury as reviewed by Rotman et al. (5). Antibody-mediated injury of peritubular capillaries results in endothelial injury, with loss of capillary patency, ischemia, and proliferation of myofibroblasts leading to progressive interstitial fibrosis (6). Presumably, all of these reactions are the result of the initial loss of blood supply. The importance of impairment of the microvascular endothelium in progressive renal disease was noted by Kang et al. (7). Similar loss of capillaries causes irreversible injury, which becomes apparent at serum creatinine values >2 mg/dl (8). Peritubular capillaries were shown to be lost progressively, with an attendant increase in serum creatinine.

HLA antibodies were shown to activate endothelial cells and stimulate proliferation of cells (9). Low doses of HLA activate the phosphoinositol 3-kinase/Akt pathway and promote expression of cell survival proteins, possibly accounting for accommodation. HLA antibodies can be eluted from needle core biopsies and detected with flow cytometry at a higher sensitivity than by ELISA (10).

Preexisting and Anamnestic Antibodies

Zachary et al. (11) studied removal and persistence of preformed antibody by plasmapheresis and cytomegalovirus intravenous Ig (IVIg). Factors that favored elimination of the antibodies were antibodies that were found by flow cytometry cross-match (weak), and cross-reacting group antibodies. Factors that favored persistence were positive complement-dependent toxicity (CDC) cross-match (strong), private donor-specific antibody (DSA) specificity, multiple transplants, and unrelated donor.

Antibodies that are present before transplantation are extremely important to characterize thoroughly, because they determine which donors can be used safely. If transplants are performed across a positive cross-match that is detected only by more sensitive methods, then acute humoral rejection is a likely consequence. For example, a 45-yr-old woman who had 0% panel-reactive antibodies (PRA) by CDC test and no history of transfusion received a CDC-negative cross-match cadaveric transplant (12). The graft was removed on day 7 after surgery with a thrombosed vein. Subsequent test of the serum with luminex technology found anti-A2 and B7 in the preoperative serum, which had not been detected by ELISA and flow PRA. The woman was found to have received a transplant from an A2 donor, whereas her husband and two sons were A2, B7 types. This indicated that 17 yr after the last immunization, low titers of the pregnancy antibodies had persisted, and a strong anamnestic antibody response had occurred within 1 wk after transplantation.

In another study, 28 patients who tested negative by flow cytometry cross-match were found to be positive by flow PRA, and 16 (57%) of 28 patients showed evidence of humoral rejection within 2 wk (13). Ishida et al. (13) suggested that these patients were “high responders,” as shown by the PRA test. Another interpretation is that because these patients increased their PRA within 1 wk, these antibodies had been restimulated and had cross-reacted with the donor antigens to produce acute rejection.

Liver transplants generally have been regarded as not susceptible to antibody-mediated rejections. However, Muro et al. (14) showed that among 14 patients with a CDC cross-match, the 1-yr graft survival rate was a very low 29%, compared with 72% for 254 negative patients. The CDC-positive cross-match was found in only 5% of the transplants, reflecting the general finding that liver transplant patients are not sensitized to as high a degree as kidney and heart transplant patients (15). C4d deposition was found in the hepatic sinusoids as well as the
portal veins and hepatic arteries in the biopsies of 20 patients with living-donor liver transplants (16).

Preformed antibodies were found in 27% of pediatric heart transplant recipients and in 50% of rejectors (patients with recurrent, refractory, or severe acute rejection in the first year after transplantation) but in only one (4%) of 23 nonrejectors. Nine cases retained and 10 developed HLA antibodies after transplantation: 63% in rejectors and 22% in nonrejectors. Preformed persistent de novo antibodies correlated with the first-year acute rejection profile (17). A review of antibody before and after transplantation in heart and lung transplants was published (18).

**De Novo Antibodies**

In contrast to the anamnestic antibodies, which tend to be of high titer, antibodies that are newly formed against a graft and found in the chronic phase seem to act differently, because their effect is not often seen as rejection of an organ until years after the appearance of the antibodies. The damage that is initiated by antibodies requires many gradual injury and repair steps, which eventually result in organ failure.

Antibodies to DP class II antibodies were found in 5.1% of 138 patients with functioning grafts and in 19.5% of 185 patients with rejected grafts ($P < 0.001$) (19). Among patients who did not have class I and DR/DQ antibodies, 13% of those who rejected a graft had DP antibodies, compared with 3.5% of patients with functioning grafts ($P < 0.05$).

In an extensive study of 39 patients who rejected a kidney graft and 26 with functional grafts, 679 postoperative serial serum samples were tested (20). IgG and IgM HLA antibodies were found in 72% of patients who rejected grafts, compared with 46% with functioning transplants ($P < 0.05$). When MICA antibodies were added to IgG HLA, 77% of failed transplants had antibodies, compared with 42% of those with functioning transplants ($P < 0.01$). When patients with IgM anti-HLA antibodies were included, 95% of patients with failed grafts had antibodies, compared with 58% of those with functioning grafts ($P < 0.01$). It was concluded that although the antibodies were not necessarily donor specific, their presence was associated with failure of the graft.

**DSA**

It often is asked whether the antibodies that appear after transplantation are donor specific. We believe that this question cannot be answered well without the use of single-antigen beads (21), in which the antisera are tested against beads that had purified specificities of a single HLA type. In an extensive study of 58 sera that were analyzed by 63 laboratories in the UCLA serum exchange, it was shown that many specificities often are missed by standard testing methods (22). We believe that the true incidence of DSA must await the use of single-antigen beads to address this problem properly.

In one study of 251 kidney transplant patients who had received a transplant >6 mo previously, 11% of the patients had HLA antibodies, but only 4.4% had DSA. Among nine patients with antibodies with biopsies, six had C4d staining, whereas in 11 patients without antibodies, none had C4d staining (23). In 1229 patients who were investigated 1 to 5 yr after transplantation, 5.5% had DSA, 11.3% had non-DSA, and 83% had no HLA antibodies (24). The presence of either DSA or non-DSA correlated with lower graft survival, poor transplantation function, and proteinuria.

Antibodies can be eluted from >70% of rejected kidneys, whereas the sera of patients before rejection may be positive in only approximately 45% of patients (25). This suggests that the antibodies could go undetected in the serum, probably because the kidney is absorbing the antibodies (26). After the graft removal, 73% of the sera contained DSA. In a study of sera of 27 patients who had rejected a kidney transplant, circulating DSA were found by cytotoxicity tests in three (11%) cases before and in 26 (97%) cases after allograft nephrectomy (27).

A patient who developed DSA between the 22nd and 30th years after transplantation is described by Weinstein et al. (28). Her serum creatinine rose from 0.7 to 1.9 mg/dl, at which time C4d was noted in the peritubular capillaries. After replacement of azathioprine with mycophenolate mofetil (MMF) and six apheresis and two infusions of IVlg, renal function stabilized at 1.9 mg/dl 33 yr after transplantation. The antibody found was against A11 and DQA1-0501, found in the donor.

It has been reported that among second graft patients, 83 patients who had nephrectomy and 157 patients without nephrectomy had similar graft survivals (29). To explain this result, after graft removal, if DSA are apparent, then a second graft that would react with these specificities probably was avoided. Conversely, if a graft is left in place after it is rejected, then DSA may become apparent once the graft becomes necrotic and is no longer able to absorb antibodies.

**Predictive Value of De Novo Antibodies**

In a prospective study of 2231 patients who were tested for HLA antibodies, 2 yr after the test, among 478 patients with antibodies, 15.1% failed, compared with a 6.8% failure in 1753 patients without antibodies ($P = 0.000000002$) (30). Among patients who had serum creatinine values of 0.5 to 1.9 mg/dl, there was only a 0.1% difference in 2-yr graft survival between those with and without antibodies. We interpret this finding to indicate that antibodies do not cause a graft failure within 2 yr in patients who have normal kidneys. However, in patients with serum creatinine values of 2 to 2.9 mg/dl at the time of testing, patients with antibodies had a 17.9 percentage points lower graft survival rate than those without antibodies. Therefore, if the patients already had some damage at the time of testing, then within 2 yr, graft survival is lower among those with antibodies. PRA testing by cytotoxicity had the greatest predictive value, possibly because this test detects antibodies that are directed to both HLA and non-HLA antigens.

Twenty-five of 71 heart transplant recipients developed de novo HLA antibodies in the first year (31). HLA antibodies were associated with cellular rejection ($P = 0.0002$) and vasculopathy ($P < 0.002$), and, most important, class II antibodies were associated with 5-yr survival ($P = 0.008$). Among 267 heart transplant patients, 20% produced IgM antibody, 54% produced IgG antibodies, and 26% did not produce HLA antibodies (32). Among those who switched isotype, 20% developed...
IgG anti-HLA class I antibodies, 40% produced IgG anti-HLA class II antibodies, and 40% made antibodies to both classes of HLA molecules. Isotype switching to class II antibodies was correlated with acute cellular rejection, transplant-related coronary artery disease, and poor long-term survival. Among 96 pediatric heart transplant patients tested, 20% had class I and 39% had class II antibodies after transplantation (33).

Antgens Other Than HLA
As mentioned earlier (20), MICA antibodies were associated with kidney transplant failures in addition to HLA antibodies. Evidence that other antigens, which unlike MICA are not linked to HLA, might be responsible for rejection was described recently by Opelz (34). Transplants into HLA-identical siblings who have a high PRA >50% had a 55% 10-yr graft survival rate, compared with 63% survival in 1 to 50% PRA patients, and a 72% survival rate in nonsensitized HLA-identical siblings. He suggested that the PRA is serving either as an indicator of higher responsiveness or as an indicator of reactions against a non-HLA antigen detected by PRA against lymphoid cells. Similar evidence that PRA that are determined by tests against non-HLA antigens was noted earlier, when the CDC assay had a higher association with acute cell rejection than PRA that were determined by ELISA or flow cytometry using purified HLA antigens on solid surfaces (30).

Treatment of Antibody-Mediated Rejection
Snanoudj et al. (35) provided a table of references on the treatment of humoral response. They noted that removal or neutralization of antibodies can be done by plasmapheresis, immunoadsorption, and IVIg, whereas a blockage of B cell proliferation can be accomplished by splenectomy, cyclophosphamide, FK506/MMF, and anti-CD20. Jordan et al. (36) provided a review of the three main avenues of treatment: (1) High-dose IVIg; (2) use of Rituximab, anti-CD20 mAb to deplete B cells; and (3) plasmapheresis with anti-cytomegalovirus IgG.

Preformed antibodies to erythrocyte anti-A and -B antigens that were expressed on endothelium were reduced by plasmapheresis and incompatible kidneys that were transplanted into 89 patients, then treated with tacrolimus and MMF, and shown to have 77% 5-yr graft survival and 56% 10-yr graft survival, regardless of antibody titer before transplantation. Although this survival was not higher overall than with a group of 78 patients who were treated with cyclosporine and azathioprine, patients with high titers survived at high rates with MMF. It was suggested that MMF prevented formation of antibodies (37).

Strategies for minimizing immunosuppression in kidney transplantation are reviewed by Kirk et al. (38). A table of adverse effects of common drugs is given. It was noted that there are no tests that detect over- or underimmunosuppression. It is our hope that detection of antibodies will be one useful test, because if graft rejection is triggered by antibodies, then their presence should indicate an increase or change in immunosuppression, and, conversely, their absence may suggest that drugs can be reduced. Of course to detect other possible causes of rejection, other tests will be required. The most important consequence of the humoral theory will be in monitoring of immunosuppression.

References