Multi-Array Antibody Screening in Detecting Antibodies to Mismatched HLA in Patients Awaiting a Second Transplant

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ABSTRACT

Effective identification of HLA specificities to which a prospective transplant recipient has antibodies depends on how effective the most sensitive assay is in detecting these antibodies. To ascertain the assay's efficacy, the results of antibody screening of patients on the waiting list for a second transplant were studied. A commercially available panel of fluoro-coded microbeads coated with multiple and single purified class I or II HLA antigens was used with flow cytometry to detect antibodies in human serum (LABScreen, One Lambda, Canoga Park, Calif, USA). A total of 112 HLA-A, B, and DR mismatches between donors and recipients were present among 34 patients. Antibodies to 56% of the mismatches were detected with 67% of the HLA-A, 38% of the HLA-B, and 63% of the HLA-DR mismatches detected, respectively. Thirty percent of the patients had antibodies to all of the mismatched HLA, 43% had antibodies to some, and 27% did not develop antibodies to any of the mismatched antigens. Among patients who developed antibodies to all of the mismatched HLA, 60% had had a transplant nephrectomy. Only 11% of patients who had no antibodies detected to mismatched HLA had had a transplant nephrectomy and 44% of them were still on immunosuppression. Using the Matchmaker program developed by Duquesnoy, the latter group of patients had a sufficient number of triplet mismatches that could have resulted in an antibody response. All of the undetected antibodies had been identified in other patients in this group. The assay used in this study to detect antibodies is considered the most sensitive one available. Nonetheless, antibodies to slightly less than half of the mismatched HLA antigens were not detected. It appears that the assay system is capable of detecting the antibodies, since in other patients with the same mismatched HLA, antibodies were detected. It is likely that the recipients could develop antibodies since there was a sufficient degree of disparity in the HLA of donors and recipients. Antibodies were more likely to be detected when there had been a transplant nephrectomy and the absence of immunosuppression. There was no way of knowing whether we were missing detecting antibodies or if they were not present. The results of this study have important implications with respect to utilizing "unacceptable antigens" in an allocation system for patients awaiting a second transplant.

RECENT EMPHASIS on the importance of identifying "unacceptable antigens," ie, HLA specificities to which a prospective transplant recipient has antibodies, raises the question as to how effective the assays are in identifying antibodies to HLA. In an effort to gain insight into the matter, we reviewed the results of antibody screening of patients on the waiting list for a second transplant, using a sensitive solid-phase assay system with single antigen microspheres.[1–3]

MATERIALS AND METHODS

A commercially available panel of color-coded microspheres coated with purified class I or II HLA antigens, and pre-optimized reagents for detection of class I and class II antibodies in human serum, was used (LABScreen, One Lambda, Canoga Park, Calif, USA). Flow cytometric technology (Luminex Co, Austin, Tex, USA) was used to detect antibodies using R-phycoerythrin-conjugated goat anti-human IgG. The results of antibody screening of 34 patients who were on the waiting list at the local organ procure-

ment organization for a second renal transplant were correlated to their clinical status in order to determine the efficacy of this assay system in detecting antibodies to mismatched HLA following rejection of the primary renal transplant.

RESULTS

A total of 112 HLA-A, B, and DR mismatches between donors and recipients were present among the 34 patients. Antibodies to 56% of the mismatches were detected with 67% of the HLA-A, 38% of the HLA-B, and 63% of the HLA-DR mismatches detected, respectively. Thirty percent of the patients had antibodies to all of the mismatched HLA, 43% had antibodies to some of the mismatched HLA, and 27% did not develop antibodies to any the mismatched antigens. Among patients who developed antibodies to all of the mismatched HLA (mean of 2.9 mismatches), 60% had had a transplant nephrectomy and had been screened for 31 months. In contrast, of patients who had no antibodies detected to mismatched HLA and had been screened for 16 months, only 11% had had a transplant nephrectomy. In addition, 44% of the patients on the waiting list with no antibodies to their mismatched HLA were still on immunosuppression with functioning (albeit poorly) grafts. Using the Matchmaker program developed by Duquesnoy,[4,5] the latter group of patients had a sufficient number of triplet mismatches that should have resulted in an antibody response. Since all of the undetected antibodies had been identified in other patients in this group, it appears that the assay system is robust enough to detect the antibodies had they been present in sufficient concentration.

DISCUSSION

The solid-phase flow cytometry-based system used in this study to detect antibodies in transplant recipients is generally considered the most sensitive one currently available.[6] Nonetheless, donor-specific antibodies to slightly less than half of the mismatched HLA antigens were not found. Antibodies to HLA-B were not detected in close to two-thirds of the patients on the waiting list for a second transplant, suggesting that HLA-B loci antigens are less immunogenic than the other loci. It does not appear that the assay system is incapable of detecting antibodies, since all of the antibodies to the HLA specificities not found were detected in other patients with the same mismatched HLA antigens. As we have observed (data not shown), and others have reported, antibodies can be found in the circulation and in kidney biopsies in 95% of patients who have rejected a renal transplant, but donor-specific antibodies are often not present among the HLA antibodies found.[7–9] It is possible that donor-specific antibodies were formed but that the assay did not detect them, for reasons that one can only speculate upon. It is possible that antibodies were present in concentrations that were below the level of assay sensitivity, especially if the rejecting transplant was still in place and absorbing the antibodies from the circulation or

the patient was still receiving immunosuppression. It is significant that among patients who had no antibodies present, only 11% had a transplant nephrectomy, whereas 60% had a transplant nephrectomy when antibodies to all of the mismatched HLA were detected. One must also consider the possibility that mismatched HLA do not necessarily result in antibody production despite the sufficient degree of disparity in the HLA of donors and recipients, as confirmed by Duquesnoy's Matchmaker system. Antigen recognition is a complicated process and there may be reactive T-cell clones that are suppressed or that were not activated due to the presence of regulatory cells. Similarly, not all transplant failures are caused by antibody-mediated rejection. Graft failure may be due to nonimmunologic processes such as drug nephrotoxicity, surgical complications, infection, or cellular mechanisms. The implications of this study with respect to using unacceptable antigens in a renal allocation system are clear. Because we cannot be sure we are detecting all the antibodies present in a patient awaiting a second transplant, they will always require a crossmatch. Most studies indicate that a flow crossmatch will predict the best outcome.[6] Unnecessary crossmatches can be avoided by using the concept of unacceptable antigens as the basis of a "virtual positive crossmatch." The detection of an antibody in a serum sample with a microsphere coated with HLA may not correspond to what takes place when the donor's lymphocytes are used as the target and may not occur in vivo and correlate with a clinical outcome. In this era of potent immunosuppressive drugs and "desensitizing" patients with positive crossmatches, it would be reassuring if we had more evidence that our in vitro assays accurately predict what will occur clinically. However, the pressure upon the transplant community to maximize the benefit of the very limited resource of deceased donor organs, in the face of a great demand, is justification for making the assumption that our assays reflect clinical outcomes with sensitivity and specificity. Establishing a renal allocation system that will result in a long-term benefit to recipients places an obligation on histocompatibility laboratories to expeditiously identify those patients who have the best chance of having a good outcome and those whose prospects of a successful transplant are less likely.

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