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see commentary on page 461

Increased intragraft rejection–associated gene transcripts in patients with donor-specific antibodies and normal biopsies

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We investigated why some donor-specific antibody–positive patients do not develop antibody-mediated rejection. Of 71 donor-specific antibody–positive patients, 46 had diagnosis of antibody-mediated rejection and 25 had normal biopsies. Fifty donor-specific antibody–negative patients with normal biopsies were used as a control group. A subgroup of 61 patients with available biopsy and 64 with blood samples were analyzed by microarrays. Both donor-specific antibody–positive/antibody-mediated rejection–positive and negative biopsies showed increased expression of gene transcripts associated with cytotoxic T cells, natural killer cells, macrophages, interferon-gamma, and rejection compared to donor-specific antibody–negative biopsies. Regulatory T-cell transcripts were upregulated in donor-specific antibody–positive/antibody-mediated rejection–positive and B-cell transcripts in donor-specific antibody–positive/antibody-mediated rejection–negative biopsies. Whole-blood gene expression analysis showed increased immune activity in only donor-specific antibody–positive/antibody-mediated rejection–positive but not negative patients. During a median follow-up of 36 months, 4 donor-specific antibody–positive/antibody-mediated rejection–negative patients developed antibody-mediated rejection, 12 continued to have donor-specific antibody, but 9 lost their donor-specific antibody. Gene expression profiles did not predict the development of antibody-mediated rejection or the persistence of donor-specific antibody. Thus, donor-specific antibody–positive/antibody-mediated rejection–negative patients had increased rejection-associated gene transcripts in their allografts despite no histologic findings of rejection but not in their blood. This was found in both biopsy and blood samples of donor-specific antibody–positive/antibody-mediated rejection–positive patients.

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The presence of donor-specific anti-human leukocyte antigen (HLA) antibodies (DSAs) is associated with an increased risk of both acute and chronic antibody-mediated rejection (AMR) in kidney allografts.[1,2] AMR has remained challenging in kidney transplantation and is the major cause of late allograft loss.[3,4] However, although some patients with DSA develop acute or chronic AMR, some do not develop AMR and demonstrate normal histopathology in their biopsies. This leads to the question of whether this represents accommodation, whether other protective mechanisms exist, or whether this is actually a state of prerejection. The term 'accommodation' is mainly used for ABO-incompatible kidney transplants where the recipients continue to have stable kidney function without allograft injury despite circulating antibodies to ABO antigens after receiving desensitization treatment.[5] Clinical evidence has not documented accommodation in HLA-incompatible transplantation, and it is suggested that the term 'protected' should be used instead of 'accommodated' if there is no allograft injury despite circulating DSA. The graft resistance to antibody-mediated damage may be due to upregulation of cytoprotective genes or complement regulatory proteins.[6] Initial studies documented upregulated expression of protective genes such as A20, Bcl-2, Bcl-xL, and heme oxygenase-1.[7]

The Edmonton group investigated the gene expression profiles of the biopsies with acute and chronic AMR by microarrays and reported that these biopsies displayed three molecular phenotypes: upregulation of endothelial-associated transcripts,[8] natural killer cell–associated transcripts,[9]

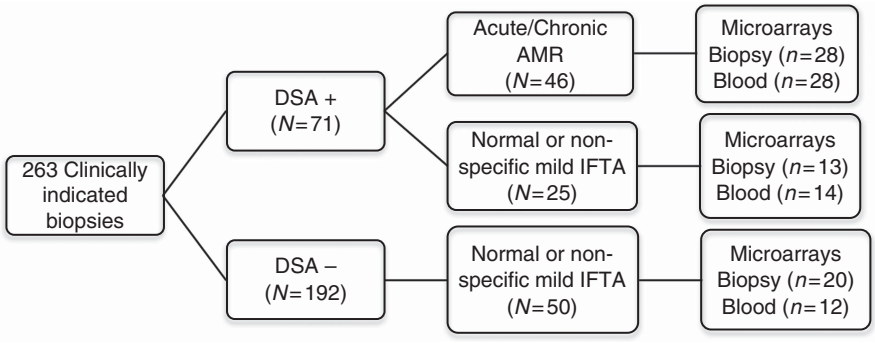

Figure 1 | Study population. AMR, antibody-mediated rejection; DSA, donor-specific antibody; IFTA, interstitial fibrosis/tubular atrophy.

and the effects of interferon-gamma.[10] They also identified 23 selective gene transcripts seen in AMR but not in cellular rejection and named these genes the DSA-specific transcripts[9] and later created AMR scores by microarrays.[11] The Mayo Clinic group showed increased intragraft gene expression associated with adaptive and innate immunity in cross-match-positive kidney transplant recipients.[12] Whole-blood gene expression profiles of DSA+ patients with AMR and also the gene expression profiles of whole-blood and kidney biopsy samples of DSA+ patients without rejection has not been investigated by microarrays.

The goal of our study was to investigate the gene expression profiles of transplant kidney biopsy and whole-blood samples of DSA+ patients without rejection compared with AMR patients by microarrays in order to elucidate the mechanisms involved in the prevention of AMR, particularly by analyzing gene transcripts associated with cytotoxic T cells, regulatory T cells, B cells, natural killer cells, and macrophages.

RESULTS

Demographic and clinical characteristics

There were 71 DSA+ and 192 DSA− patients (Figure 1). Of the 71 DSA+ patients, 46 had biopsy diagnosis of acute AMR ($n=9$) or chronic AMR ($n=37$), and 25 had normal histopathology or minimal nonspecific interstitial fibrosis/tubular atrophy. Of the 192 DSA− patients, 50 patients with normal histology and/or mild nonspecific interstitial fibrosis/tubular atrophy were used as a control group. There were no significant differences in age, sex, race, type of transplantation, immunosuppressive treatment, and history of previous transplantation between the three study groups (Table 1). A previous history of acute rejection was higher in DSA+/AMR+ (24%) and DSA+/AMR− (16%) groups compared with the DSA− group (6%; $P=0.047$). A previous history of acute AMR was 13%, 4%, and 0% in DSA+/AMR+, DSA+/AMR−, and DSA− groups, respectively. Previous biopsies were performed at a median of 24 (range18–50), 27 (range 8–72), and 48 (range 24–60) months before the current biopsies in DSA+/AMR−, DSA+/AMR+, and DSA−/AMR− groups, respectively. The time to biopsy after transplantation was earlier in DSA+/AMR− (median 0.3 years (range 0.1–1.5)) and DSA− (median 0.5 years (0.2–1.8)) groups when compared with the DSA+/AMR+ patients who underwent kidney biopsies at a median of 4.1 (1.8–8.4) years after transplantation ($P<0.001$).

Sensitization

Patients with DSA+/AMR+ were significantly more likely to have higher median class II panel reactive antibody (63% vs. 9%, $P=0.004$), class II DSA frequency (70% vs. 44%, $P=0.04$), and median class II DSA mean fluorescence intensity (MFI) values (4958 vs. 0, $P=0.04$) when compared with DSA+/AMR− patients (Table 1). There were no significant differences in class I panel reactive antibody and/or DSA frequency between the two groups. Seventeen DSA+/AMR− patients had available pretransplant Luminex results. Of these patients, 11 had pretransplant DSA and received a transplant with antithymocyte globulin and intravenous immunoglobulin induction. The remaining six patients developed *de novo* DSA. In the DSA+/AMR+ group, most transplants were performed before the introduction of Luminex technology; only six had a pretransplant Luminex test performed and four showed DSA.

Allograft injury by Banff scores

As expected owing to a histopathologic diagnosis of acute or chronic AMR, DSA+/AMR+ biopsies had higher mean glomerulitis (0.72 ± 0.75), peritubular capillaritis (1.28 ± 1.1), interstitial inflammation (1.3 ± 0.92), and chronic glomerulopathy (0.89 ± 1.04) scores when compared with DSA+/AMR− and DSA− biopsies ($P<0.001$; Table 2). Although the DSA+/AMR− group had slightly higher mean glomerulitis (0.24 ± 0.6 vs. 0.08 ± 0.27), peritubular capillaritis (0.42 ± 0.77 vs. 0.22 ± 0.62), and interstitial inflammation (0.64 ± 0.81 vs. 0.38 ± 0.60) scores when compared with DSA− biopsies, respectively, the difference was not statistically significant. Microvascular inflammation scores (glomerulitis + peritubular capillaritis) >1, which could be a signature for AMR, were seen in 56% of DSA+/AMR+ patients but were observed in only three DSA+/AMR− patients (12%), similar to the DSA− group (9%). In all, 68% of DSA+/AMR− group had both glomerulitis and peritubular capillaritis score of zero. There were no statistically