Differences in pathologic features and graft outcomes in antibody-mediated rejection of renal allografts due to persistent/recurrent versus *de novo* donor-specific antibodies

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Antibody-mediated rejection (ABMR) of renal allografts occurs in two forms. Type 1 ABMR results from persistence and/or a rebound of preexisting donor-specific antibodies in sensitized patients and usually occurs early post-transplantation. Type 2 ABMR is associated with *de novo* donor-specific antibodies and usually occurs over one year post-transplantation. It is generally accepted that types 1 and 2 also differ with regard to certain pathologic features including the frequencies of C4d positivity and concurrent cell-mediated rejection. However, direct comparison of pathologic, serologic, and clinical features of types 1 and 2 ABMR is lacking. Here we compared these features in 80 cases of ABMR (37 type 1, 43 type 2) diagnosed at our center. Compared with type 1, type 2 ABMR occurred later post-transplantation, was more often associated with donor-specific antibodies against Class II HLA, and was associated with more interstitial fibrosis/tubular atrophy and more frequent cell-mediated rejection, although these did not differ with respect to C4d positivity. By univariate analysis, graft survival was lower with type 2 than type 1 ABMR with borderline significance. Still, among these 80 patients, all but one treated for ABMR following diagnosis, the only two independent predictors of graft failure were at least moderate interstitial fibrosis/tubular atrophy and failure of the donor-specific antibody relative intensity scale score, a measure of the combined strength of all donor-specific antibodies present, to decrease in response to therapy.

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Antibody-mediated rejection (ABMR) is a major cause of renal allograft failure.[1–4] Active ABMR is manifest morphologically as microvascular inflammation (MVI), primarily glomerulitis and peritubular capillaritis.[1–3,5–12] If unrecognized or not successfully treated by measures including the removal of donor-specific antibodies (DSAs), acute ABMR leads to chronic allograft damage, including transplant glomerulopathy (TG), arterial intimal fibrosis, and interstitial fibrosis/tubular atrophy (IF/TA).[11–16] TG in particular is strongly associated with increased rates of graft loss.[17–19] Historically, ABMR has been under-recognized in renal allografts for 2 reasons. First, it may be subclinical and lead to chronic damage, including TG, before a detectable rise in serum creatinine occurs.[9,12,20,21] Second, it was not until 2009 that evidence began to appear indicating that ABMR may occur in the absence of complement deposition in the microcirculation,[12,22] and prior to the most recent (2013) Banff classification for ABMR[5] complement deposition, in the form of C4d staining within peritubular capillaries (ptc), was a requirement for diagnosis of ABMR in renal allograft biopsies.[23] Furthermore, acute/active ABMR may occur at any time after transplantation, and late-onset ABMR due to *de novo* DSA is a major determinant of late renal allograft failure.[1–4]

ABMR of renal allografts occurs in the following 2 forms: type 1, resulting from persistence and/or a rebound of preexisting DSA in sensitized patients, and type 2, associated with *de novo* DSA. It is generally accepted that type 1 ABMR usually occurs early after transplantation, whereas type 2 ABMR most often occurs at least 1 year after transplantation and not infrequently much later.[24–26] While studies directly comparing these 2 forms of ABMR are lacking, there exists "conventional wisdom" regarding these forms of ABMR based in part on differences between early and late ABMR and in part on individual studies focused on ABMR in highly

sensitized patients and ABMR associated with *de novo* DSA. In a study of 234 indication biopsies from 173 non-highly sensitized patients, cases of ABMR diagnosed during the first year after transplantation were usually "pure" ABMR without concurrent cell-mediated rejection (CMR), acute/active (without TG), and associated with DSA against either HLA class I or class II. By contrast, cases diagnosed more than 12 months after transplantation were more frequently mixed ABMR/CMR and chronic, active, and mainly associated with anti-class II DSA.[2] Other studies had previously shown a strong association between anti-class II DSA and TG, and that lesions of TG evident by light microscopy are infrequently seen during the first year after transplantation.[15,17,18,27] In the DeKAF study[4] that also focused on indication biopsies of non-highly sensitized patients, only 57% of cases of late ABMR (mean ~7 years after transplantation) were C4d-positive, although C4d-positive ABMR was associated with a worse graft survival after biopsy than C4d-negative ABMR. A similar high fraction of C4d-negative cases of type 2 ABMR, and worse outcome for C4d-positive ABMR, was earlier reported by Sis *et al.*[22] using gene transcripts associated with endothelial activation to define MVI. In highly sensitized patients with ABMR, there is no apparent association with DSA against HLA class I versus class II,[28] and limited evidence suggests that most cases of ABMR in these patients, particularly in the early post-transplant period, are C4d-positive.[15,29]

Our center performs renal transplants in both highly sensitized and nonsensitized patients, giving us the opportunity to directly compare types 1 and 2 ABMR. In this study, we compare pathologic and serologic features and graft outcomes in 80 patients having an initial diagnosis of ABMR (37 type 1, 43 type 2) made during and after January, 2010, an era at our center in which biopsies showing MVI led to testing for DSA and, if positive, treatment for ABMR, whether or not C4d staining yielded positive results.

RESULTS
Morphologic and serologic features of type 1 versus type 2 ABMR

Features of types 1 and 2 ABMR are compared in Tables 1 and 2. Nearly three-fourths of cases of type 1 ABMR were "pure" ABMR, with no concurrent CMR or borderline infiltrate. By contrast, the majority of cases of type 2 ABMR showed CMR or borderline lesions (Figure 1). When we did not consider borderline infiltrates and lesions of intimal arteritis without concurrent tubulo-interstitial rejection (isolated v-lesions), the latter in some instances possibly reflecting ABMR rather than CMR,[25] only 4 of 37 (11%) type 1 biopsies showed mixed ABMR/CMR, compared with 16 of 43 (37%) type 2 biopsies (*P* = 0.009; Table 1). Patients with type 1 ABMR were more likely to have received deceased donor (as opposed to living donor) transplants compared with those having type 2 rejection. As would be expected, a greater fraction of patients with type 1 ABMR had received 1 or more previous renal transplants (Table 1).

Not surprisingly, biopsies showing an initial diagnosis of type 2 ABMR were generally done later after transplantation than those showing an initial diagnosis of type 1 ABMR, although diagnosis of the latter occurred as late as 3.5 years after transplantation, while type 2 ABMR occurred as early as 9 months after transplantation. The indication for biopsy in patients with type 1 ABMR was acute graft dysfunction in nearly 70% of cases, while in those with type 2 ABMR the most frequent indication was progressive graft dysfunction (Table 1), consistent with a more indolent onset of the latter following initial development of *de novo* DSA. Consistent with the longer post-transplant interval and the less acute onset, median scores for chronic glomerulopathy (cg), maximum number of peritubular capillary basement membrane layers, and IF/TA (evaluated as Banff ci + ct scores) were all significantly greater in biopsies of type 2 ABMR, and 62% of cases of type 2 ABMR were either chronic, active (60%), or chronic (2%), compared with only 30% of cases (all chronic, active) of type 1 ABMR (Table 1 and Figure 1). However, parameters reflecting recent interaction of antibody with the microvascular endothelium — glomerulitis, peritubular capillaritis, and ptc C4d deposition — were not different in types 1 and 2 ABMR. Indeed, similar fractions of biopsies of type 1 (70%) and type 2 (74%) ABMR showed diffuse (C4d score 3) or focal (C4d score 2) C4d staining in peritubular capillaries.

As previously noted,[15,29] type 1 ABMR is associated with DSA against either HLA class I or class II, or DSA against class I and class II. By contrast and also as previously reported,[2,24] type 2 ABMR is only infrequently associated with anti–class I DSA alone, and the majority of such cases are associated with anti–class II alone (Table 2). In our patient population, 8 of 43 patients (19%) with type 2 ABMR and no patients with type 1 ABMR had documented (in the patient chart) nonadherence with immunosuppressive medications prior to diagnosis of ABMR (Table 1), consistent with previous observations that nonadherence contributes to development of *de novo* DSA.[24]

Graft outcomes in type 1 versus type 2 ABMR

The median follow-up was 21 months (IQR: 10–30 months; total range: 1–74 months); for patients not developing graft loss, the median time from biopsy to last follow-up was 27 months (IQR: 13–36 months; total range: 4–74 months). Two patients died with functioning grafts. All but 1 of 80 patients (with chronic, inactive ABMR) were treated with high-dose i.v. Ig (IVIG), with rituximab, plasmapheresis, or both in 68 patients following diagnosis of ABMR. Among these patients, 6 of 37 (16%) with type 1 ABMR and 16 of 43 (37%) with type 2 ABMR lost their grafts. Kaplan–Meier analysis of death-censored graft survival showed a worse survival in patients with type 2 ABMR; this was borderline significant (*P* = 0.047 by log-rank [Figure 2], but *P* = 0.054 by univariate Cox analysis; Table 3). Among patients that did not develop graft loss, the median post-biopsy follow-up interval and the mean serum