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Review Article

The specificity of acute and chronic microvascular alterations in renal allografts

Filippone EJ, Farber JL. The specificity of acute and chronic microvascular alterations in renal allografts.

Abstract: The diagnosis of an antibody-mediated rejection (AMR) is made when there is evident histologic injury in the presence of detectable donor-specific alloantibodies (DSA) and diffuse peritubular capillary C4d staining (C4d-pos). In the presence of only detectable DSA or C4d-pos, the tissue injury is currently considered "presumptive" for antibody causation. In acute antibody-mediated rejection (AAMR), diagnostic morphologic features include microvascular inflammation (MVI), specifically glomerulitis and peritubular capillaritis. In the case of chronic active AMR (CAAMR), these inflammatory lesions have progressed to chronic microvascular injury, transplant glomerulopathy (TG) and peritubular capillary basement membrane multilayering (PTCBMML). Either TG or PTCBMML is sufficient morphological evidence for a diagnosis of CAAMR. Unfortunately, these lesions are not specific. MVI, TG, and PTCBMML are found in the setting of cell-mediated immunity, as well as in association with non-alloimmune mechanisms. The available treatments for AMR and CMR are different, and it is important to ascertain the dominant mechanism when approaching an individual patient. At present, no gold standard exists to establish the specific pathogenesis in the more ambiguous cases. We detail here the differential diagnosis of MVI, TG, and PTCBMML.

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In renal transplantation, donor-specific alloantibodies (DSA) cause hyperacute rejection in minutes, acute antibody-mediated rejection (AAMR) in days, or destroy grafts by chronic active antibody-mediated rejection (CAAMR) over a longer time course (1). Hyperacute rejection is largely obsolete, owing to current cross-matching and desensitization techniques. The diagnosis of AAMR or CAAMR by the Banff criteria depends on the triad of specific histologic findings, DSA, and peritubular capillary (PTC) staining for C4d (2). When either DSA or C4d is present, evident tissue injury is only "presumptive" of antibody-mediated rejection (AMR). Alternatively, DSA or C4d can occur alone or together in the absence of histologic lesions. Further complicating matters, Banff criteria for antibody-mediated tissue injury are not specific. In this review, the differential diagnosis of several of these signs of tissue injury is discussed: acute microvascular inflammation (glomerulitis and peritubular capillaritis) and its chronic sequelae, transplant glomerulopathy (TG) and PTC basement membrane multilayering (PTCBMML), Banff criteria for the diagnosis of AAMR and CAAMR, respectively.

Acute humoral rejection and microvascular inflammation (MVI)

In the early days of renal transplantation, preexisting DSA caused hyperacute rejections within minutes to hours with destruction of the graft (3). Histologic evaluation disclosed glomerulitis (g) and PTCitis (ptc) that involved predominantly polymorphonuclear leukocytes (PMNs). With the advent of the T-cell cross-match in 1969, hyperacute rejections became rare, and the emphasis became the prevention and treatment for cell-mediated rejections (CMR). In 1990, Halloran et al. (4) placed the focus back toward antibodies by describing seven patients with very early acute rejections (AR) in the presence of positive T-cell cytotoxic cross-matches. These rejections were viewed as very early AAMR and ascribed to preformed class I DSA. Histologically, all seven biopsies had both g and ptc. The main inflammatory cell appeared to be the PMN, as in hyperacute rejection (4,5).

With the discovery of PTC C4d staining as a marker of alloantibody injury and its application in acute and chronic situations, it became evident that mononuclear cells were more prominent than PMNs in biopsies diagnosed with AAMR (6). Current Banff criteria for diagnosing AAMR include DSA, C4d, and the morphological alterations summarized in Table 1 that include MVI, g and/or ptc (2). If only DSA or C4d are positive, MVI allows a diagnosis of presumptive AAMR. Hence, a biopsy with any degree of MVI (theoretically one inflamed glomerulus) with detectable DSA or C4d has presumptive AAMR. With regard to g (Table 2) (7), current Banff criteria do not detail the minimum number of cells/glomerulus required, but mononuclear cells and endothelial cell swelling are specified. Regarding ptc (Table 3) (8), ≥10% of PTCs must be inflamed with at least three to three inflammatory cells.

As noted above, g in hyperacute rejections and very early AAMR may involve PMNs. Otherwise the predominant cell type is mononuclear. Although a criterion for the diagnosis of AAMR, the specificity of g is not 100%. Sund et al. (9) studied very early (one wk) protocol biopsies. Seven patients with AR and positive PTC C4d staining (C4d-pos) had significantly higher glomerular monocytes (Mo) and PMNs, as compared to 10 with negative staining (C4d-neg) AR and 10 with no AR. Similarly, Magil and Tinckham compared 23 early biopsies (less than six months) with diffuse C4d-pos AR with acute tubular injury to 28 with C4d-neg CMR (10). PMNs were detectable in the glomeruli of 96% and 93% of both groups, as well as 100% of PTC of both groups. Distinguishing features included presence of g (57% in C4d-pos vs. 11% in C4d-neg) and the presence of glomerular Mo (96% vs. 43%). Subsequently, Magil studied 42 patients with g and AR in the first six months (11). In the 20 C4d-pos patients, Mo predominated, and in the 22 C4d-neg cases, T cells (TC) did. The mean glomerular Mo/TC ratio was >1.0 in 75% of C4d-pos vs. 14% of C4d-neg rejections ($p < 0.0001$). Studying 96 patients with AR in the first 12 months, this group again noted this correlation: 83% of biopsies with (mean) ≥1 Mo/glomerulus (deemed high) were C4d-pos vs. 11% of those with a ratio <1, and 78% vs. 18% had Banff criteria for g, respectively (12). A high Mo count was predictive of one- and three-yr eGFR by multivariable analysis, and this obtained whether C4d-pos or C4d-neg. These data indicate that using C4d as a marker of humoral activity, a strong but not absolute correlation exists between antibodies and g, especially g involving Mo in early (less than one yr) biopsies. Cell-mediated mechanisms as evidenced by CD3-positive staining, however, result in g in C4d-neg cases and may contribute in C4d-pos ones.

In a study of 240 biopsies obtained after one yr or more, Papadimitriou et al. found those in the upper quartile of maximum number of Mo in the

Table 1. Morphologic evidence of AAMR (type/grade)

I. ATN-like with minimal inflammation
II. Capillary and/or glomerular inflammation (ptc/g>0) and/or thromboses
III. Arterial inflammation (v3)

AAMR, acute antibody-mediated rejection. Adapted from (2).

Table 2. Banff quantitative criteria for glomerulitis ("g") score

g0: no glomeruli involved
g1: glomerulitis in <25% of glomeruli
g2: glomerulitis in 25–75% of glomeruli
g3: glomerulitis in >75% of glomeruli

Minimum number of cells required for consideration not specified. Adapted from (7).

Table 3. Banff quantitative criteria for peritubular capillaritis ("ptc")

ptc 0: <10% of PTCs with any inflammation
ptc 1: ≥10% of cortical PTCs with capillaritis, with maximum 3–4 luminal inflammatory cells
ptc 2: ≥10% of cortical PTCs with capillaritis, with maximum 5–10 luminal inflammatory cells
ptc 3: ≥10% of cortical PTCs with capillaritis, with maximum >10 luminal inflammatory cells

PTC, peritubular capillaries.
Note the composition (mononuclear vs. neutrophils) and extent: ≤50% (focal) vs. >50% (diffuse). Adapted from (8).

most involved glomerulus (>12) were more likely to have DSA, C4d-pos, TG, and the worst allograft survival (GS). Indeed, the maximum Mo count was the only factor significant for GS on multivariable analysis (13). Acute CMR in this late cohort did not affect the composition of cells in g. Unlike previous data from earlier biopsies (11), the Mo/TC ratio was not different in C4d-pos vs. C4d-neg cases. In a later study of 1101 biopsies, pure CMR did not have more g (or ptc) than DSA negative (DSA-neg)/C4d-neg biopsies without any rejection (combined g + ptc >0 in 6% vs. 0.6%) (14).

Others have reported different results. Batal et al. (15) studied 111 biopsies with at least interstitial inflammation obtained at a mean of 504 d. Using strict criteria (minimum ≥5 leukocytes/glomerulus to be considered positive), 44 were graded g0 by Banff. Even with this stricter criterion than Banff's, 47% of DSA-neg, C4d-neg CMRs had g>0, and 62% of DSA-neg, C4d-neg borderline CMRs did as well. Similarly, Aiello et al. (16) noted g in 40% of 90 biopsies with AR. Of the 36 with g>0, 22 had C4d-neg CMR and 10 had CMR that was suspicious for AAMR (C4d-pos). These data reinforce those of Magil (11) that CMR in the absence of detectable antibodies can produce g.

Gibson et al. (17) studied ptc in 688 allograft biopsies (181 for an indication). They found ptc >0 in 26% (46% of indication biopsies). Overall, most cases were moderate (ptc 2), focal (involving 10–50% of PTC) and mononuclear predominant. Only 8% had a majority of PMNs. There was a strong correlation of ptc with g (if g >0, then ptc >0 in over 80%) and C4d (75% had ptc >0 if C4d-pos vs. 24% if C4d-neg). Interestingly, 68% of 76 biopsies with ≥1A CMR had ptc >0 as did 45% of 105 with borderline rejections. Furthermore, 14% of biopsies without any rejection (AAMR or CMR) had ptc≥ 1. Fahim et al. (18) studied 42 biopsies, comparing 10 with pure CMR in the absence of g with 32 having g: 13 C4d-neg and 19 C4d-pos. No significant difference in PTC total cell count, mononuclear count (Mo + TC), or PMN count existed between the three groups. The PTC Mo/TC ratio was significantly higher in C4d-pos g cases (2.3) than C4d-neg g cases (1, $p = 0.0008$) and pure CMR ones (0.9, $p = 0.0014$), results mirroring the glomerular findings of Magil (11), but unlike those of Papadimitriou (13). These data support the concept that cell-mediated mechanisms produce MVI without the help of antibodies.

Several groups have studied the prognostic significance of MVI. Among 878 transplants, Cosio et al. (19) found by multivariable analysis that C4d-neg AR (presumably CMR) was only associated with reduced GS if g or ptc was present. In the absence of MVI, there was no difference compared with transplants without rejection. de-Kort et al. (20) studied 58 conventional risk transplants with development of de novo DSA (dnDSA) who had indication biopsies. Despite dnDSA and an indication for the biopsy, 26 had no detectable MVI (g + ptc = 0). Six (23%) of the 26 were C4d-pos and none had CMR. As the degree of MVI increased (g + ptc = 1 or 2, or g + ptc ≥3), C4d-pos increased (29% and 64% respectively), as well as CMR (33% and 73%), suggesting significant contributions from both arms of adaptive immunity in the development of MVI. Using MVI as a predictor of GS, the area under the curve in ROC analysis was 0.84, better than that for C4d ($p = 0.006$). Sis et al. (21) used MVI to predict the presence of DSA in 329 indication biopsies. MVI (g + ptc >0) in early biopsies (less than one yr) was non-specific, being found in AAMR, CMR, and ATN. Only in the presence of C4d-pos was MVI likely to indicate DSA at this early time. In later biopsies, C4d-pos was not required for this determination. Furthermore, the MVI sum score strongly predicted GS on multivariable analysis. This group has also demonstrated a role for NK cells in antibody-mediated cases (22), an observation supported by experimental studies (23).

In summary, MVI is found in AAMR. In hyperacute rejections and some that occur very early, PMNs predominate. Most commonly the inflammatory infiltrate, however, is mononuclear, especially Mo and possibly NK cells. Such MVI can be ascribed to AMR in the presence of both C4d-pos and DSA-pos. Acute CMR results in MVI as well, both g and/or ptc. In the presence of CMR and the absence of C4d and DSA, MVI is presumably cell-mediated. Non-alloimmune mechanisms such as ATN may result in MVI in the absence of any rejection. In the future, identification of pathogenesis-based transcripts (PBTs) specific for the type of injury may determine the predominant cause (24, 25), along with cell type (Mo vs. TC, perhaps NK-cell). In any case, MVI is not synonymous with antibody causation.

Chronic microvascular injury: TG and PTCBMML

Microvascular injury and inflammation result in chronic changes, including TG and PTCBMML. These chronic changes are considered synonymous with antibody causation and either is considered sufficient tissue injury required for diagnosis of CAAMR by Banff. Similar to MVI, however, these lesions are non-specific.

Transplant glomerulopathy is diagnosed by light microscopy when 10% of capillary loops in the

Table 4. Banff quantitative criteria for transplant glomerulopathy (cg)

cg0: no glomerulopathy. Double contours in <10% of peripheral capillary loops in most severely affected glomerulus
cg1: double contours affecting up to 25% of peripheral capillary loops in the most affected non-sclerotic glomeruli
cg2: double contours affecting 26–50% of peripheral capillary loops in the most affected non-sclerotic glomeruli
cg3: double contours affecting >50% of peripheral capillary loops in the most affected non-sclerotic glomeruli

Number of glomeruli and percentage sclerotic. Adapted from (7).

most affected glomerulus have double contours (Table 4) (7). By EM, at least three capillary loops should show GBM reduplication (13), and probably over 90% have associated PTCBMML (26). Although it may be clinically silent, clinical manifestations include declining GFR, proteinuria, and hypertension (27). In a large series of conventional transplants undergoing protocol biopsies, TG was detected in 4% at one yr, increasing to 20% by five yr (28). In higher-risk, live donor transplants performed against a positive cross-match, the one-yr incidence was 22% (29).

Evidence indicates that chronic antibody-mediated injury results in TG. Preformed HLA antibodies, especially if DSA and class II, correlate with future development of TG. Among 582 transplants, the presence of class II antibodies increased the hazard ratio (HR) for TG by 5, as compared to those without any class II antibodies. The HR increased to 10 if DSA-pos (28). Furthermore, TG is associated with C4d-pos and DSA. The frequency of their occurrence, however, has varied considerably in the published series. Regele et al. (6) detected C4d in PTC in 67% of 58 biopsies with TG. Subsequent studies have found a lesser incidence. These include 0% of 22 biopsies (30), 15% of 36 (31), 24% of 68 (32), 36% of 53 (26), 48% of 25 (33), 55% of 58 (34), and 67% of 59 (6). Where assessed, these C4d-pos cases had detectable DSA, as did some of the C4d-neg ones. Overall, 25–60% of these TG cases, however, lacked any evidence of antibody mediation (both HLA/DSA-neg and C4d-neg) (26, 30, 31).

Other potential causes of TG include CMR, thrombotic microangiopathy (TMA), and chronic hepatitis C virus (HCV) infection. As noted above, CMR results in g and ptc. Such MVI precedes or coexists with TG. Whereas both AAMR and subclinical AMR (35) can significantly increase the risk of TG, most cases are not preceded by such events (32). In one series of 73 patients, 7% had prior AAMR (32). Furthermore, acute CMR more commonly precedes TG than does AAMR (26, 28), although the exact significance of this remains uncertain (see below). For example, in the series of 582 transplants noted above, prior CMR predominated (15, including nine borderline) compared with AAMR (three cases) (28). In the series of Sis et al. (26), prior AR occurred in 20 of 37 patients with rejection history. Among 35 biopsies in these 20, CMR existed in 74% (only one of which was borderline), as compared to 26% with AAMR.

Other evidence exists to support cell-mediated immunity in the pathogenesis of TG. Akalin et al. (36) compared six biopsies with TG to 17 with "chronic allograft nephropathy" (CAN, now referred to as interstitial fibrosis/tubular atrophy, or IF/TA). All TG biopsies had glomerular staining for the costimulatory molecule ICOS and the chemokine receptor CXCR3 with its ligand Mig, findings that indicate the presence of activated T cells. All CAN-only biopsies were negative. Homs et al. (37) found increased mRNA expression for interferon-gamma (InfG), the Th1 transcription factor T-bet, and the cytotoxic T-lymphocyte cytokine granzyme-B in the glomeruli of 22 patients with TG as compared to 18 with CAN alone. Similarly, Sun et al. (38) demonstrated significantly increased T-bet expression by immunohistochemistry in glomeruli and PTC of 32 patients with TG, as compared to 23 with IF/TA and 15 stable grafts. T-bet expression correlated strongly with CD4, CD8, and CD68 infiltration. Dean et al. (39) compared intragraft gene expression in 22 positive cross-match transplants with TG to 10 conventional DSA-neg controls. Despite the antibody involvement, gene expression profiles of PBTs associated with cell-mediated immunity were significantly enriched, including those for cytotoxic T lymphocytes and InfG. Finally, we retrospectively studied 50 consecutive biopsies with TG at Thomas Jefferson University Hospital. Only 45% had detectable DSA by Luminex solid phase assay (SPA), and only 14% were C4d-pos. Surprisingly, 49 of 50 had lymphocytic infiltration of fibrous intimal thickening, thereby satisfying Banff criteria for chronic active CMR (Filippone et al. manuscript in preparation).

Pathologic overlap exists between TG and TMA. In chronic TMA, double contours are found by light microscopy (LM), and the ultrastructural changes are also similar to TG (33, 40, 41). For example, Wavamunno et al. (42) compared the ultrastructural changes of protocol biopsies of seven simultaneous kidney–pancreas (SPK) patients who developed TG to those of eight SPK patients that did not. Within one yr, swelling and vacuolization of endothelial cells with loss of their fenestrations was observed in those destined to develop TG.

They also had a widened subendothelial space with accumulation of flocculent electron-lucent material. Eventually, there were new layers of GBM and mesangial cell interposition. Haas and Mirocha (43) noted similar early changes (endothelial cell swelling, subendothelial widening, and GBM duplication) in three-month indication biopsies, many of which demonstrated TG on subsequent biopsies. These early and later changes of TG are also those of acute and chronic TMA.

Post-transplantation TMA may represent recurrent disease, for example inherited complement regulatory protein abnormalities, or arise de novo (40). These latter cases may develop very early (even within one wk) and have been ascribed to calcineurin-inhibitor (CNI) toxicity (44). A possible association with mTOR inhibitor use has been reported as well (45). Post-transplantation TMA is often graft limited, but about 25% of cases have systemic features, including those of a microangiopathic hemolytic anemia (41). An association of TMA with AMR is reported. Satoskar et al. (41) studied 59 patients with de novo TMA and found diffuse C4d-pos in 55%. Of note, 13% of C4d-pos biopsies evidenced TMA compared with 3.6% of C4d-neg ($p < 0.0001$). No significant difference in the severity of TG between C4d-pos and C4d-neg groups was noted, although greater MVI was observed in the former. In 37 TMA cases (of 1101 biopsies), only six were C4d-pos. The incidence of TMA was the same in C4d-pos and C4d-neg biopsies overall (3.4% of each) (46). If the biopsy occurred at ≤90 d, however, the probability of observing TMA in C4d-pos biopsies was significantly greater than in C4d-neg ones. By contrast, after 90 d, TMA was less frequent in C4d-pos biopsies (46).

Baid-Agrawal et al. (33) studied 25 cases of TG. They found 48% to be C4d-pos; 32% had TMA, and 36% were HCV positive. Significant overlap existed between the presence of TMA and HCV: five had both TMA and HCV, as compared to three with just TMA and four with just HCV. A significant association of HCV and TG was found by others (28, 47), although not all (48). Cosio et al. (47) found positive serology for HCV in 29% of 41 patients with g and 33% of 27 patients with TG, as compared to 1.8% of 105 patients with neither ($p = 0.0004$ for both comparisons). Gloor et al. (28) noted a significant association by multivariable analysis between HCV and development of TG in 582 conventional transplants with a HR of 2.9 ($p = 0.01$).

PTCBMML frequently coexists with TG, being found in over 90% of such cases. PTCBMML alone represents sufficient tissue injury for a diagnosis of CAAMR per Banff if both DSA-pos and C4d-pos. The minimum requirements to diagnose PTCBMML, however, are not well specified by Banff. In an important paper, Ivanyi et al. defined the "cpc lesion" (49). This required circumferential involvement (>75% of the circumference of a PTC) with the most involved capillary having ≥7 layers or the three most involved capillaries having 5–6 layers. Such a lesion had a sensitivity for chronic rejection (defined as arterial fibrous intimal thickening or TG) of 59%, a number similar to the 57% reported in an earlier series (50). Notably, LM evidence of acute CMR was found in 78% of cases, which suggested that cpc is attributable to slowly progressive, smoldering CMR. More recently, Liapis et al. (51) studied 360 native kidney and 187 transplant biopsies. Severe PTCBMML (equivalent to cpc) was rarely found in native kidneys with the exception of late TMA. In transplants, this lesion lacked specificity, as it was found in 20% of C4d-neg acute CMR, 36% of cases felt to have CNI toxicity, and 67% of C4d-neg chronic active CMR. Finally, cpc was found in approximately half of antibody-mediated cases (AAMR/CAAMR). Hence, although PTCBMML is usually found together with TG, neither of these lesions is specific for antibody causation.

Discussion

The immunologic destruction of a renal allograft involves a complex interplay between innate and adaptive immune systems (recent review 52). The methodology for antibody detection is constantly evolving, and consensus guidelines have recently been published (53). Here, we have detailed the lack of specificity of histologic and ultrastructural features elicited by humoral injury, although part of the discrepancies noted relate to variability between laboratories in the methodology and proficiency in determining DSA and C4d. In addition, interobserver discrepancy in the diagnosis of cell-mediated rejection is significant. For example, with three pathologists evaluating the same 245 biopsies, agreement between any two was only 45% with regard to a diagnosis of TCMR or mixed T-cell- or antibody-mediated rejection (25). These issues reduce both the sensitivity and specificity of associated morphologic features of any diagnostic category.

Alloantibodies are considered the prime mediators of chronic rejection. Both cell-mediated immunity and antibodies, however, are capable of producing the acute and chronic lesions discussed above (Table 5). Treatment of cell-mediated and antibody-mediated rejections differ. In the former, steroids with the possible addition of thymoglobulin

Table 5. Relationship of histologic features to specific diagnostic category

	Diagnostic category				
Histology	Acute antibody-mediated rejection (AAMR)	Acute cell-mediated rejection (ACMR)	Chronic active antibody-mediated rejection (CAAMR)	Chronic active T-cell-mediated rejection (CACMR)	Thrombotic microangiopathy (TMA)
g	Banff criterion	**Present** (15,16,21) **(38%)**[b]	Present (uncertain %)	Present (uncertain %)	Present (uncertain %)
ptc	Banff criterion	**Present** (17,21) **(49%)**[b]	Present (uncertain %)	Present (uncertain %)	Present (uncertain %)
TG	**TG preceded by AAMR in 8.6%** (26,32)	**TG preceded by ACMR**[b] **in 32%** (26,28)	Banff criterion	**CACMR may coexist in 50%**[a]	**Indistinguishable**
PTCBMML	Not present	**Present** (52) **(20%) ACMR: Present in up to 78% of PTCBMML** (50)	Banff criterion	**Present (67%)** (52)	**Found in TMA even in native kidneys** (52)
C4d$^+$	Banff criterion	Not present	Banff criterion **(35% of reported cases of TG are C4d$^+$)** (26,30–34, 6)	Not present	**Frequently found (40%)** (42, 47)

Abbreviations as per text. Percentages represent means unless single study. Notable findings in bold. Online numbers with in parentheses represent references from which data were derived.
[a]Filippone et al. Manuscript in preparation.
[b]Includes borderline ACMR.

would be in order, possibly with increased chronic therapy. In the latter, and despite a dearth of high-level evidence (54), plasmapheresis, IVIg, and steroids are used, with consideration of rituximab, bortezomib, thymoglobulin, eculizumab, and possibly splenectomy depending on the circumstances. More difficult is the case with features of both, such as a C4d-neg 1A CMR with significant MVI and DSA. The same issues obtain with TG. As outlined above, TG is not automatically an antibody-mediated lesion, even with associated PTCBMML.

At the present time, no gold standard exists to identify the predominant pathophysiologic mechanism of allograft injury. Alloantibodies are commonly detectable prior to chronic alloimmune loss of a renal transplant (55). DSA alone, however, does not establish causality. Such antibodies may simply indicate a more aggressive alloimmune diathesis and are not causally related to the injury in a particular case. The presence of C4d staining strengthens a likely pathogenic role. DSA frequently exist, however, without C4d, and the reverse may occur. Among late (seven yr) biopsies for allograft dysfunction, 40 were double positive, 74 cases double negative, but 28 had C4d alone, and 31 had DSA alone. The presence of C4d-pos correlated with reduced GS, whereas DSA alone did not (56).

C4d could occur in the absence of DSA for several reasons. The DSA may be against untyped donor antigens (e.g., DP). They may be present but below the detectability of current assays. They may be absorbed by the graft and, thus, not detectable. They may have been present earlier as initiators of disease but not required to sustain the injury. Non-DSA HLA antibodies could react with the graft through epitope spreading. Finally, pathogenic antibodies may be directed against non-HLA antigens. Such a situation exists in CAAMR for antibodies against agrin (57), glutathione-S-transferase T1 (58), and peroxisomal-trans-2-enoyl-coA-reductase (59).

DSA can occur in the absence of C4d for several reasons and still be pathogenic. C4d staining may be fleeting, with cases changing from positive to negative in days to weeks (60). Alternatively, DSA could mediate tissue injury without fixing complement. Experimental and clinical evidence indicates that antibodies via Fc receptors recruit NK cells and macrophages that injure tissue (23). Antibodies bind to the cell membranes and induce functional changes. For example, HLA antibodies can induce endothelial cell exocytosis of P-selectin and induce inflammation in the absence of complement (61). Non-HLA angiotensin receptor antibodies induce proinflammatory responses by direct binding (62).

More importantly, in the absence of C4d, DSA may be present but not pathogenic. The current trend is to label such cases as C4d-neg antibody-mediated rejection (63), although by Banff they would be "presumptive" AMR. Given the non-specificity of MVI and TG/PTCBMML, it is critical to determine whether DSA are pathogenic in a particular patient. Preformed DSA, even at low levels detectable only by SPA, are associated with adverse outcomes (64). This is not always the case, however, as they can frequently wane or disappear

over time, either spontaneously (65) or after desensitization (66). For example, Kimball et al. (65) prospectively followed 69 non-desensitized flow cross-match (FCXM)-positive patients with SPAs. Within one yr, two-thirds showed elimination of FCXM positivity and had no DSA by SPA. Three-yr graft survival was 95%. Akalin et al. desensitized 35 patients with positive cross-matches (66). Within one yr, 52% lost DSA, and another 30% had reductions in the number or strength of their DSAs. When persistent, DSA may not always cause detectable harm (67). Bartel et al. (67) compared 34 non-desensitized patients with excellent graft function after one yr to 130 patients with decreased GFR and/or proteinuria. Nine of the 34 were flow PRA-positive, including five with DSA. All five maintained excellent graft function (eGFR >60) for at least five more yr, including one with persistingly positive FCXMs (both T- and B-cell). At present, the best evidence for the pathogenicity of a given DSA, aside from its ability to fix complement (C4d+), is a rising and high titer: prior to transplantation a high titer predicts a positive cross-match (68); in the early post-transplant period, rising titers predict AAMR (69); and later, they predict graft failure (70).

As well as being preformed, DSA may develop de novo (dnDSA) in up to 25% of cases (71). This may occur late (after one yr) (71–73), although earlier appearance has been reported (74–76). The predominant dnDSA appears to be class II, especially DQ (75–77). Some of the very early cases may represent activation of memory B-cells detectable at the time of transplantation by HLA-specific tetramer staining in the absence of circulating antibody (78). Development of dnDSA correlates with reduced GS in many studies (71–76). A significant number of patients tolerate their presence, however, without obvious adverse outcomes. As with TG, dnDSA may follow CMR (73, 74). In one study with protocol screening, dnDSA rarely preceded AR (3 of 19 with AR), but usually occurred concurrently or following the rejection (74). Furthermore, detection of dnDSA had a positive predictive value of only 6% for subsequent AR. Do these antibodies detectable following CMR then assume prime importance for further graft injury? Or are they merely epiphenomena with smoldering CMR the real culprit? dnDSA are most troublesome when found in the setting of late graft dysfunction in a non-adherent patient, where AAMR and CMR may coexist (63, 73).

Other methods are currently being explored to predict the toxicity of DSA. The ability to detect the complement fixation capability of DSA in vitro by SPA (e.g., C1q or C4d positivity on Luminex beads) may help predict development of AAMR or more chronic issues such as TG and late graft failure (79–81). Most promisingly, the ability to differentiate the specific cause of issue injury by evaluation of PBTs may allow determination that antibodies are the proximate cause (22, 24, 25). Unfortunately, this kind of analysis, as spearheaded by the Edmonton group and others, is not generally available and still requires confirmation.

In conclusion, both acute MVI (g and ptc) and chronic microvascular changes (TG and PTCBMML) can clearly be caused by alloantibodies. Both cell-mediated and non-alloimmune mechanisms, however, similarly result in these acute and chronic lesions, either together with antibodies or as the primary process. The pathogenicity of these alternatives to AMR needs to be considered for appropriate stratification in the design of therapeutic clinical trials and obviously for consideration of treatment in the individual patient.

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