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Transplant Glomerulopathy With Glomerular C3 Deposits: Why the Worse Outcome?

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Transplant glomerulopathy (TG) is a morphologic pattern of glomerular injury in kidney allografts, defined by duplication or multilayering of the glomerular basement membranes (GBMs). TG is the pathologic finding most closely associated with chronic antibody-mediated rejection (ABMR), and is strongly associated with donor-specific antibodies (DSAs), especially DSAs directed against human leukocyte antigen class II.[1] However, GBM duplication is not specific for chronic ABMR. Studies have shown that approximately 75% of renal allograft biopsies with GBM duplication are associated with concurrent or prior DSA, and/or peritubular capillary C4d staining, although such GBM duplication also may be the result of chronic thrombotic microangiopathy (e.g., resulting from recurrent disease or calcineurin inhibitor nephrotoxicity) or hepatitis C virus infection.[2] In each case, it is postulated that persistent or repetitive injury to the glomerular endothelium results in separation of the endothelium from the underling GBM with subendothelial electron-lucent widening, followed by new basement membrane formation with or without mesangial interposition; these changes may be seen by electron microscopy during the first weeks to months posttransplantation in grafts exposed to DSA, well before GBM double contours are evident by light microscopy.[3] TG as a manifestation of chronic ABMR may be the result of complement-mediated injury to the graft endothelium, as evidenced by peritubular capillary C4d deposition, but may also occur in the absence of C4d. Clinically, TG is manifested by low-grade to nephrotic-range proteinuria with progressive allograft dysfunction and has an extremely poor prognosis, resulting in graft loss in a large fraction of affected patients.[1]

Although most descriptions of TG do not mention immune deposits other than C4d associated with the endothelium, an early description of Habib and coworkers[4] noted that in most cases examined by immunofluorescence, glomeruli showed segmental deposits of IgM and fibrin sometimes associated with trace amounts of C3. Although such deposits, including those of C3, are often (as they were by Habib *et al.*[4]) attributed to nonspecific trapping within areas of glomerular capillary remodeling, a recent study[5] showed that complement cascade genes are upregulated in grafts with TG undergoing allograft failure compared with those remaining functional. However, the clinical significance of demonstrable glomerular C3 deposition in TG has been unknown.

In the current issue of *Kidney International Reports*, Panzer *et al.*[6] carefully analyzed the association of glomerular complement C3 deposition with allograft failure in a cohort of 111 patients with TG, and report that glomerular C3 deposition is an independent risk factor for allograft failure. In this study, 72 (65%) of the allografts with TG failed a median of 3 years after diagnosis; allograft failure was seen in 36 of 46 (78%) grafts with glomerular deposition of C3 versus 36 of 65 (55%) grafts without C3. Glomerular C3 deposits were often associated with deposits of IgM and C1q, and, importantly, these deposits were noted to be granular in all cases and were associated with immune complex deposits by electron microscopy in 53% (as opposed to hyaline aggregates in just 11%), indicating that in most cases, the C3 was a component of immune complexes. In a multivariable analysis including clinical, serologic, and morphologic parameters, Panzer *et al.*[6] found that there were 3 independent predictors of graft loss in their cohort of patients with TG: arteriolar hyaline thickening, a chronicity score (sum of Banff scores for chronic glomerulopathy, interstitial fibrosis, tubular atrophy, and arterial intimal fibrosis), plus

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the presence of glomerular C3 deposits. The latter findings clearly raise interest in the potential mechanisms by which the C3 became deposited in the glomeruli, and the possible role of immune complexes in the pathogenesis and progression of chronic ABMR.

The first and most obvious question is whether the C3-positive TG cases do indeed represent chronic (or chronic active) ABMR, as opposed to other etiologies, most notably hepatitis C–related disease. As noted previously, TG is not specific for ABMR, with hepatitis C being one documented alternative etiology[2]; furthermore, in the study of Baid-Agrawal *et al.*,[2] hepatitis C–positive patients with TG had poorer graft survival than those who were hepatitis C–negative. However, among the 111 patients with TG studied by Panzer *et al.*,[6] only 2 (both C3-positive) were hepatitis C–positive. Still, although 71% of the patients with TG of Panzer *et al.*[6] were DSA-positive, it is worth noting that this fraction was significantly lower in the C3-positive group (50%) compared with the C3-negative group (77%), raising the question of whether more of the former had an underlying etiology other than ABMR. To this end, according to Banff 2017 criteria, although 90% of C3-negative biopsies had a diagnosis of chronic active ABMR (65%), chronic ABMR (20%), or suspicious for chronic active ABMR (5%), this fraction was only 76% (57%, 15%, and 4% in the respective subcategories) in the C3-positive group. Perhaps because of this, only 6% of patients with C3-positive biopsies received treatment for ABMR with i.v. Ig ± rituximab, as compared with 38% of patients with C3-negative biopsies. Thus, differences in treatment might have contributed to the worse outcomes in C3-positive patients, although Panzer *et al.*[6] found no significant association of ABMR treatment with graft failure in their cohort of patients with TG.

Might C3 deposition in glomeruli reflect the complement-activating properties of DSA present? It is well documented that such properties of DSA affect outcomes in ABMR.[7,8] Of the 4 IgG subclasses, IgG3 is the strongest activator of complement via the classical pathway, and Lefaucheur and

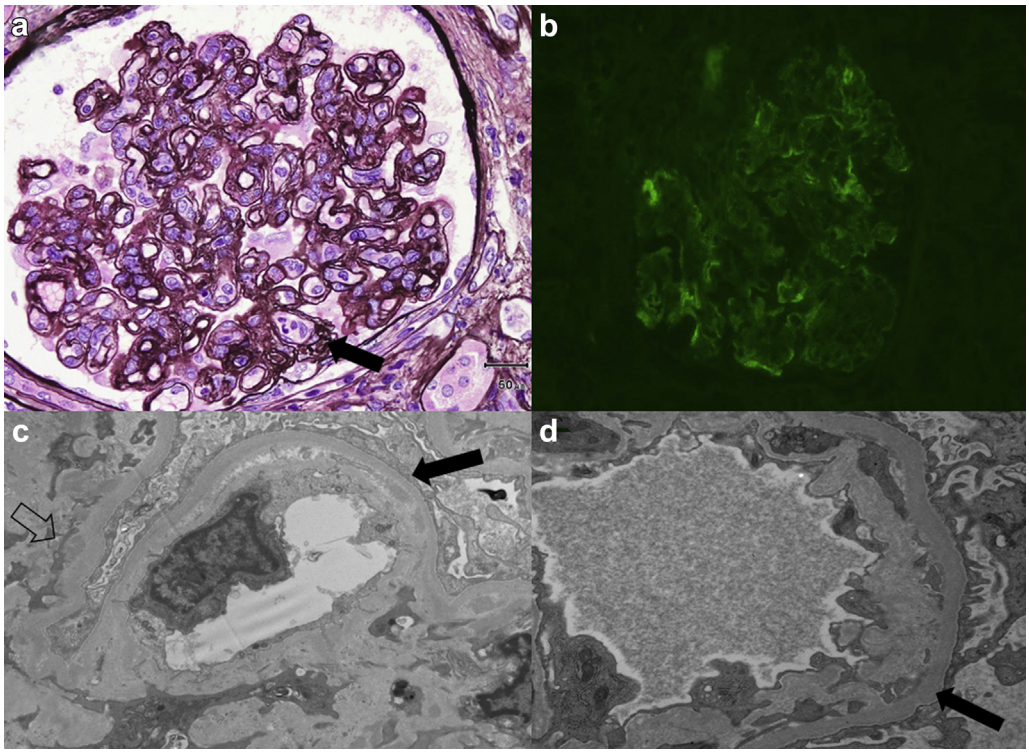

Figure 1. A case of transplant glomerulopathy with C3 and immune complex–type, electron-dense deposits. (a) A glomerulus shows global, prominent double contours of glomerular capillary basement membranes on Jones methenamine silver stain. The arrow indicates segmental glomerulitis with a capillary occluded by leukocytes and swollen endothelium (original magnification ×400). (b) Immunofluorescence shows modest, granular to globular staining for C3 in glomerular capillary walls and more segmentally in mesangial areas (fluorescein isothiocyanate–conjugated anti-human C3, original magnification ×400). (c) Ultrastructural study shows subendothelial electron-lucent widening with a newly formed, duplicated glomerular basement membrane; the glomerular endothelium exhibits swelling with loss of fenestrations. Electron-dense deposits are seen segmentally in the subendothelial space and mesangial region (arrows). There is moderately extensive but incomplete podocyte foot process effacement (uranyl acetate and lead citrate stain, original magnification ×10,000). (d) Another glomerular capillary shows glomerular basement membrane duplication and small, subendothelial electron-dense deposits (arrow) (original magnification ×14,000).

colleagues[7] have shown that the presence of an immunodominant DSA that is of the IgG3 subclass and/or is C1q binding is an independent risk factor for kidney allograft loss. Furthermore, in a more recent study, Lefaucheur *et al.*[8] showed, in 116 renal transplant recipients having 1 or more DSA present at the time of transplantation, that prophylaxis using eculizumab, an anti-C5 monoclonal antibody, decreased the incidence of biopsy-proven ABMR in patients with a complement-activating DSA, but not in patients with non–complement-activating DSAs. Although Panzer *et al.*[6] did not report the IgG subclasses or C1q binding properties of DSAs in their patients, it is possible that the presence of complement-activating DSAs may have played a role in the worse allograft survival in those patients whose biopsies showed glomerular C3 deposition.

There is in fact evidence from animal studies that alloantibody may cause immune complex deposition. Grau *et al.*[9] reported findings from an experimental model in which kidneys from Fischer-344 rats were transplanted into Lewis rats without immunosuppression. Their findings recapitulated many morphologic features of human active ABMR, and in addition revealed at 26 weeks posttransplantation GBM double contours and segmental immune complex–type deposits in subendothelial and mesangial locations, without associated changes of proliferative glomerulonephritis.[9] By immunofluorescence, the deposits contained IgG, IgM, and C4d. The authors also examined human renal allograft biopsies with TG by electron microscopy as well as immunohistochemistry performed on paraffin sections; after exclusion of patients with recurrent or *de novo* glomerulonephritis, 8 of 46 biopsies showed small numbers of subendothelial and mesangial immune complex–type deposits.[9] Immunohistochemistry done on 6 cases showed glomerular capillary wall deposits in all cases plus mesangial deposits in 4, with the deposits composed of IgM plus variable amounts of C3 and C1q, similar to the findings of Panzer *et al.*[6] We have similarly observed immune complex deposits in a minority of our cases of TG associated with DSA; 1 such case is illustrated in Figure 1. Grau *et al.*[9] proposed a putative mechanism of immune complex formation in their animal model, with circulating antibodies reactive against MHC antigens expressed on glomerular endothelial cells as well as non-MHC antigens within the GBM, such as perlecan and components of type IV collagen leading to *in situ* immune complex formation with subsequent activation of complement.

In summary, the study of Panzer *et al.*[6] raises our awareness that TG is not always indicative of chronic (or chronic active) ABMR, that immune complex deposits within glomeruli may occur in TG, and the presence of the latter does not necessarily rule out ABMR as the etiology of the TG, although it should prompt us to consider other possible glomerular lesions, such as a glomerulonephritis related to hepatitis C. The findings of this study are also potentially important because they identify glomerular C3 deposition as an independent risk factor for allograft failure in patients with TG. At this point, the mechanisms by which immune complexes containing C3 become deposited in glomeruli, and the reason(s) why the presence of such complexes is associated with worse graft outcomes, is not clear. The latter may be as straightforward as correlation with complement-fixing DSA, and additional studies examining the possibility of such a correlation are needed. The C3 deposits within glomeruli may also signify that 2 additive, immune mechanisms might be ongoing to produce injury to the graft in those TG cases in which such deposits are present. It is hoped that the study of Panzer *et al.*[6] stimulates further investigation into the immunologic processes underlying the development of TG and the mechanisms by which TG exerts its well-documented deleterious effect on renal allograft survival.

DISCLOSURE

All the authors declared no competing interests. MH serves as a paid consultant on pathology adjudication committees for 2 industry-sponsored clinical trials: Shire ViroPharma, Treatment of Acute Antibody-Mediated Rejection, and AstraZeneca, Treatment of Proliferative Lupus Nephritis. He has also received honoraria for serving as a speaker and advisor for CareDx. None represent a conflict of interest relevant to any of the material presented in this article.

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