Emerging role of B cells in chronic allograft dysfunction

Robert B. Colvin[1], Tsutomu Hirohashi[2], Alton B. Farris[3], Francesca Minnei[4], A. Bernard Collins[1] and R. Neal Smith[1]

[1]*Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA;* [2]*Transplantation and Cardiac Surgical Divisions, Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA;* [3]*Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia, USA and* [4]*Department of Anatomia Patológica, Ospedale Pediatrico Bambino Gesù, Rome, Italy*

B cells have many possible mechanisms by which they can affect allograft survival, including antigen presentation, cytokine production, immune regulation, and differentiation into alloantibody-producing plasma cells. This report reviews the last mechanism, which the authors regard as most critical for the long-term survival of allografts, namely, the promotion of chronic rejection by alloantibodies. Chronic humoral rejection characteristically arises late after transplantation and causes transplant glomerulopathy, multilamination of peritubular capillary basement membranes, and C4d deposition in PTCs and glomeruli. Circulating antidonor human leukocyte antigen class II antibodies are commonly detected and may precede the development of graft injury. Prognosis is poor, especially when recognized after graft dysfunction has developed. Improved detection and treatment are critically needed for this common cause of late graft loss.

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Correspondence: *Robert B. Colvin, Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Thier-831, Boston, Massachusetts 02114, USA. E-mail: colvin@helix.mgh.harvard.edu*

A series of seminal papers have led to our current appreciation of the important role of alloantibodies in late graft loss. Alloantibodies were first implicated in chronic rejection of human renal allografts by Paul Russell and colleagues[1] in 1970, who reported that chronic allograft arteriopathy arose only in patients who developed *de novo* antidonor antibodies (human leukocyte antigen (HLA)). Subsequent studies by Paul Terasaki *et al.*[2] and several other investigators detected an association of circulating HLA antibodies with an increased risk of long-term graft loss, but without demonstrating direct connection with antibodies and graft pathology.[3] Halloran and colleagues[4,5] showed that acute renal allograft rejection in patients with donor-specific anticlass I HLA antibodies had distinct pathological features (for example, neutrophils in capillaries), but again provided no direct link of the pathology with the antibodies. A breakthrough discovery was the demonstration of the complement fragment C4d in peritubular capillaries (PTCs) in patients with acute rejection by Helmut Feucht.[6] This was tied to circulating donor-specific antibodies and graft pathology by Collins and colleagues[7,8] and confirmed by many others, leading to the introduction of the diagnosis 'acute antibody-mediated rejection' in the Banff classification. Mauiyyedi *et al.*[9] then connected the dots and discovered that glomerulopathy or arteriopathy was linked to C4d deposition in PTCs and donor-specific alloantibody (DSA). For this condition, they proposed the new term 'chronic humoral rejection' (CHR). Regele *et al.*[10] independently made similar observations and extended the features of CHR to include capillaritis and basement membrane multilamination in PTCs. After other groups had confirmed these findings, it was clear that about 50% of patients with transplant glomerulopathy or arteriopathy have C4d deposition in PTCs, but the frequency varied considerably by center.[7,11–15] Studies by Scornik *et al.*[13] showed that only acute rejection and chronic allograft nephropathy had positive PTC staining, arguing that these are the two major forms of antibody-mediated rejection. Most if not all cases with C4d+ antibody-mediated rejection, even if it is subclinical, have detectable circulating antibodies.[16]

CHR

In 2005, the Banff consensus conference added a new category 'chronic active antibody-mediated rejection' (a.k.a. CHR) to its classification with the criteria given in Table 1.[17] Recent studies have indicated that CHR is common in unselected indication biopsies, found in one 10-year series in 9.3% of 771 cases.[18] The onset is typically late, after the first year, and the prevalence rises progressively to about 20% in the fifth year. Proteinuria is common but not invariable ($\sim$50% have $>$1 g per day proteinuria). Renal function is often abnormal, but can remain stable for considerable time periods (years).[19] The strongest risk factor identified to date is the presence of pretransplant donor-specific antibodies,[20] but most cases arise in patients without a history of presensitization or even an episode of acute humoral rejection. Serologically, the most intriguing aspect of CHR is the strong association with class II DSA,[11,20] which is not a feature of acute humoral rejection.

The major features of CHR are duplication of the glomerular basement membrane ('transplant glomerulopathy'), multilamination of PTC basement membranes, mononuclear cells in glomeruli and PTCs, and loss of normal glomerular capillary endothelial fenestrations ('dedifferentiation'; Figure 1).[21] All these features were well described in transplant pathology before they were known to be due to antibody-mediated injury.[22] The cells in glomerular and PTCs are primarily macrophages (CD68+)[23] that express the FcγRIII receptor.[18] Leukocytes in glomeruli also express T-bet, a transcription factor related to interferon-γ production.[24] Glomerular endothelial cells display increased plasmalemmal vesicle-associated protein-1, indicating altered vesicle physiology.[25] In addition to multilamination of basement membranes, loss of PTCs has been demonstrated in patients with chronic graft injury and this correlates inversely with serum creatinine.[26] It is possible that the loss of capillaries is related to endothelial–mesenchymal transition as described by Kalluri and colleagues.[27] In any case, loss of PTCs can affect the extent of C4d positivity and contribute to the lower density of C4d+ PTCs often observed in CHR.[28]

One difficulty in the recognition of CHR is that not all diagnostic components may be present at any particular time. For example, studies by Sis *et al.*[11] showed that, although PTC multilamination is almost always present (91%) in patients with transplant glomerulopathy, detectable DSA in the circulation and C4d deposition in the graft are less common (70 and 32%, respectively). Overall, about 26% of patients with transplant glomerulopathy have no DSA or C4d. The question is whether these represent another form of glomerular injury, such as thrombotic microangiopathy, or whether the lesions are a residue of a past episode of antibody-mediated injury. We have seen several instances in which previous or subsequent biopsy results of patients with transplant glomerulopathy and C4d+ showed glomerulopathy and no C4d deposition.[28] It is also possible that those cases with transplant glomerulopathy and DSA but without C4d are due to complement-independent antibody-mediated injury.

Plasma cells are present in increased frequency in late allografts with rejection and C4d+ PTCs.[29] Indeed, allograft biopsy samples, regardless of diagnosis, have increased levels of immunoglobulin gene transcripts as a function of time after transplantation.[30] It is debatable whether these particular plasma cells, through the antibodies they synthesize, are related to the late graft injury or are an epiphenomenon. One argument in favor of a pathogenetic role is the provocative demonstration by Thaunat *et al.*[31] of synthesis of class II DSA by infiltrating plasma cells cultured from nephrectomy specimens. Others have shown that a variety of

Table 1 | Banff criteria for chronic antibody-mediated rejection (CHR)[a]

Morphological features[b]
Duplication of glomerular basement membrane (cg1–3)
Multilaminated PTC basement membrane
Arterial intimal fibrosis without elastosis
Interstitial fibrosis with tubular atrophy with or without PTC loss
Diffuse C4d positivity along PTC
Presence of donor-specific antibody

Abbreviations: CHR, chronic humoral rejection; PTC, peritubular capillary.
[a]All three major criteria are required. These parallel those that are used in the Banff schema for acute antibody-mediated rejection. The presence or absence of graft dysfunction determines whether rejection is clinical or subclinical, as in other forms of rejection.
[b]Other morphological features commonly observed are aggregation of mononuclear inflammatory cells in PTC,[16] transplant glomerulitis,[19] and a plasma cell infiltrate in the interstitium.[24]

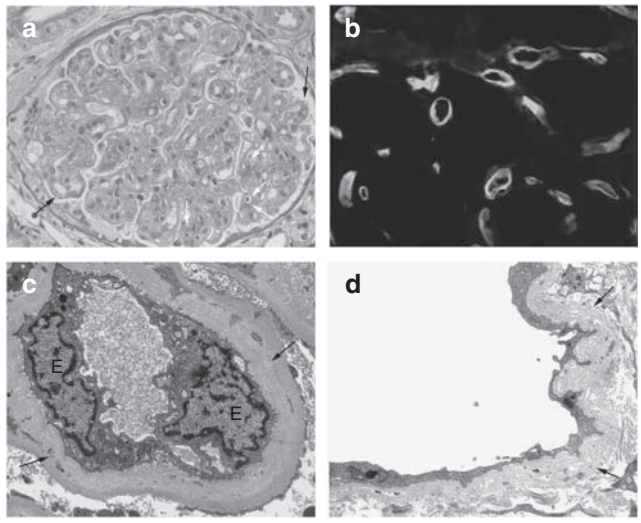

Figure 1 | Chronic antibody-mediated rejection. (**a**) Light microscopy of a case of transplant glomerulopathy shows a glomerulus with widespread duplication of the glomerular basement membrane (GBM) evident on periodic acid-Schiff stain (black arrows). Mononuclear cells are present in glomerular capillaries (glomerulitis) indicated with white arrows. (**b**) Prominent staining of peritubular capillaries for C4d is evident in a cryostat section of the same case. (**c**) Electron microscopy of another case shows reactive endothelial cells (E) that have lost their fenestrations and multilamination of the GBM (arrows). The original basement membrane is at the point of the arrows, the inner layers are newly formed. (**d**) Peritubular capillaries show similar multilamination (arrows) and loss of fenestrations.