B cells in renal transplantation: pathological aspects and therapeutic interventions

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Abstract
B cells are vital in renal transplantation. B2 cells are part of the adaptive immune system. Activated B cells mature into plasma cells or memory B cells: their life spans can be prolonged by niches. B cells have a wide variety of functions: antibody production, antigen presentation, cytokine production and shaping of the splenic architecture. These functions play a vital role in graft rejection, both T cell-mediated rejection and antibody-mediated rejection. Markers of B cell activity include intragraft B cell infiltration, C4d deposition and circulating donor-specific antibodies. Many therapeutic options target B cells or plasma cells. As greater understanding is gained of their appropriate use, and new agents are developed, we should see prolonged graft survival and reduced graft rejection.

Keywords: B-lymphocytes; Graft rejection; Humoral; Immunity

Introduction
For many years the predominant focus of research into both transplant rejection and autoimmune diseases has been T cells. Over the last decade, with the growing interest in humoral rejection, B cells have emerged as an important factor in renal transplantation. In this article we aim to review B cell development, their role in the mechanisms of rejection and the therapeutic options that exist to modify these effects.

B cell development
Two lineages of B cells exist: B1 cells are part of the innate immune system and develop during fetal and perinatal life; B2 cells are part of the adaptive immune system and develop during postnatal life.

B1 cells are self-renewing and form part of the ‘natural memory’ [1]; B2 cells develop initially from within the bone marrow: pluripotent haemopoietic stem cells develop into pro-myeloid lymphoid cells, which undergo maturation to eventually become pro-B cells, then pre-B cells. At this stage IgM is expressed, forming B cell receptors (BCRs) [2], and immature B cells are formed. These remain in the bone marrow for 1–3 days, then migrate to the spleen and differentiate, via a transient ‘transitional’ B cell phase, into follicular or marginal zone B cells [3] (see Figure 1).

B cells are activated by antigen stimulation. Those cells with high affinity for the antigen undergo extrafollicular differentiation into plasma cells; those with lower affinity enter germinal centres and undergo affinity maturation [4] via somatic hypermutation of the BCR (subsequently differentiating into either memory B cells or plasma cells). This ensures that the initial humoral response is mediated by those plasma cells with the greatest affinity, and therefore the highest chance of success, while those with weaker binding between the BCR and antigen modify their BCRs to ensure greater affinity. After activation, isotype switching occurs, producing immunoglobulin other than IgM, ‘providing antibodies with the same antigen specificity but distinct effector capacities’ [5].

B cell signalling
The generation of memory B cells and plasma cells from naïve precursors is primarily controlled by STAT3 (Signal Transducer and Activator of Transcription 3) in response to IL-21 and, to a lesser degree, IL-10 stimulation [8].

B cell survival is promoted by a number of factors, including BAFF (B cell-Activating Factor) and APRIL (A PRoliferation-Inducing Ligand). BAFF binds strongly to BAFF-R (BAFF Receptor) and TACI (Transmembrane Activator and CAML-Interactor), and weakly to BCMA (B Cell MAtruration). APRIL binds strongly to TACI and
BCMA, but not with BAFF-R at all. Binding to BAFF-R and BCMA promotes B cell survival; TACI activation can induce apoptotic signals; both BAFF-R and TACI are also responsible for isotype switching [6].

As well as BAFF-mediated pathways, functional BCR-mediated pathways are vital for maintenance of peripheral B cell homeostasis [7].

**B cell niches**

The life-span of B cells and plasma cells can be prolonged by niches. The distribution of these niches is still being investigated; it is recognized that plasma cell niches are found within bone marrow [9]—stromal cells expressing CXCL12 maintain long-lived plasma cells [10]. The number of these stromal cells limits the ‘memory plasma cell’ population, although they are sufficient to sustain a large number of specificities.

As well as these physiological niches, there is increasing evidence of the existence of tertiary lymphoid organs: ‘the progressive organization of chronic inflammatory infiltrates to form functional ectopic germinal centres’ [11] by lymphoid neogenesis. Inflammatory nodules can form in chronically inflamed tissue, and indeed in allografts, with T cells, B cells and plasmacytoid cells in close approximation to newly formed lymphatic vessels [12].

**Functions of B lineage cells**

The functions of B cells include [13]:

(i) Antibody production (indirectly, via maturation into the antibody-producing plasma cells)

(ii) Antigen presentation

(iii) Production of cytokines to trigger polarization of naïve T lymphocytes into Th1 or Th2 cells

(iv) Shaping of the splenic architecture (with lymphotoxin expression playing a vital role [14])

**Regulatory B cells**

The functions of B cells described above all lead to activation of the immune response; there is mounting evidence that specific strains of B cells can also act as negative regulators [15]. The mediator for this in mice is IL-10 production: this may also be important in humans [16]. This negative regulation may also affect the development of graft tolerance.

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Fig. 1. B cell development pathway. Originating from the pluripotent haemopoietic stem cell (PHSC). Affected cell types have been marked with the targets of drugs described in the text.
Mechanism of graft rejection

There are two main pathways of allore cognition [17]: direct and indirect. This occurs in the two ways by which T cells are activated after recognizing antigens from the graft. The ‘direct’ pathway involves intact donor Major Histocompatibility Complex (MHC) on the surface of donor antigen-presenting cells (APC) interacting with the T cell receptor (TCR) on recipient T cells to initiate T cell activation. This is possible because of the inherent ability for TCR to recognize intact allogeneic MHC. The ‘indirect’ pathway involves donor antigens being taken up by recipient APC, processed into peptidic fragments, and presented to recipient T cells in the grooves of recipient MHC. After transplantation of a renal graft, donor APCs—mainly dendritic cells—migrate from the graft and move towards the recipient’s secondary lymphoid organs. On the way, they will stimulate recipient T cells via the direct pathway. These will then migrate to the allograft and initiate an inflammatory reaction. Once donor APCs have been exhausted, recipient APCs maintain the immune response by processing and presenting antigens shed from the graft (the indirect pathway). B cells can act as these APCs [18] and have unique properties [19]:

(i) Due to their antigen-specific receptor, they can extract and present antigen even if membrane tethered or in minimal quantity.
(ii) They have the capacity for clonal expansion, so can become the numerically dominant APC.

Once the T cell response against the graft has been initiated, rejection mechanisms may be triggered—termed T cell-mediated rejection (TCMR) or antibody-mediated rejection (AMR). Both the cognate and non-cognate functions of B cells aid the TCMR response. B cell differentiation into plasma cells, with resultant antibody production, is responsible for AMR.

Antibody-mediated rejection

AMR is described as occurring in 5–7% of patients and 12–37% of biopsies taken for acute rejection [20].

The concept of AMR was formally recognized in the Banff criteria at the 2001 meeting [21] (reported in 2003), with three cardinal features:

(i) Morphologic evidence of acute tissue injury
(ii) Immunopathologic evidence for antibody action
(iii) Serologic evidence of circulating antibodies to donor HLA or other anti-donor endothelial antigens.

This was further sub-divided into acute and chronic AMR at the 2005 Banff meeting [22] (reported in 2007).

Markers of B cell activity in rejection

A number of indicators of B cell activity have been examined for correlation with rejection episodes: specifically B cell or plasma cell infiltration in graft biopsies, C4d deposition on peritubular capillaries (PTCs) and circulating donor-specific antibodies (DSAs).

B cell and plasma cell infiltration

Two distinct B-lineage cell types can be found in renal biopsies for acute rejection: CD38+ cells (plasmablasts and plasma cells) and CD20+ B cells. The appearance of CD38+ cells correlates with both the presence of circulating DSA and C4d staining in PTC [23]. In contrast, the presence of CD20+ graft infiltrates correlates with neither DSA nor C4d deposition.

Nevertheless, CD20+ lymphocytic infiltrates within biopsies from paediatric patients with episodes of rejection appear to correlate with increased graft loss [24]. Similarly, Hippen et al. [25] examined a separate group of patients with ACR and found the presence of CD20+ infiltrates to be significantly associated with both steroid-resistant rejection and graft loss within 4 years of follow-up. However, after only 1 year of follow-up, the presence of B cell infiltration was not associated with worsening of renal function [26].

C4d deposition

The diffuse deposition of C4d in renal transplants is considered to be a result of the antibody-mediated activation of the classical complement pathway. However, not all AMR involves C4d deposition: C4d-negative AMR has been described. Indeed, it has been claimed that two-thirds of AMR is C4d negative [27]. There is a significant association between C4d staining and BAFF expression in renal biopsies of patients with acute rejection [28], suggesting that in those patients with complement deposition, BAFF signalling (and therefore B cell activation) plays a role.

A retrospective study of 40 patients with acute rejection after at least a year following transplantation [29] found no significant difference in the rate of graft loss or renal function between those with C4d-positive biopsies and those with negative biopsies. (However, the study authors excluded all patients with multiple acute rejection episodes within the first year of transplant, who may well be expected to be more likely to have C4d deposition.) In a separate study, C4d deposits were found in 10/11 biopsies from kidneys with chronic transplant glomerulopathy, and only 2/13 controls [30]. In a study of 75 ABO-incompatible graft biopsies and 244 HLA-incompatible graft biopsies [31], it was suggested that C4d deposition does correlate with graft injury in HLA-incompatible grafts, but does not in ABO-incompatible grafts.

It is unclear exactly what benefit the detection of C4d deposition adds to the prediction of graft dysfunction. Reflecting this uncertainty, the 2007 update to the Banff criteria [32] (published in 2008) has included a new diagnosis of ‘C4d deposition without morphological evidence of active rejection’.

Circulating DSA

The presence of circulating DSA correlates strongly with the presence of transplant glomerulopathy, a histopathological appearance that is typical of chronic rejection. This has led to the belief that the majority of transplant glomerulopathy represents late AMR [33].
Sis et al. [34] found 33/47 patients with transplant glomerulopathy had circulating anti-HLA antibodies, 28 of which were donor specific. More specifically, de novo DSA appears to hold more danger for the graft: a similar longitudinal study [35] found that in those patients with identified de novo HLA antibodies (i.e. patients in whom no antibodies were found for 6 months post-transplant who subsequently developed antibodies), 6/11 grafts failed, as opposed to 4/22 who did not develop antibodies. In an acute episode of rejection, those patients with identified DSA have a significantly worse rate of 5-year graft survival than those without DSA (42 ± 16% versus 89 ± 6%) [36]. Moreover, those patients with DSA who were defined as being unsuccessfully treated (i.e. did not have a reduction in DSA of >50% within 14 days of treatment by whatever means) experienced a significantly worse rate of graft loss than those successfully treated (27.9 months median allograft survival compared with no grafts lost after >4 years).

**B cell therapeutics**

There are a number of therapeutic options available that modify B cell or plasma cell responses, many of which have been initially used in oncology or rheumatology before transfer to transplantation (see Table 1).

**Alemtuzumab (Campath)**

Alemtuzumab is a humanized anti-CD52 monoclonal antibody, which depletes both T and B cells. When given intraoperatively, it has been shown to deplete the B cell population almost completely [37]—the cells returned to baseline levels by Month 6. When used as an induction agent, it is effective at reducing TCMR [38]; much less is known about its effects on AMR.

**IVIG**

IVIG is a commercial preparation of IgG derived from pooled human plasma of 50 000–100 000 or more screened donors [39]. Jordan et al. [40] categorize the proposed mechanisms of action of IVIG on AMR as:

(i) modification of antibody levels
(ii) inhibition of cytokine gene activation and anti-cytokine activity
(iii) anti-T cell receptor activity
(iv) Fc receptor-mediated interactions with APCs to block T cell activation
(v) anti-CD4 activity
(vi) stimulation of cytokine receptor antagonists
(vii) inhibition of complement activity

*In vitro*, IVIG does not affect B cell proliferation, but does inhibit T cell proliferation: its effects on B cell function are proposed to be due to “indirect effects on T cells and/or interactions with circulating antibodies and complement factors” [41].

IVIG has been used both for reducing anti-HLA antibodies prior to transplantation and for the treatment of AMR.

For desensitization, the administration of IVIG in combination with rituximab has led to a significant reduction in panel reactive antibodies [42] (from 77 ± 18% to 44 ± 30%). Six of the patients in this study subsequently received a transplant from a deceased donor, waiting between 2 and 18 months after desensitization (having waited between 60 and 324 months beforehand).

Its effective use in AMR is in combination with other treatments since, in one study, using IVIG alone for AMR led to a 50% graft survival rate at 36 months post-rejection, as opposed to 91.7% using combination plasmapheresis, IVIG and rituximab [43]. A comparison of patients with AMR within the first year of transplant treated with plasmapheresis alone, or plasmapheresis with IVIG, showed a significantly higher rate of 1-year graft survival in the combination group (90.9 vs 30.8%) [44]. IVIG in combination with rituximab for AMR has led to a 1-year graft survival rate of 86% and a 2-year graft survival rate of 58% [45].

**ATG**

Anti-thymocyte globulin (ATG) has traditionally been viewed as a treatment for TCMR. There is some evidence that it also affects B cells and plasma cells. *In vitro*, it has a
been shown to induce apoptosis in these cells in a dose-dependent fashion (at concentrations between 1 and 1000 μg/mL) [46]. However, at a dose of 100 μg/mL (again in vitro), Perry et al. [47] found no evidence of plasma cell apoptosis. Nevertheless, ATG has been found to be effective at treating episodes of AMR in combination with plasmapheresis [48].

**Rituximab**

Rituximab is a chimeric anti-CD20 monoclonal antibody that rapidly depletes circulating B cells, but leaves plasma cells (and therefore antibody production) untouched [49] (as plasma cells do not express CD20). Once bound to the CD20 marker on the B cell, it causes its effects in at least three ways [50]:

(i) Activation of the complement cascade, leading to complement-mediated cytotoxicity,

(ii) macrophage recognition, leading to phagocytosis and antibody-dependent cell-mediated cytotoxicity (ADCC) and

(iii) natural killer cell interaction, also leading to ADCC.

**Rituximab on induction.** The timing of rituximab administration may be important for reasons that remain as yet unexplained. Although the drug causes reduction in B cell numbers within 1–3 days [51], it was first used in ABO-incompatible transplantation with a single dose of 375 mg/m² being given 2–4 weeks before immunoadsorption, followed by tacrolimus, MMF and prednisolone. This protocol has shown equivalent results to ABO-compatible transplantation [52]. The same group has recently published the results of a randomized controlled trial [53] comparing a single dose of rituximab with placebo, given within 24 h before revascularization of the donor (ABO compatible) kidney. They found a non-statistically significant decrease in episodes of rejection: based on results from their ABO-incompatible donor programme [52], they were expecting a greater (significant) decrease, and ascribed this to the difference in timing of rituximab administration.

There is currently an ongoing randomized controlled trial of rituximab administered during transplant surgery. Van den Hoogen and Hilbrands report their early results in a letter to the *New England Journal of Medicine* [54]. Their analysis of the first 65 patients showed a relative risk of acute rejection in the rituximab group of 0.53 (95% CI 0.21–1.32).

The use of rituximab at induction has not always been successful. Clatworthy et al. [55] have reported the early results of a trial comparing rituximab with daclizumab (an anti-CD25 monoclonal antibody) as induction therapy in patients undergoing renal transplantation. This study was stopped after the recruitment of 13 patients because five out of six patients in the rituximab group were found to have acute rejection on renal biopsy within the first 3 months. The rituximab group appear to have been under-immunosuppressed: they did not receive daclizumab induction and were steroid-free after induction (as were the control group). As above, rituximab was given at induction (rather than 2–4 weeks prior to transplantation).

**Rituximab for acute rejection.** A retrospective review of renal transplant recipients with AMR [56] compared 26 patients treated with plasmapheresis and rituximab with 28 treated with plasmapheresis alone. The 2-year graft survival for the rituximab group was 90% compared with 60% in the plasmapheresis alone group, although both groups showed evidence of renal impairment post-treatment. This promising effect of rituximab is perhaps unexpected—plasma cells should not be affected directly by rituximab administration, which leads to the hypothesis that the other functions of B cells (particularly antigen presentation and cytokine production) are vital in the development of AMR.

**Rituximab for chronic rejection.** A study of 87 paediatric renal transplant recipients [57] found six developed CAMR—all with C4d deposition. They were treated with four weekly doses of IVIG followed by a single dose of rituximab: four out of six patients experienced stabilization or improvement of their GFR after treatment; one patient did not respond and the last had stabilization of GFR but then lost the graft from progressive chronic rejection. These last two were found to have the highest degree of transplant glomerulopathy and the highest degree of C4d deposition in peritubular capillaries.

Two other reports have described the successful use of rituximab for CAMR in adult renal transplant recipients. In the first [58], four patients with progressive graft dysfunction in the presence of DSA and histological features consistent with CAMR received rituximab with IVIG and all four showed improvement in graft function. In the second [59], 7/14 patients with transplant glomerulopathy who received rituximab showed improvement in graft function at a median of 30 months post-treatment. Importantly, 4/14 patients experienced severe infection, in the context of profound B cell lymphopenia.

In the UK, a randomized controlled trial (the RituxiCAN-C4 trial) is currently recruiting patients to determine the efficacy and side effect profile of rituximab in this patient group.

**Rituximab and intragraft CD20+ cells.** Rituximab certainly reduces the circulating B cell population; it also affects intragraft B cells. When given at induction, Genberg et al. [51] found that 25/26 renal biopsies taken from patients who received rituximab were negative for CD20+ cells, compared with 3/24 who did not receive rituximab. Similar results have been found in treatment for biopsy-proven vascular rejection [60].

Unsuccessful treatment with rituximab may correlate with a lack of reduction in intragraft B cells: a study of two transplant patients unsuccessfully treated with rituximab for CAMR [61] found persistent B cell nodules within the graft, albeit with lower levels of intragraft B cells than patients who did not receive rituximab.

**BAFF expression with rituximab.** As discussed above, BAFF is one of the key mediators of B cell survival. Twelve weeks after rituximab administration, there is a significant increase in BAFF levels due to two separate mechanisms [62]. The first is due to the reduction in B cells. The result-
ant decrease in BAFF-R numbers frees BAFF into the circulation. The second is due to increased BAFF production, following transcription regulation. In one paper, BAFF was found to be overexpressed in two chronically rejected grafts: the inflammatory cells infiltrating the graft were the source [61].

**Side effects of rituximab.** Rituximab appears to be a generally safe drug, with minor effects arising from administration (such as a cytokine release syndrome [63]). However, a recent paper [64] has raised the issue of infectious complications: although the overall rate of infection was similar in transplant patients treated with rituximab to those not treated, those that did develop infections had a significantly higher rate of infection-related death (9.09% compared with 1.55%). From the data presented, it is difficult to ascribe this difference to rituximab in whole or part, as there are a number of problems with the study samples, including previous adjunctive treatments such as ATG and the use of historical controls [65].

Thaunat et al. [19] have suggested that rituximab (and, in fact, all B cell depletion therapies) could cause a ‘paradoxical immune stimulatory effect’: that depending on the time of administration, it can have a deleterious rather than beneficial effect due to the depletion of regulatory B cells mentioned above. This theory offers another explanation for the increased rejection seen in Clatworthy et al.’s trial [55].

**Bortezomib**

Bortezomib, a proteasome inhibitor, has been used successfully in the treatment of multiple myeloma. It promotes apoptosis of plasma cells (which are not targeted by any current therapy), therefore preventing antibody secretion. The proteasome is responsible for protein processing and degradation in mitosis, and the disruption of this process leads to activation of the unfolded protein response and subsequent apoptosis [66]. The fact that protein synthesis is extremely active in plasma cells (they secrete thousands of IgG molecules each second) is likely to make them more susceptible to proteasome inhibition than other cells [47].

**Bortezomib for DSA reduction.** Studies of bortezomib use in renal transplantation are in the early stages. Bortezomib has been used in an attempt to desensitize patients with DSA: antibody titres are not reduced to a level suitable for transplantation. However, it has been shown to decrease donor-specific plasma cells and memory B cells [67] and, separately, anti-HLA antibody-triggered C4d deposition [68].

Trivedi et al. [69] examined the use of bortezomib and plasmapheresis in 11 live donor transplant recipients with anti-HLA antibody elevation in the absence of acute rejection, following the use of a stimulation/deletion regimen. They found a reduction in anti-HLA Abs in 9/11 patients.

**Bortezomib for acute rejection.** A further case series examined the use of bortezomib in six patients with mixed ACR and AMR [70]. There was a reduction in DSA levels and reversal of rejection episodes (based on renal biopsy results and improvement in eGFR), although two patients developed early chronic rejection 1–2 weeks post-bortezomib therapy. The same group has subsequently used bortezomib for early acute AMR, in combination with plasmapheresis and rituximab, with encouraging results [71]. Used on its own, for subacute AMR, bortezomib had no effect on DSA levels in four patients [72].

**Splenectomy**

Splenectomy has been used pre-emptively in ABO-incompatible transplantation, for example by Sawada et al. [73], although it now seems that replacing splenectomy with anti-CD20 therapy is at least as effective in preventing AMR [74] and avoids the operative side effects.

Splenectomy may be important in the treatment of AMR post-transplant, although only case series have been published on this to date. Locke et al. [75] report on five patients who developed acute severe AMR after HLA-incompatible renal transplantation, treated with open splenectomy after plasmapheresis and IVIG. All five showed improvement in renal function, DSA levels and C4d deposition post-splenectomy. Similarly, Kaplan et al. [76] performed laparoscopic splenectomy on four patients with AMR following unsuccessful treatment with plasmapheresis and IVIG, leading to a post-operative improvement in renal function. However, all four patients received a dose of rituximab the day before surgery, so the relative contribution of true and ‘chemical’ splenectomy cannot be differentiated.

As has been discussed previously, the spleen is not the only repository of memory B cells and plasma cells; bone marrow and inflammatory niches play a significant part, so the exact mechanism of the effectiveness of splenectomy is unclear—it may relate to debulking, i.e. removing a large proportion of the active B cell population.

**Atacicept**

Atacicept (TACI-Ig) blocks B cell stimulation by both BAFF and APRIL. It has been used in autoimmune disease [77] and B cell malignancies [78], but not in transplantation as yet.

**Conclusion**

B cells play many roles in the development of graft rejection — not only plasma cell development and production of antibody but also via cognate interactions with T cells and non-cognate functions. T cells and B cells have traditionally been viewed as operating in isolation, the former responsible for TCMR and the latter for AMR. It is likely that, rather than being discrete mechanisms of rejection, these two form ends of a spectrum, with cells of the immune response working together in ways that are only now becoming understood. The realization of the importance of B cells opens up whole new fields of research, such as the importance of B cell niches and tertiary lymph-

oid organs. As we gain greater understanding of various biomarkers of B cell activation (such as CD20+ cell infiltration, C4d deposition and circulating DSA), we will be able to more effectively, target new therapies and new methods of using existing therapies, to prolong graft survival and prevent graft rejection.

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