TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

REVIEW

B-cell regulation and its application to transplantation

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Keywords
alloantibody, antibody-mediated rejection, B cells, BAFF, regulatory B cells, rituximab.

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Conflicts of interest
The author has no conflict of interest to disclose.

Received: 9 February 2013
Revision requested: 8 April 2013
Accepted: 8 July 2013
Published online: 4 August 2013

doi:10.1111/tri.12160

Summary

There has been increasing interest in the role played by B cells and their associated antibody in the immune response to an allograft, driven by the need to undertake antibody-incompatible transplantation and evidence suggesting that B cells play a role in acute T-cell-mediated rejection and in acute and chronic antibody-mediated rejection. This review focuses on the molecular events, both activating and inhibitory, which control B-cell activation, and considers how this information might inform therapeutic strategies. Potential targets include the BAFF (B-cell-activating factor belonging to the tumour necrosis factor family) and CD40-CD40L pathways and inhibitory molecules, such as CD22 and FcγRIIB. B cells can also play an immunomodulatory role via interleukin (IL)10 production and may contribute to transplant tolerance. The expansion of allograft-specific IL10-producing B cells may be an additional therapeutic goal. Thus, the treatment paradigm required in transplantation has shifted from that of simple B-cell depletion, to that of a more subtle, differential manipulation of different B-cell subsets.

Introduction – Some B cell basics

B cells are cells of the lymphoid lineage characterized by the surface expression of antibody/immunoglobulin (Ig) as the B-cell receptor (BCR). They also variably express a range of other surface markers at different times during their development, for example, CD20, CD19, CD27 and CD32B (FcγRIIB), which frequently reflect adaptations to facilitate their function or maturation. The most obvious and well-studied function of B cells is as precursors to antibody-producing plasma cells. The lineage provides antibodies reactive not only to protein and peptide antigens (T-dependent antigens) but also (in contrast to T-cell responses) to carbohydrate/polysaccharide antigens (T-independent antigens). B cells have additional functions in promoting an immune response, including T-cell activation via antigen presentation [1,2] and the orchestration of the microarchitecture of secondary lymphoid organs and mucosal-associated lymphoid tissue [3–5]. More recent data also suggest a potential role for some B cells in enhancing innate immune responses [6].

B cells in transplantation – why do we care?

In recent years, there has been increasing interest in how B cells, plasma cells and their associated antibody respond to allografts [7]. This focus was initially driven by a very pragmatic issue, that of organ shortage, which prompted a re-exploration of the feasibility of antibody-incompatible transplantation. In addition to the challenges to short- and long-term graft survival encountered when transplanting individuals with pre-formed ABO or human leucocyte antigen (HLA) antibodies [8], it is now well-established that the appearance of de-novo donor-specific antibodies (DSA) is frequently associated with acute antibody-mediated rejection (AMR) and its deleterious effects on the transplant [9]. Such antibodies, and indeed some non-HLA antibodies, may also contribute to the long-term allograft attrition, in the guise of chronic AMR [10]. Outside of their remit of antibody production, there is an appreciation that B cells may play a role in acute cellular rejection, also known as T-cell-mediated rejection (TCMR). This concept is supported by a number of lines of evidence, including

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the fact that patients with pre-formed DSA have an increased frequency of TCMR compared with nonsensitized patients [11], and the observation that the presence of B cells within allografts with TCMR can portend a worse prognosis in some patients [12–14].

In response to this heightened awareness of the deleterious effects of B cells and antibody on allograft outcome, a number of B-cell-targeted immunosuppressive agents have been utilized in transplantation. However, there is now a growing body of evidence demonstrating that B cells can not only act as immune effectors but may also negatively regulate or modulate immune responses [15,16], rendering approaches dependent on B-cell depletion inadequate. In support of the importance of the immunomodulatory potential of B cells are studies showing a B-cell signature in microarray studies of tolerant transplant recipients [17,18] and an upregulation of B-cell biomarkers in rejection-free transplant recipients [19]. Thus, the therapeutic paradigm required in transplantation has shifted from that of simple B-cell depletion, to that of a subtler, variable manipulation of different B-cell subsets.

B-cell subsets

The B-cell lineage contains two broad types of cell, B1 and B2 cells with differing function, location and surface markers. B1 cells reside principally in the peritoneal and pleural cavities [20,21]. B1 cells produce low-affinity, polyreactive, natural antibodies to T-independent antigens as a first line of defence. Recent data suggest that, in contrast to B2 cells, B1 cells also have phagocytic and microbiocidal activity [22] strengthening the view of these cells as both innate and adaptive players. The natural antibodies produced by these cells include those which are protective in atherosclerosis [23], which may be of relevance to chronic vasculopathy in transplantation.

B2 cells are formed in the bone marrow, released as immature B cells, and circulate through secondary lymphoid organs (spleen and lymph nodes) as naïve B cells (expressing IgM and IgD). There are two distinct populations of B2 cells, follicular B cells and marginal zone B cells, which are responsible for T-dependent and T-independent responses, respectively. Marginal zone B cells (as their name suggests) are located within the marginal zone of the spleen, an area specifically adapted to filter blood-borne antigens. In humans, marginal zone B cells express CD21(CR2) and the memory marker CD27 [24].

It has recently been appreciated that sub-populations of B cells may act as immunoregulatory cells. These regulatory B cells were first described in the mouse and subsequently in humans [15,16,25,26] and mediate their immune inhibitory function via the production of interleukin (IL)-10, leading some authors to describe them as B10 cells [15]. In mice, several groups have identified IL-10 producing B cells within a number of B-cell compartments (Fig. 1). Ding *et al.* demonstrated that T-cell Ig domain and mucin domain (TIM)-1 is expressed by most IL-10-producing murine B cells across all subsets [27]. IL-10-producing B cells have also been identified in humans and comprise around 5% of circulating B cells, although cells with the potential to produce IL-10 may be found at higher frequencies. Human IL-10-producing B cells are characterized by surface expression of CD24, CD38, CD5 and CD1d, as well as the memory marker CD27 (like human marginal zone B cells) in some reports [25,26]. This discrepancy in CD27 expression between reports may be explained by recent data showing that both $CD24^{hi}38^{hi}$ transitional B cells and $CD24^{hI}27^{+}$ marginal zone-like cells are capable of IL-10 production. Of note, the former produced more IL10 on stimulation *ex vivo* and were more effective in inhibiting T cells *in vitro*, suggesting that B cells with more potent regulatory potential reside within this more naïve compartment [28].

B-cell activation

When B cells encounter an antigen that binds their BCR, they may become activated. The outcome of this interaction depends on the affinity of the BCR for the antigen, but also on the environmental context, which provides additional co-activating or inhibitory signals to B cells (Fig. 2). The BCR (surface Ig) forms the antigen recognition part of the BCR complex, but has no intrinsic signalling capacity. Thus, it must associate with Igα (CD79a) and Igβ (CD79b), which mediate signalling by virtue of immunoreceptor tyrosine-based activation motifs located within their cytoplasmic domains [29,30]. Following BCR aggregation, these tyrosines are phosphorylated, initiating a signalling cascade which includes the generation of signalling intermediates such as phosphatidylinositol-(3,4,5)-trisphosphate (PIP3), via the action of phosphatidylinositol-3 kinase (PI3K) [31]. The B cell expresses additional co-receptors that can lower the threshold for B-cell activation. CD19 is one such co-receptor that has a number of tyrosines within its cytoplasmic domain that may also be phosphorylated by src tyrosine kinases, and subsequently act as docking sites for SH2-containing proteins such as PI3K [32]. B-cell activation can also be facilitated by the presence of complement components (C3b and C3d binding to CD21/CR2). CD21 amplifies BCR signalling through its association with CD19 and its augmentation of PI3K-dependent signalling [33,34]. B cells also express pathogen pattern recognition receptors, in humans toll-like receptors (TLR) 1, 2, 6, 7, 9 and 10 [35]. Pasare and Medzhitov showed that in murine B cells, TLR signalling was required, in addition to CD4 T cell help, for B-cell activation and

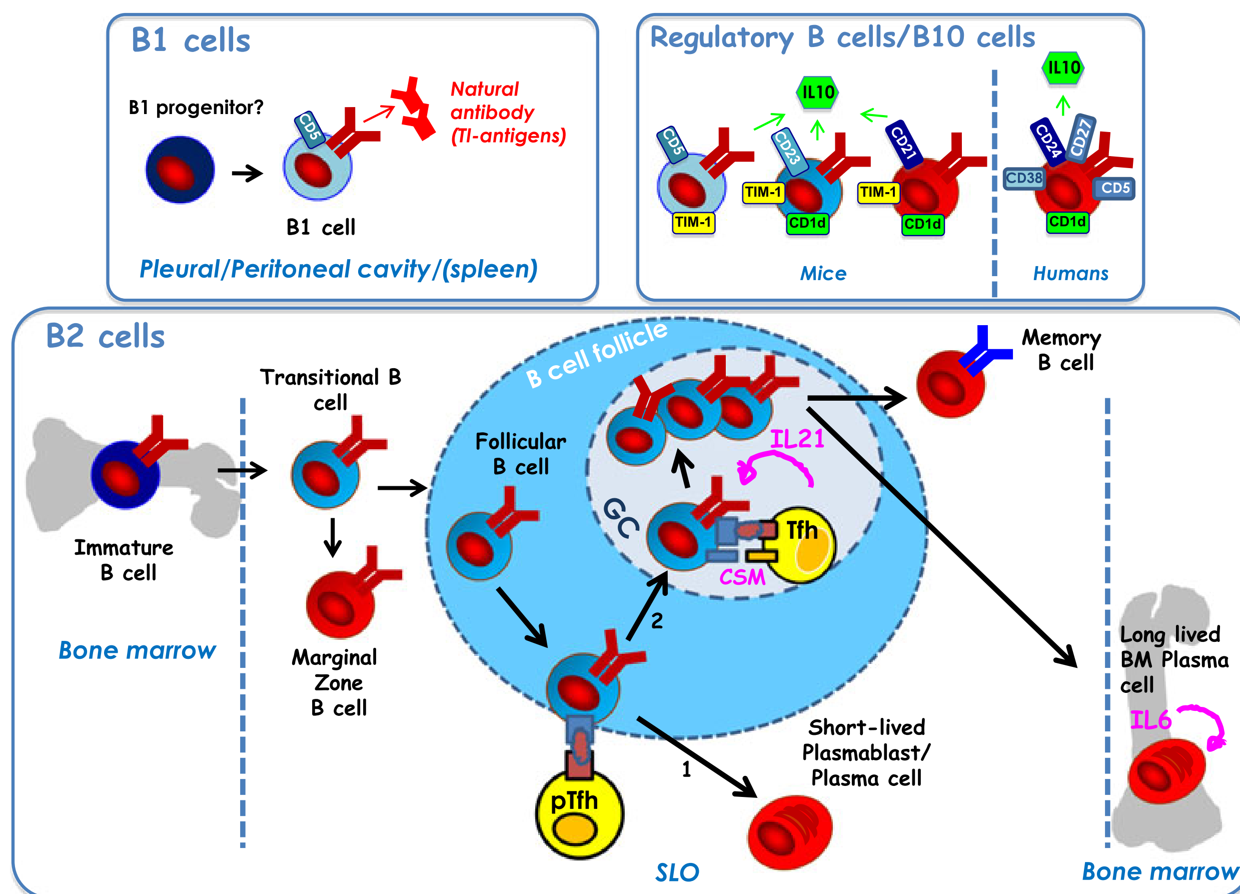

Figure 1 B-cell subsets. B1 cells reside within pleural and peritoneal cavities (and in smaller numbers in the spleen). They produce low-affinity natural antibody to T-independent antigens. B2 cells are formed in the bone marrow and develop from pro-B cells, to pre-B cells, through to immature B cells which are released into the periphery. Following antigen encounter follicular B cells move to the T–B border within secondary lymphoid organs (SLO) and present antigen to a cognate CD4 T cell, known as a pre-T follicular helper cell (pTfh). Following this interaction, the fate of the B cell lies between two pathways, either (1) to become a short-lived extrafollicular plasmblast or (2) to enter the germinal centre, undergo rounds of somatic hypermutation and selection to generate class switched memory B cells or plasma cells. This process requires the presence of IL-21-producing T follicular helper cells (Tfh). The interactions between Tfh and B cells also requires costimulatory molecule (CSM) interactions including CD40L/CD40, ICOS/ICOS-L, and CD28/CD86. Some B cells have the capacity to produce the immunoregulatory cytokine IL10, and have been dubbed 'regulatory B cells' or 'B10 cells'.

antibody production *in vivo* in response to T-dependent antigens [36]. B cells may also receive pro-activation and survival signals via cytokines, including BAFF (B-cell-activating factor belonging to the tumour necrosis factor family, also known as BLys, TALL-1, and THANK) [37,38]. BAFF exists in both membrane-bound and soluble forms and is produced by monocytes, macrophages and dendritic cells. There are three BAFF receptors; BAFF-R (also known as BR3), TACI (transmembrane activator and calcium modulator and cyclophyllin ligand interactor) and BCMA (B-cell-maturation antigen). These are principally expressed on follicular, germinal centre and memory B cells, but BCMA is preferentially expressed on plasma cells whilst BAFF-R is also expressed on activated T cells and regulatory T cells [38]. Over-expression of BAFF is sufficient to drive abnormal B-cell survival, hypergammaglobulinaemia and a lupus-like autoimmune disease in mice which occurs independent of T cell help but requires TLR signalling [39].

If the BCR aggregation is sufficient to induce signalling, the B cell internalizes bound antigen, processes it and presents in the context of surface major histocompatibility complex (MHC) class II molecules. At the border between the B-cell follicle and T cell zone of secondary lymphoid organs cells, the B-cell MHC-peptide complex may be recognized by a cognate T cell via its T-cell receptor [40]. B cells are important antigen-presenting cells, because of their ability to clonally expand, and efficiently take up antigen via their BCR [41]. B cells can also produce cytokines which support T cells [42] (Fig. 2). Hence, B cells are critical for optimal T-cell activation [43] and memory [44] in alloimmune responses.

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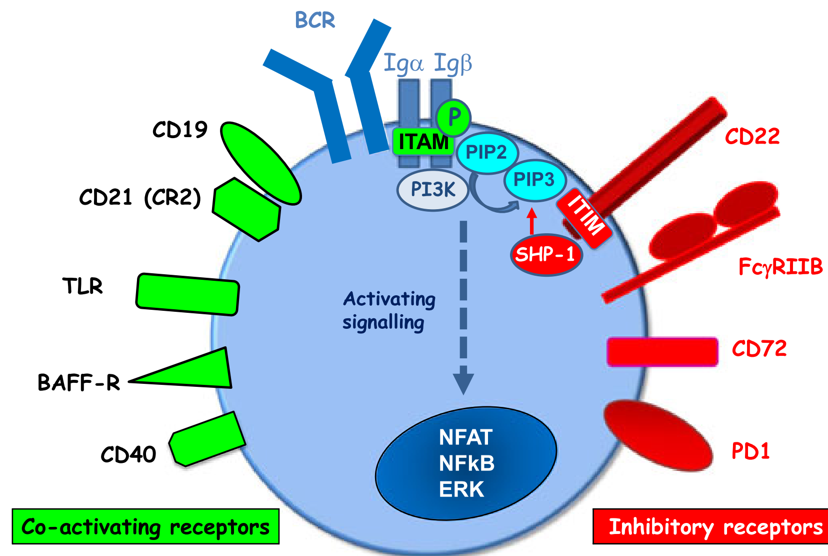

Figure 2 B-cell activation and signalling. B cells recognize antigen via surface Ig (B-cell receptor, BCR) but signal via Igα and Igβ which have immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic domains. Following BCR aggregation, the ITAM tyrosines are phosphorylated by src kinases, initiating a signalling cascade which includes the generation of signalling intermediates such as phosphatidylinositol-(3,4,5)-trisphosphate (PIP3), via the action of phosphatidylinositol-3 kinase (PI3K). The B cell expresses additional co-receptors that can lower the threshold for B-cell activation, including CD19 and CD21. CD19 augments signals delivered through the BCR by lowering the signalling threshold for B-cell activation. It also has tyrosine residues in its cytoplasmic domain, which once phosphorylated, act as a docking site for several proteins, including PI3K. Additional activating signals come from toll-like receptors (TLR) and via the cytokine BAFF. BCR signalling is also negatively regulated by the src kinase Lyn through its ability to phosphorylate ITIMs (tyrosine-based inhibitory motifs) in inhibitory cell surface receptors such as CD22 and FcγRIIB. This allows the recuitment of phosphatases such as SHP-1, which breakdown PIP3, terminating activatory signalling.

Activated B cells may form extrafollicular plasmablasts, which are short-lived (7–14 days) and produce early, non-mutated antibody with specificities encoded in the primary repertoire [45]. In mice, this extrafollicular antibody response requires the presence of Bcl-6-expressing CD4 T cells [46]. Inappropriate, prolonged survival of extra-follicular plasmablasts contributes to autoantibody production in some murine models [47]. In humans, these cells may be responsible for early alloantibody in *de novo* responses, but their role in long-term alloantibody production is less clear. The importance of this distinction lies in the fact that these plasmablasts express different surface molecules to bone marrow plasma cells (e.g., CD19) and may therefore be more susceptible to targeted therapies.

The alterative fate for B cells, given an adequate T–B interaction, is to return to the follicle to form the germinal centre, where they undergo rounds of somatic hypermutation and class switch recombination. Here, B cells with a higher affinity for antigen are positively selected and differentiate into either memory B cells or plasma cells [48]. Recent studies have demonstrated that a subset of CD4 T cells located within B-cell follicles and characterized by expression of the transcription repressor Bcl-6 [known as T follicular helper (Tfh) cells] are essential for the development of germinal centre B cells [49,50]. Germinal centre formation may be reduced by inhibiting Tfh-B-cell interactions, for example, by ICOS-ICOSL, CD40-CD40L, SAP-SLAM or IL21 blockade. Specific inhibition of Tfh cells may represent a possible strategy in future attempts to inhibit humoral alloimmunity. A small proportion of plasma cells arising from the germinal centre become established as long-lived plasma cells in the bone marrow. They reside within a number of limited niches, do not proliferate, but act as long-term antibody factories, producing IgG. Plasma cells have also been described in inflamed tissues in autoimmunity and within allografts [51–53], suggesting that inflammatory lesions can provide additional niches for plasma cells. Furthermore, tertiary lymphoid organs have been observed in animal models of transplantation [51] and in human renal and cardiac allografts [51–53]. B cells can produce lymphotoxin-β and VEGF-A, and may therefore play a role in orchestrating the development of these structures within allografts [54].

Controlling B-cell activation

As detailed above, the B cell expresses a number of co-activating receptors which augment BCR-mediated activation. Given the potent and widespread effects of B-cell activation, it is critical that there are also stringent control measures to prevent inappropriate B-cell responses. To this end, the B cell also expresses a number of inhibitory

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receptors, for example FcγRIIB, CD22, CD72 and PIR-B [55]. These receptors are characterized by the presence of ITIMs (immunoreceptor tyrosine inhibitory motifs) within their cytoplasmic domain. Ligation of an inhibitory receptor results in phosphorylation of the ITIM tyrosine and the recruitment of a phosphatase (SHIP, SHP-1 or SHP-2). The phosphatase subsequently dephosphorylates activatory intermediaries, such as PIP3, thus negatively regulating B-cell activation.

FcγRIIB is the sole inhibitory member of the FcγR family and binds the Fc portion of IgG. It is expressed on B cells as well as on plasmablasts and long-lived plasma cells. FcγRIIB is co-ligated with the BCR in the presence of IgG-opsonised antigen and is therefore important in terminating GC responses once sufficient antigen-specific IgG has been generated [56]. In addition, *in vitro*, immune complex cross-linking of FcγRIIB on plasma cells mediates apoptosis [57]. Manipulation of this receptor in mouse models emphasizes its importance in immune modulation; FcγRIIB-deficient mice are prone to antibody-associated autoimmune disease [58,59] but are protected from some infections [60,61]. In contrast, transgenic over-expression of FcγRIIB on B cells results in reduced autoimmunity [62]. In humans, a single nucleotide polymorphism, rs1050501, has been identified in the *FCGR2B* gene which is associated with receptor dysfunction [63,64] and is found at increased frequency in patients with systemic lupus erythematosus [65].

CD22 is a glycoprotein of the Ig superfamily in the sialoadhesin subclass. The ligand for CD22 is Siaα26Galβ1–4GlcNAc, a glycosylated sialic acid residue expressed at high levels on lymphocytes and inflamed endothelial cells [66]. CD22 is constitutively associated with the BCR providing a level of tonic inhibition to signalling. Coligation of CD22 to the BCR reduces B-cell activation while sequestering CD22 away from the BCR, as would occur if CD22 bound its ligand on adjacent cells, results in B-cell hyperactivity. CD22-deficient mice have an expanded B1 cell population, increased serum IgM, and their B cells are hyper-responsive to stimulation through the BCR. With ageing, they develop high-affinity isotype-switched autoantibodies but not overt autoimmune disease [67].

Moving from basic B-cell biology to the bedside

Can these studies of basic B-cell biology (many of them in mice) inform our search for novel therapeutics to modulate B cells in transplantation? Possible strategies to target B cells include the following:

i B-cell depletion
ii Reducing or blocking effector B-cell activation
iii Enhancing the generation of regulatory B cells
iv Depleting plasma cells or limiting their production of antibody
v Removing or blocking the effector function of antibody

A number of recent reviews have discussed strategies (iv) and (v) [7,68,69], which include proteosome inhibition with bortezomib, the blockade of antibody-induced complement activation with the C5a monoclonal antibody eculizumab [70–72], or the use of intravenous immunoglobulin (IVIG) to modulate effector cell responses to antibody [73,74]. I will therefore focus on strategies (i)–(iii).

(i) B-cell depletion

The most basic strategy to target B cells is to deplete them. This has largely been achieved through splenectomy or via the administration of cytotoxic antibodies which bind antigens expressed on the surface of B cells. Agents currently used for B-cell depletion are the anti-CD52 antibody, alemtuzumab (CAMPATH-1H), anti-thymocyte globulin (both of which deplete T cells in addition to B cells), and the anti-CD20 antibody rituximab. Rituximab is a chimeric murine-human monoclonal antibody directed against the B-cell surface molecule CD20. CD20 is not found on pro-B cells or mature plasma cells (the latter produce 90% of circulating IgG), thus, rituximab eliminates circulating B cells without preventing the regeneration of B cells from precursors, and does not directly affect Ig levels. A number of reports also suggest that rituximab may deplete B cell aggregates within allografts [75], although not those residing in lymph nodes [76]. Despite a lack of effect against long-lived plasma cells, and therefore serum IgG, some [77,78] (but not all [75]) groups have reported that ritxuimab treatment is associated with a reduction in DSA titres. This may indicate that the alloantibody-producing cell in such cases is not a long-lived plasma cell and continues to express CD20, but more likely reflects the effects of high dose IVIG which was used concomitantly with rituximab in some of these studies [77,78].

Rituximab has been used to good effect as part of a desensitization strategy in ABO-incompatible transplantation [79–81]. These studies confirm a reasonably good safety profile, with no excess of infection or other adverse events such as TCMR [82]. Rituximab has also been utilized for the treatment of AMR (reviewed in [75]) although a recent randomized controlled trial (RITUX ERAH) from Lebranchu and colleagues suggests no additional benefit when added to a regimen of plasmapheresis, IVIG, and corticosteroids [83]. Rituximab has also been used in conjunction with IVIG for the prevention of AMR [78,84]. In such patients, rituximab-mediated depletion may well prevent the generation of additional alloantibody producing cells from the naive B-cell pool, and may also target short-lived

plasma cells still expressing CD20. More recently, there has been interest in the use of rituximab for the treatment of chronic AMR in renal transplantation [75,77,85], and a randomized control trial is currently on-going in the UK to test its efficacy in this context (http://clinicaltrials.gov/ct2/show/ NCT00476164).

Data linking B cells with TCMR, as well as acute and chronic AMR have prompted the use of rituximab as an induction agent in transplantation in nonsensitized patients. Three randomized controlled trials have been undertaken for this indication [86–88]. Tyden et al. used a single dose of rituximab in combination with steroids, tacrolimus and mycophenolate mofetil and showed a reasonable rate of TCMR (11.6% at 6 months compared with 17.6% in the control group) [86]. Longer term (3 year) follow-up demonstrated no significant difference in AMR or TCMR, nor in the development of DSA [89]. We undertook a similar trial, but were forced to halt recruitment, because of an excess rate of TCMR in the rituximab group (83% vs. 14% in the control group) [87]. In contrast to the study by Tyden *et al.*, we used two doses of rituximab, at day 0 and day 7 and did not use corticosteroids for maintenance. The explanation for the observed differences may be that the timing of B-cell depletion is critical in determining whether regulatory or effector B cells are targeted and that regulatory B cells were inadvertently depleted. This study emphasizes the concept that B cells may play an important role in modulating the immune response to an allograft, and that pan-B cell depletion is therefore a potentially risky strategy. Recent data showing accelerated allograft rejection, and enhanced CD4 T-cell activation in a skin graft model following CD20 antibody-mediated B-cell depletion supports such a view [90]. In addition to the removing the direct beneficial effects of regulatory B cells on an alloimmune response, B-cell depletion may also negate the indirect effect of B cells in expanding regulatory T cells (reviewed in [42]). The effects of B-cell depletion on immune regulatory cells is likely to explain the less well-publicized, paradoxical, exacerbation of existing autoimmunity or the occurrence of new autoimmune disease in patients treated with rituximab [91]. The results of the third randomized controlled trial, the largest to date, were presented by Hilbrands and colleagues at the recent American Transplant Congress [88]. They randomized 280 renal transplant recipients to receive a single dose of rituximab or placebo at induction followed by tacrolimus, mycophenolate and corticosteroids (in a similar regimen to that employed by Tyden et al.). Overall, there was a similar rate of biopsy-proven acute rejection between groups (15.9% in those receiving rituximab, vs. 21.8% in the placebo group, $P = 0.15$). However, when excluding low risk patients and analyzing a subset of patients who were sensitized pretransplant (panel reactive antibodies of >5% or re-transplants), rituximab significantly reduced the incidence of acute rejection (17.9% vs. 41.1%, $P = 0.039$). These data support the notion that B cells make a significant contribution to TCMR.

Other B-cell depleting agents which may be useful in transplantation include a potentially more potent anti-CD20 antibody, ofatumumab [92] and anti-CD19 monoclonal antibodies [93], the latter showing promise in the treatment of some autoimmune diseases. CD19 is expressed earlier in the B-cell lineage than CD20, and in memory B cells, short-lived plasma cells and B1 cells, thus, CD19-targeted therapy has the advantage of depleting additional B-cell subsets, including 50% of murine bone-marrow resident, long-lived plasma cells [90,93]. Thus, in contrast to CD20 antibody treatment, a single treatment with a CD19 monoclonal antibody, resulted in a reduction in circulating allograft-specific IgG in an acute cardiac allograft model [90]. Furthermore, in a murine model of renal chronic allograft rejection (DBA/2 to C57Bl/6), B-cell depletion using a CD19 antibody significantly enhanced allograft survival, was associated with a reduction in IgM and IgG alloantibody titres, and was shown to reduce alloantibody titres in sensitized animals [90]. As recent data suggest that rituximab fails to deplete lymph node-resident, CD27+ (memory marker) B cells [76], it would be of interest to investigate how well CD19 antibodies targets such cells.

One potential way to increase the efficacy and enhance the specificity of B-cell-depleting antibodies is to utilize bispecific antibodies, in which each arm of the variable component is engineered to recognize a different antigen. Such agents have been developed in haematoncology for the treatment of B-cell malignancies and include CD20–CD22 antibodies [94] and CD19–CD22 antibodies [95]. This technology may have future utility in transplantation, providing a means to deplete specific subsets of B cells.

(ii) Modulation of B-cell activation and survival

Antigen-specific activation of B cells via the BCR can be modulated by the engagement of co-stimulatory receptors, such as CD19 and CD21 (CR2), or B-cell inhibitory receptors, such as FcγRIIB and CD22. Therefore, inhibiting co-activators or inducing inhibitory molecules may potentially be sufficient to prevent the initial steps of B-cell activation. One attractive target in this regard is the BAFF pathway, and is supported by animal models of transplantation. In a murine cardiac allograft model, BAFF-deficient recipients had extended transplant survival [96] whilst in an islet allograft model, BAFF blockade with a monoclonal antibody (in combination with rapamycin at induction) resulted in long-term survival of MHC-disparate allografts [97]. In humans, BAFF has been identified by immunohistochemistry in renal transplant biopsies with acute rejection [98]

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and elevated BAFF mRNA and protein [99,100] and BAFF-R positive B and T cells have been noted in renal allografts with chronic rejection [96]. More recently, Thibault-Espitia *et al.* reported that patients with higher circulating soluble BAFF had a significantly greater risk of developing DSA, and that in patients with stable graft function, high BAFF-R mRNA in peripheral blood mononuclear cells was associated with the development of graft dysfunction [101]. We have shown that in patients undergoing antibody-incompatible renal transplantation, elevated serum BAFF levels at baseline (prior to both antibody removal/desensitization and transplantation) are associated with a heightened risk of subsequent AMR [102]. A pilot study to assess the use of belimumab (a humanized monoclonal antibody that inhibits BAFF) as monotherapy to reduce antibody levels in sensitized patients was terminated after recruiting eight patients because of the lack of efficacy (ClinicalTrials.gov Identifier: NCT01025193). Additional clinical studies will be required to determine whether alternative patient groups/clinical endpoints in transplantation may benefit from BAFF-blockade, perhaps in combination with other immunosuppressive agents, since these agents have already been used in the treatment of autoimmune disease [103,104].

The class 1A PI3K enzymes are heterodimers comprised of a regulatory subunit (p85) and a catalytic subunit (p110). As detailed above, PI3K plays an important role in generating PIP3, a signalling intermediate generated downstream of BCR signalling, which may also be required for BAFF-mediated B-cell survival [105]. The p110δ subunit is relatively leucocyte specific, offering a potential therapeutic target. Mice lacking p110δ exhibit reduced T-independent antibody responses and p110δ inactive B cells have reduced proliferative responses to anti-IgM, anti-CD40 and IL-4 stimulation [106,107]. Furthermore, TLR9-mediated antibody class switching is blocked by p110δ inactivation [108]. Consistent with an important role for PI3K in B-cell homeostasis *in vivo*, patients with B-cell malignancies have responded well to the p110δ-selective inhibitor CAL-101 [109]. There is considerable interest in the use of similar agents for the treatment of autoimmune diseases [110], and they may well have efficacy in transplantation.

As detailed above, an interaction between B cells and Tfh cells is required for a germinal centre reaction. Thus, inhibition of some of the survival signals provided by Tfh cells to B cells may have utility in limiting the progression of B cells through the germinal centre reaction, thereby reducing memory B and plasma cell formation. Potential targets include co-stimulatory pathways (e.g., CD40:CD40L interactions) and the cytokine IL21. Indeed, recent data suggest that both CD40 ligation and IL21 are required for the upregulation of the transcription factor Blimp-1 and the subsequent development of plasma cells from B cells [111]. Ligation of CD40 is also required for IL6 production by B cells, leading to recombinant activating gene expression [112]. Initial studies targeting CD40L (CD154, expressed on CD4 T cells), were abandoned because of a high rate of thromboembolic events associated with CD154 expression on platelets. However, efforts to block the B cell side of the equation with nondepleting anti-CD40 antibodies have met with considerable success in a number of nonhuman primate studies pioneered by Kirk and Larsen [113,114]. This therapeutic strategy has been moved into man, with a phase 1 study confirming the safety of an anti-CD40 monoclonal antibody, ASKP1240, in renal transplant recipients. There is now a Phase 2a randomized controlled clinical trial underway to compare the efficacy of ASKP1240 (in combination with basiliximab at induction and mycophenolate and corticosteroid maintenance) with a standard regimen of basiliximab, followed by tacrolimus, mycophenolate and steroids (ClinicalTrials.gov Identifier: NCT01780844). The study aims to enrol 126 patients and should finish recruiting by 2014. It will be of interest to determine if B–cell-specific endpoints are affected by this strategy.

In mouse models, IL21 production by Tfh is required for optimal progression of the germinal centre reaction [115–117]. IL21 also acts as a survival factor for plasma cells within secondary lymphoid organs [118]. An IL21R-fusion protein has been shown to ameliorate disease in a mouse model of lupus [119]. In a human xenogeneic graft versus host disease model, a neutralizing anti-human IL-21 monoclonal antibody given prophylactically significantly reduced disease severity [120]. Thus, IL21 blocking agents are in development and represent another potential therapeutic avenue for B-cell inhibition.

The cytokine IL6 is required for B-cell differentiation into plasma cells and is also a survival factor necessary for the persistence of short- and long-lived plasma cells within niches [111,121]. A mouse anti-human IL6 receptor blocking antibody (tocilizumab) has been developed and trialled for use in some autoimmune and autoinflammatory diseases [122] and may well have utility in targeting humoral responses in transplantation.

Theoretically, increasing inhibitory input into B cells may also offer a therapeutic strategy in B cell control. There is some evidence to support the concept of targeting the IgG receptor FcγRIIB. In murine cardiac allograft models, chronic arteriopathy and autoantibody production were increased in FcγRIIB-deficient recipients [123]. A monoclonal antibody against FcγRIIB (hu2B6-3.5) has been used to direct monocyte- or macrophage-induced cytotoxicity against lymphomatous B cells [124] and plasma cells from patients with systemic light-chain amyloidosis [125]. In addition, FcγRIIB cross-linking on B cells, plasma cells and myeloma cell lines [57] can induce apoptosis *in vitro*.

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Epratuzumab, a humanized anti-CD22 antibody, induces some depletion of naive and transitional B cells, producing a 35% reduction in total B cell numbers, but can also inhibit B-cell activation and proliferation [126]. Epraztuzumab has shown some efficacy in treating lupus and appears to have a reasonable safety profile [127] and awaits study in transplantation. There is some evidence that IVIG may ligate CD22 and inhibit BCR signalling *in vitro*, an effect dependent on Fc sialylation [128]. However, a more recent study suggests that the anti-inflammatory effect of IVIG *in vivo* is independent of both B cells and CD22 [129].

(iii) Enhancing the generation of regulatory B cells

The identification of B cells with regulatory potential raises the question of whether these cells might be enhanced *in vivo* to reduce allograft-specific responses or whether it may be possible to generate these cells *ex-vivo* for subsequent use therapeutically. These cells may have the potential to promote transplant tolerance, given the evidence of a B-cell signature in microarray studies of tolerant transplant recipients, and the presence of a higher percentage of B cells expressing CD1d and CD5 in the peripheral blood of these patients [17,18]. In mice, transplant tolerance can be induced by the administration of an anti-CD45RB antibody. This phenomenon is dependent upon B cells, but not on IL-10 [130]. Thus, there are likely to be additional regulatory B-cell subsets which suppress in an IL-10 independent manner, via production of TGF-β, or via contact-dependent inhibition [15].

One barrier to generating B10 cells *in vivo*, is that *in vitro* studies suggest that the stimuli which induce their differentiation are those which will also stimulate effector B cells, for example, CD40 ligand, IL21, TLR ligands, and BAFF [15,131,132]. Administration of BAFF in mice was shown to increase the number of IL-10-producing B cells in the marginal zone [131]. In support of a similarly important role for BAFF in the survival of B10 cells in humans is the observation that patients with multiple sclerosis treated with Atacicept (an antagonist of BAFF and APRIL), developed an unexpected increase in inflammatory activity [133]. In mice, TIM-1 is expressed by most IL-10 producing B cells, and *in vivo* its ligation with an agonist monoclonal antibody increased such B cells and was associated with long-term islet allograft survival [27]. Whether this marker identifies B10 cells in humans and might potentially be targeted *in vivo* in transplant recipients remains to be determined.

Concluding comments

There is firm evidence demonstrating that B cells play an important role in the immune response to an allograft beyond antibody production. It is also clear that some B-cell subsets can potentially inhibit or regulate immune responses. There are now an increasing number of therapeutic agents available which target B cells, and a willingness to use them in transplantation, as demonstrated by the widespread application of rituximab. However, a more detailed understanding of B cell biology in humans is required to design and implement therapies which reduce the harmful effects of effector B cells whilst promoting immunosuppressive, tolerance-enhancing B-cell subsets.

Funding

The authors have declared no funding.

Acknowledgements

Dr Clatworthy is supported by the NIHR Cambridge Biomedical Research Centre.

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