Effector and regulatory B cells: modulators of CD4+ T cell immunity

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Preface

B cells are essential for humoral immunity, but the role that B cells have in regulating CD4^{+} T cell responses remains controversial. However, new data showing that transient depletion of B cells potently influences the induction, maintenance and reactivation of CD4^{+} T cells and the recent results identifying antibody-independent functions of B cells have reinvigorated interest in the many roles of B cells in both infectious and autoimmune diseases. In this Review, we discuss recent data showing how effector and regulatory B cells modulate CD4^{+} T cell responses to pathogens and autoantigens.

Immunity to pathogens often requires both B cell-dependent humoral immune responses and T cell-dependent cellular immune responses, which cooperate to clear infectious organisms. Although CD4^{+} T cells clearly participate in humoral immune responses by providing help to B cells and can enhance cellular immunity by producing cytokines, the converse possibility, that B cells participate in both types of immune response, is still not widely accepted. Some early studies of B cell-deficient mice indicated that the absence of B cells adversely affected both CD4^{+} T cell[1–4] and CD8^{+} T cell responses[5, 6]. However, other studies showed that B cells were dispensable for the generation and maintenance of antigen-specific T cell responses[7–10]. These conflicting results were further clouded by data showing that mice lacking B cells during embryonic development exhibit immunological abnormalities, including defects in Peyer's patch organogenesis[11], loss of follicular dendritic cells (FDCs)[12, 13] and gp38-expressing stromal cells in the spleen[14], alterations in splenic dendritic cell (DC) homeostasis[15] and decreased T cell numbers in the thymus[16] and spleen[14]. Given that many of the developmental and architectural defects observed in B cell-deficient mice are likely to influence T cell responses, it has been difficult to unambiguously assign a role for B cells in regulating cellular immune responses to either pathogens or autoantigens.

The question of whether B cells have a role in cellular immune responses is now receiving renewed interest with the emergence of clinical data showing that B cell depletion is an effective treatment for several T cell-mediated autoimmune diseases Multiple Sclerosis (MS)[17], Type 1 Diabetes (T1D)[18] Rheumatoid Arthritis (RA)[19] and others[20, 21]. Indeed, studies in both humans and mice show that the clinical efficacy of B cell depletion therapy does not necessarily correlate with changes in the levels of circulating autoantibody, suggesting that B cells may contribute to autoimmunity independently of autoantibody production[22, 23]. Importantly, transient B cell depletion studies that distinguish the role of B cells during development from their roles during the course of an immune response have

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provided convincing evidence that B cells do regulate T cell-mediated immune responses. Furthermore, new mouse models, in which B cells are present but cannot secrete antibody, show that B cells can regulate T cell-mediated immune responses via antibody-independent mechanisms.

In this Review, we focus on emerging data from patients and mouse models showing that B cells modulate CD4^{+} T cell responses. Specifically, we discuss the roles that B cells have in regulating the development, proliferation and maintenance of CD4^{+} effector and memory T cells and the data suggesting that B cells also modulate the number of regulatory T cells. We review experiments showing that recently described "effector" and "regulatory" B cell subsets modulate the function of T cells by presenting antigen, by providing co-stimulation and by producing cytokines that direct the proliferation and effector functions of responding T cells. Collectively, these data show that B cells are not simply the passive recipients of T cell help, but actively participate in cellular immune responses by directing the magnitude and quality of the T cell response to foreign and self-antigens.

Effects of B cell depletion on T cells in autoimmunity

Decreased CD4^{+} T cell effector responses

Rituximab, a mouse/human chimeric antibody that binds to human CD20, induces B cell depletion via FcR-mediated antibody dependent cell cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) mechanisms[24, 25]. Rituximab treatment depletes naïve and memory B cells from peripheral blood[26]. However, it is less effective in depleting tissue residing marginal zone and germinal center B cells[24, 27] and does not affect CD20^{neg} long-lived plasma cells[26].

Given the relative effectiveness of B cell depletion by Rituximab, the drug has been tested in a wide variety of diseases. It is approved to treat non-Hodgkin's lymphoma and RA in patients with disease that is refractory to anti-tumour necrosis factor (TNF) therapy. Rituximab is also being evaluated for the treatment of other autoimmune diseases, including systemic lupus erythamatosus (SLE), type 1 diabetes, idiopathic thrombocytopenic purpura (ITP), pemphigus vulgaris (PV), mixed cryoglobulinemia vasculitis (MCV), T1D, MS and others[20]. Most of the clinical studies to date have focused on the extent of B cell depletion and clinical correlates of disease remission. Interestingly, autoantibody titres to some self-antigens declined following B cell depletion while others did not[22, 23], suggesting that some autoantibody responses are continuously initiated by new cohorts of CD20-expressing B cells while others are likely being maintained by long-lived CD20^{neg} plasma cells. Regardless, in many studies B cell depletion was clinically effective even in patients that did not have decreased autoantibody titres[22, 23], indicating that B cells must also contribute to pathology by other mechanisms. Given that a number of T cell-mediated autoimmune diseases are being effectively treated with B cell depletion, investigators have begun examining whether Rituximab treatment alters the subset composition, activation or function of T cells (Tables 1 and 2). For example, T cell responses were monitored in Rituximab-treated patients with pemphigus vulgaris (PV)[28]. Although B cell depletion therapy did not change the proportion of total T cells in these patients, the frequency of desmoglein-specific (autoreactive) T cells in peripheral blood that produced interferon-γ (IFNγ) or interleukin-4 (IL-4) declined significantly following B cell depletion[28]. By contrast, Rituximab treatment did not affect the number of IFNγ-producing tetanus toxoid specific T cells[28]. Patients who remained in complete remission for more than one year exhibited the greatest decline in desmoglein-reactive T cells, whereas autoreactive T cells reemerged in patients who relapsed[28]. Interestingly, the titres of autoantibodies specific for desmoglein correlated with the frequency of autoreactive T cells; Rituximab-treated patients with lower numbers of desmoglein-reactive T cells also had lower desmoglein-specific antibody titres, whereas

relapsed patients whose autoreactive T cell numbers increased also showed a rebound in desmoglein autoantibodies28. These data suggested that B cells contribute to PV both directly through autoantibody production and indirectly through regulation of autoreactive T cells.

Similar results were observed in patients with idiopathic thrombocytopenic purpura (ITP)[29]. Although autoantibodies do contribute to the pathogenesis of ITP, patients with ITP often present with monoclonal or pauciclonal repertoires of T cells, which are preferentially skewed toward the production of T helper 1 (T_H1)-type cytokines such as IFNγ[30]. In addition, T cells from patients with ITP express higher levels of the anti-apoptotic protein BCL-2, and lower levels of the pro-apoptotic protein BAX and are more resistant to apoptosis than T cells from healthy controls[30]. Each of these T cell abnormalities was reversed in ITP patients who responded positively to B cell depletion therapy, but was unaffected in non-responders[29], suggesting that the efficacy of the B cell depletion therapy in ITP was associated with changes in T cell repertoire and function. Thus, B cells from patients with PV and ITP, as well as other human and mouse autoimmune diseases (see Tables 1 and 2), appear to enhance autoreactive T cell responses.

Increased regulatory T cell development

T cells were also phenotypically altered in Rituximab-treated patients with SLE. In one study, Rituximab treatment reduced the number of CD40L-expressing CD4+ T cells in the peripheral blood of SLE patients but did not alter the expression of other activation markers like HLA-DR, CD69 or CD25[31]. In a follow up study, the investigators showed that Rituximab treatment decreased CD40L mRNA expression in T cells from the responder patients with SLE[32]. Interestingly, investigators analyzing two separate cohorts of patients with SLE reported that the frequency of CD4+CD25hi T cells in peripheral blood was significantly increased after B cell depletion[33, 34] and these T cells expressed FOXP3[34]. Furthermore, the proportion of CD4+ T cells spontaneously producing IL-10 or transforming growth factor-β (TGFβ) was increased in the Rituximab-responsive patients[33]. Increased expression levels of mRNAs associated with regulatory T cells, including CD25, FOXP3, cytotoxic T lymphocyte antigen 4 (CTLA4) and glucocorticoid induced TNF-receptor related protein (GITR) were observed in the Rituximab-responsive patients with SLE32. Moreover, FOXP3 levels remained low in patients who did not respond to therapy and declined to baseline levels in patients who relapsed32. Together, these data are consistent with the idea that successful Rituximab treatment not only curtails T cell effector responses but also promotes the expansion of regulatory T cell populations.

In support of this hypothesis, Rituximab treatment of patients with mixed cryoglobulinemia vasculitis (MCV) also resulted in changes to effector and regulatory T cell populations. Patients with MCV normally present with increased systemic levels of T_H1 cytokines and greater numbers of activated T cells and activated, clonally expanded memory B cells[35]. After Rituximab treatment, both B and T cell abnormalities were reversed in a subset of MCV patients[36]. The clinical efficacy of the B cell depletion correlated best with reductions in systemic levels of IFNγ and IL-2 and a decrease in the size of the clonally-expanded B cell population[36]. In addition, investigators observed a notable increase in the frequency of CD4+CD25+FOXP3+ regulatory T cells in Rituximab-responsive MCV patients, but not in non-responder patients or patients who relapsed in less than one year[36]. These data suggested that elimination of the clonal (and presumably autoreactive) B cells reduced inflammation and favoured the development or proliferation of regulatory T cells.

Similar increases in regulatory T cells were reported in a B cell-depleted autoimmune mouse model. Early Rituximab treatment (before the onset of clinical disease) of non-obese diabetic (NOD) mice expressing a human CD20 transgene[27] led to less insulitis and delayed

the onset of diabetes32. Moreover, Rituximab treatment after the onset of hyperglycemia, combined with therapeutic administration of insulin, led to long-term remission in 36% of the animals and ultimately ameliorated the requirement for daily insulin injections[37]. Mice that were B cell depleted before disease onset had increased numbers of Foxp3^{+} T cells and CD4^{+} T cells from these animals suppressed diabetes in adoptive transfer experiments[37]. In addition, the B cells that repopulated Rituximab-treated animals were able to suppress diabetes development when transferred with autoreactive T cells into NOD/severe combined immunodeficient (SCID) hosts[37]. This result suggests that depletion of the pathogenic B cell population facilitated the establishment of both B cell and T cell regulatory populations and indicated that at least some B cells can suppress autoimmune disease.

An increase in the number of regulatory T cells was also observed in NOD mice treated with B cell maturation antigen (BCMA)-Fc to block B cell-activating factor (BAFF)- and a proliferation-inducing ligand (APRIL)-mediated survival signals for B cells[38]. B cell depletion with BCMA-Fc in 9-week-old NOD mice (after the onset of insulitis) prevented hyperglycemia and islet destruction, even though the BCMA-Fc treatment protocol did not effectively deplete all of the B cells in the pancreas and diabetogenic T cells were still present and sufficient to cause disease when transferred into NOD/SCID recipients[38]. Thus, the remaining lymphocytes appeared to be held in check in the BCMA-Fc treated animals. Indeed, T cells from spleen and pancreatic lymph nodes of BCMA-Fc treated mice were less activated as measured by reduced CD40 expression on T cells and decreased systemic levels of IL-17[38]. Furthermore, BCMA-Fc treatment also promoted a gradual increase in the number of CD4^{+}CD25^{+}Foxp3^{+} regulatory T cells in the spleen and pancreatic LN[38]. These regulatory T cells seemed to be important for ameliorating disease, as CD25 blockade after B cell depletion resulted in recurrence of diabetes[38].

Likewise, another group reported that the frequency of Foxp3^{+} regulatory T cells in the pancreatic lymph nodes and islets increased in NOD mice after treatment with CD22-specific monoclonal antibodies to deplete B cells[39]. Similar to the results of Hu et al[37], the B cells that repopulated the CD22-specific antibody-treated mice prevented the development of diabetes when transferred with diabetogenic T cells into NOD/SCID hosts[39]. Transcriptome analysis of these "protective" B cells revealed significant changes in gene expression with almost 200 genes downregulated in the newly emerging B cells compared to B cells from normoglycemic 10-week-old NOD mice[39]. The B cells that repopulated the hosts were also less effective in presenting antigen to T cells and inducing inflammatory cytokine production by the T cells[39].

Collectively, the data suggest a model (Fig. 1) in which a positive feedback loop between autoreactive effector B cells and T cells is established, leading to exacerbated inflammation and tissue damage. B cell depletion, when effective, blocks the further proliferation or maintenance of autoreactive T cells that may prevent the repopulating potentially autoreactive B cells from being selected and expanded in the periphery. Depletion of autoreactive B cells also prevents the formation of new plasmablasts, resulting in a decrease in autoantibody titres specific for some, but not all, autoantigens. Together, the reductions in autoreactive B and T cells lead to a decline in organ-specific and systemic inflammation, favoring the emergence of regulatory T and B cells that can prevent the reactivation of remaining autoreactive cells. In the next sections we will discuss experimental data that describe at a mechanistic level how B cells might regulate CD4^{+} T cell responses.

B cells regulate CD4+ T cell mediated immune responses

Not required for CD4+ T cell homeostasis

The clinical data evaluating T cell responses in Rituximab-treated patients are limited by the types of experiments that can be carried out with peripheral blood cells. However, mechanistic studies are possible when evaluating T cells in B cell-depleted mice. For example, a recent study used B cell depletion to show that B cells do not control normal T cell homeostasis[40]. In fact, T cells and T cell subsets were present in typical numbers in the thymus and secondary lymphoid tissues of B cell-depleted naïve mice[40]. In addition, the phenotypic and transcriptional profiles of the peripheral T cells in B cell-depleted naïve mice were identical to those of T cells from non-depleted mice[40]. Furthermore, the T cells from B cell-depleted naïve mice responded normally to polyclonal stimuli such as treatment with combined CD3- and CD28-specific antibodies[40]. Thus, these data suggest that the effects of B cell depletion on T cell function are probably due to changes in T cells responding to antigen.

Activation and maintenance of CD4+ T cells

Consistent with the idea that B cells modulate T cell responses to antigen, numerous reports indicate that B cells regulate the initial proliferation of CD4+ T cells after encounter with antigen[41−45] and have roles in CD4+ T cell memory responses[44, 46−50]. For example, the number of IL-4-producing T cells responding to the intestinal nematode *Heligmosomoides polygyrus* in B cell-deficient (and B cell-depleted) mice was reduced ten-fold compared with that observed in normal mice, even as early as 3 days after infection[50]. Furthermore, despite the continued presence of antigen, antigen-specific T cell responses remained depressed in B cell-deficient mice for weeks, suggesting that B cells are required for the initiation and expansion of the primary CD4+ T cell response to *H. polygyrus*. B cells were also required to maintain or reactivate *H. polygyrus*-induced memory T helper 2 (T_H2) cells[50]. Interestingly, although antibody was essential for successful immunity to *H. polygyrus*, antibody was completely dispensable for the primary and memory CD4+ T response[50]. However, primary and secondary Th2 cell responses to *H. polygyrus* did require antigen-specific antigen-presenting B cells[50].

Similar results were reported for CD4+ T cell responses to lymphocytic choriomeningitis virus (LCMV)[51]. In these experiments, the number of LCMV-specific IFNγ-producing CD4+ T cells was reduced by 60% in B cell-deficient mice on day 8 after primary infection, and was dramatically reduced (by 95%) on day 70 post-infection[51]. LCMV-specific memory CD4+ T cells were not reduced in mice in which B cells were unable to produce antibody[51], which indicates that although B cells were required to maintain CD4+ T cell memory, antibody–antigen complexes were not needed. Interestingly, in B cell-deficient mice LCMV-specific memory CD4+ T cells disappeared relatively early after primary infection (within 1 month), during the contraction phase of the T cell response[51], suggesting that B cells play a role in the establishment or early maintenance of LCMV-specific memory CD4+ T cells.

B cells were also required for the reactivation of memory T cells after herpes simplex virus (HSV) reinfection[52]. In this model, mice were vaccinated with attenuated HSV and then challenged with virulent HSV-2. Protection depended on IFNγ-producing CD4+ T cells that were reactivated by MHC class II-expressing, non-infected antigen presenting cells (APCs)[52]. When vaccinated mice were depleted of B cells alone, or depleted of either DCs or macrophages, they were still protected after re-infection[52]. However, T_H1 cell-mediated protection against HSV was completely abrogated when both B cells and DCs were depleted

before the challenge infection[52]. Thus, B cells, in combination with DCs, maximize the memory CD4^{+} T cell recall response to this virus.

Suppression of CD4^{+} T cells

In addition to activating T cells, B cells may also modulate regulatory T cell development, proliferation or survival. In support of this possibility, one recent study showed that culturing CD19^{+} human B cells with CD4^{+}CD25^{+} alloreactive T cells plus IL-2 and CD28-specific antibody induced a 40-fold expansion of regulatory T cells[53]. The expanded T cells expressed FOXP3 and were highly suppressive in *in vitro* assays[53]. Interestingly, the antigen specificity of the regulatory T cells was determined by the stimulating B cells[53].

In a second study, CD40L-activated B cells were used to induce regulatory T cells[54]. Co-culture of CD40L-stimulated human B cells with naïve CD4^{+}CD45RA^{+}CD45RO^{−}CD25^{−} T cells resulted in the expansion of a population of CD4^{hi} T cells that also expressed CD25, FOXP3, CTLA4 and GITR[54]. These T cells suppressed the proliferation of T cells responding to the original target alloantigens expressed on the B cells[54]. Importantly, no exogenous stimuli were added to the co-cultures, indicating that the CD40L-activated B cells, which expressed high levels of MHC class II, CD80 and CD86 and secreted substantial amounts of IL-2[54], provided all of the necessary signals to induce regulatory T cell development.

B cells were also required for regulatory T cell-dependent tolerance induction[55]. Ovalbumin-specific regulatory T cells can be induced in normal mice by transferring naïve ovalbumin-specific transgenic CD4^{+} T cells into recipient hosts and then repeatedly administering ovalbumin coupled to cholera toxin B subunit through the sublingual, nasal or oral route[56]. This tolerance protocol was significantly less effective when the naïve T cells were transferred into B cell-deficient hosts and resulted in fewer Foxp3^{+} regulatory T cells and poorly suppressed ovalbumin-specific effector T cell responses[55]. However, transfer of naïve B cells plus naïve ovalbumin-specific CD4^{+} T cells to the B cell-deficient hosts rescued their ability to generate suppressive ovalbumin-specific Foxp3^{+} regulatory T cells[55]. Moreover, B cells from the ovalbumin-tolerized mice expressed higher levels of IL-10 mRNA than did B cells from mice treated with phosphate-buffered saline[55] and transfer of the B cells from orally tolerized mice to new hosts significantly suppressed naïve CD4^{+} T cell responses to vaccination with ovalbumin and adjuvant[55]. Together, these results suggest that a subset of IL-10-producing B cells, activated in response to a tolerogenic antigen, drove the development of regulatory T cells.

All of these studies, when taken together, show that B cells participate in inducing primary T cell proliferation and generating, maintaining and reactivating T_H1 and T_H2 memory CD4^{+} T cells via antigen-specific but antibody-independent mechanisms. The data also show that B cells can modulate regulatory T cells. Therefore, the data predict that B cells have dual and potentially opposing roles in T cell-dependent immune responses. This hypothesis was elegantly tested in recent experiments in which mice were B cell-depleted either before or after the initiation of experimental autoimmune encephalomyelitis (EAE) with myelin oligodendrocyte glycoprotein (MOG) peptide immunization. The severity of clinical disease was significantly higher in mice depleted of B cells seven days prior to immunization with MOG peptide than in non-depleted mice[57]. Moreover, the number of autoreactive, cytokine-producing T cells in the central nervous system increased significantly in mice depleted of B cells before immunization[57]. In striking contrast, if B cells were depleted 14 days after immunization with MOG, clinical disease was ameliorated and the numbers of total CD4^{+} T cells, MOG-specific T cells and IFNγ or IL-17-producing CD4^{+} T cells in the CNS were reduced[57].

These mouse model data clearly showed that B cells can both suppress and enhance T cell responses. Likewise, emerging results from patient studies examining the effect of Rituximab treatment to prevent acute rejection of solid organ transplants indicates that B cells have the ability to play opposing roles in T cell-mediated immune responses. Acute cellular rejection of transplanted organs is largely held to be T cell-mediated[58]. However, it is clear, at least in the case of kidney transplants, that CD20$^+$ B cells can be found in the rejecting kidney[59] and that alloantibodies, particularly in pre-sensitized (already immune) individuals contribute to the cellular rejection process[60]. Treatment of sensitized individuals with Rituximab appears to reduce alloantibody titres and, when combined with other therapies such as corticsteroids and plasmapheresis, can be highly effective in suppressing T cell mediated rejection of the organ following transplant[58]. By contrast, depletion of T cells from non-sensitized (naïve) individuals appears to greatly enhance acute T cell-mediated rejection[61], suggesting the possibility that depletion of a regulatory population of B cells enhances the destructive T cell-mediated alloresponse. Thus, experimental and clinical data predict that there must be multiple functionally distinct populations of regulatory and effector B cells that can modulate T cell responses (Fig 2).

Functions of effector and regulatory B cells in T cell-mediated immunity

Antigen presentation and co-stimulation

Many of the studies described above show that B cells modulate effector, memory and regulatory T cell responses by antibody-independent mechanisms. One way in which B cells can modulate T cell responses independently of antibody is by presenting antigen to T cells. Although it is often assumed that antigen-presenting dendritic cells are not only necessary but also sufficient for CD4$^+$ T cell activation, proliferation and differentiation[62], it is clear that antigen-presenting B cells can be important for the proliferation and differentiation of T cells[1, 42, 44, 45, 50, 63−66] and that B cells are particularly effective APCs when antigen is limiting[67−69]. Thus, B cells may become increasingly relevant as antigen-presenting cells when antigen load is low. Consistent with this idea, a recent study using B cell depleted mice showed that B cells are essential for CD4$^+$ T cell responses when mice are immunized with limiting amounts of antigen, but are dispensable when larger quantities of antigen are used[40].

In addition to antigen presentation by B cells, evidence also suggests that co-stimulatory molecule expressing B cells modulate T cell responses[43, 70−72]. For example, experiments using mice in which B cells, but not other APCs, are selectively unable to express CD80 and CD86 (B-costimulatory-deficient mice) showed that expression of these costimulatory molecules by B cells was required for the activation and/or proliferation of autoreactive CD4$^+$ T cells in a mouse model of arthritis[72]. Indeed, CD4$^+$ T cells from the B-costimulatory deficient mice proliferated less following *in vitro* restimulation with arthritis-inducing cartilage proteoglycan autoantigens than did T cells from the wild-type mice[72]. Moreover, T cells from B-costimulatory deficient mice with proteoglycan autoantigen-induced arthritis were significantly less effective in inducing severe arthritis when transferred into SCID recipient animals[72]. Although these experiments did not address whether B cell-dependent co-stimulation is required for memory CD4$^+$ T cells responses to cartilage proteoglycan autoantigens, B cells that lack CD80 and CD86 were far less effective than wild-type B cells in activating CD4$^+$ memory T cells *in vitro*[72], suggesting that B cells likely provide co-stimulatory signals to T cells during both primary and secondary responses to antigen. In follow up experiments, the same group reported that transiently depleting B cells four days after a secondary challenge with cartilage proteoglycan autoantigens reduced the number of IFNγ− and IL-17-producing autoantigen-specific CD4$^+$ T cells[73]. In addition, T cells from B cell-depleted mice were unable to transfer disease to SCID recipients[73].

Thus, antigen-presenting, co-stimulatory molecule-expressing B cells are required to activate and expand effector and memory T cell populations.

Cytokine production

In addition to presenting antigen and providing co-stimulation to T cells, B cells secrete cytokines and chemokines either constitutively or in response to antigen, TLR ligands, T cells or combinations of these stimuli[74, 75]. Although it has been known for decades that B cells can make cytokines[76], the concept that the cytokines made by B cells are involved in modulating the activation or function of CD4+ T cells is relatively new[74, 75]. However, the evidence is growing that cytokine-producing B cells have relevant roles in humoral and cellular immune responses. For example, one group recently showed that Foxp3+ regulatory T cells proliferate in co-cultures containing splenic B cells and anti-CD3 stimulated T cells and that the proliferation of regulatory T cells depends on the production of TGFβ3 by the B cells[77]. Similarly, TGFβ-expressing B cells, induced *in vivo* in a model of local inhalation tolerance, were sufficient to promote the conversion of CD25−CD4+ T cells into Foxp3+ regulatory T cells[78]. Thus, cytokines made by B cells can either directly suppress T cell immune responses or can enhance the development and maintenance of regulatory T cells which subsequently suppress effector T cell proliferation and function.

B cells can functionally sub-divided based on their cytokine profile. Indeed, prior work from our lab revealed that B cells primed by T cells as well as antigen and/or TLR ligands in the presence of T_H1-type cytokines secrete IFNγ and IL-12p40 (mouse) or IFNγ and IL-12p70 (human). These B cells, which we have operationally termed B effector-1 cells (Be-1 cells), do not secrete significant amounts of IL-4, IL-13 or IL-2 but can secrete IL-10, TNFα and IL-6[79, 80]. By contrast, B cells primed by T cells and antigen in the presence of T_H2-type cytokines (referred to as Be-2 cells by our lab) secrete IL-2, Lymphotoxin, IL-4 and IL-13 but make minimal amounts of IFNγ and IL-12[80, 81]. Be-2 cells can also secrete IL-10, TNFα and IL-6[80]. Finally, regulatory B cells (also referred to as B10 cells by the Tedder group[82]) produce IL-10 upon stimulation with combinations of antigen, CD40L and TLR ligands[83−86].

Regulatory IL-10 producing B cells suppress CD4+ T cell responses and can prevent the induction of autoimmune disease in several mouse models[83−85]. IL-10 producing B cells are found in both B1 and B2 lineage cells and recent data suggests that there are multiple phenotypically distinct populations of IL-10 producing B cells that can function in a regulatory capacity[83−86]. Experiments characterizing one population of regulatory B cells showed that B cells expressing markers of transitional T2 B cells produce IL-10 and can prevent the development of autoimmunity[87, 88]. Other studies suggested that follicular B cells[89] or B cells expressing high levels of CD1d[90, 91] can also produce IL-10 and function as regulatory B cells. In the most recent study, a CD5+CD1dhi IL-10 producing regulatory B cell population was assessed in more detail[82]. Transfer of these IL-10-producing B cells into CD19-deficient mice or B cell-depleted animals blocked the CD4+ T cell-dependent inflammatory response in a contact hypersensitivity model[82]. Importantly, the suppressive function of CD5+CD1dhi B cells was antigen dependent, as regulatory B cells from mice primed with one antigen could not suppress the activity of T cells primed with a second antigen[82]. Furthermore, the regulatory function of these B cells was absolutely dependent on their ability to produce IL-10[82]. Together, these experiments showed that an antigen-specific IL-10-producing B cell subset can down-modulate T cell activation.

In addition to suppressing T cell responses, cytokine-producing B cells can also enhance T cell mediated immune responses[48, 50, 80, 92−95]. For example, *in vitro* generated effector B cells that produced either T_H1- or T_H2-type cytokines were shown to promote the activation and differentiation of naïve T cells into effector T_H1 and T_H2 cells, respectively, through a

cytokine-dependent mechanism[80]. Similarly, IL-12-producing human B cells were shown to promote T_H1 cell differentiation *in vitro*[48, 92] and IL-4-producing human B cells were reported to enhance T_H2 cell responses[95]. These *in vitro* experiments were recently confirmed with *in vivo* data showing the importance of B cell cytokines in promoting T cell responses. In one set of experiments, TNF production by B cells was shown to be required for the generation of an optimal T_H1 cell response to *Toxoplasma gondii*[94]. In another set of experiments, the generation of a protective T_H2 memory response to *H. polygyrus* was shown to depend on IL-2-producing B cells[50].

In summary, the current evidence suggests that B cells influence CD4^{+} T cell responses by multiple antibody-independent mechanisms. B cells can enhance T cell responses by presenting antigen and by providing co-stimulation. In addition, phenotypically distinct populations of cytokine-producing B cells can have opposing roles and either enhance or suppress T cell responses (Fig. 3).

Conclusions and perspectives

Antibody is vital for protective immunity to many pathogens and antibody production by B cells is unarguably the most important effector function of these cells and their *raison d'être*. However, B cells also carry out a variety of other effector and regulatory functions during the course of an immune response. The recent use of B cell depletion therapy is now distinguishing the multiple roles that B cells have during the course of immune responses, independent from antibody production. These experiments reveal that B cells interact in a complex duet with CD4^{+} T cells to modulate the cellular and humoral immune responses to pathogens and autoantigens. B cells together with DCs, mediate optimal proliferation of CD4^{+} T cells and can enforce or stabilize the differentiation of T cells into polarized effector cell subsets. B cells also influence the contraction of the CD4^{+} T cell response and the development of T cell memory. Finally, B cells appear to play an important role in the reactivation of memory CD4^{+} T cells.

In addition to promoting effector and memory CD4^{+} T cell responses, B cells influence the development, expansion and survival of regulatory T cell populations. The evidence suggests that activated effector B cells enhance effector T cell responses in autoimmune settings, while simultaneously blocking the survival or expansion of regulatory T cell subsets. Although B cell depletion experiments do not discriminate whether the activated effector B cells directly inhibit regulatory T cells or whether the B cells contribute to the inhospitable inflammatory milieu that prevents the survival or expansion of regulatory T cells, other experiments suggest that under some conditions, naïve and appropriately activated B cells can provide signals that actually facilitate tolerance and the development and expansion of regulatory T cells. Thus, B cell depletion may promote the development of a more tolerogenic environment in autoimmune disease through multiple mechanisms. First, the depletion of autoreactive effector B cells can block the B cell–T cell feed-forward loop, preventing the further proliferation and reactivation of pathogenic T cells and thereby reducing organ-specific and systemic inflammation. Second, the decrease in inflammatory cytokine production will favour the development and survival of regulatory T and B cells that can further dampen the autoimmune response. Finally, the transitional and naïve B cells that initially repopulate the depleted host seem to express a distinct transcriptional profile and may be particularly effective in inducing or expanding regulatory T cell populations. Thus, B cell depletion therapy, when most effective, likely resets the balance between effector and regulatory populations of both B and T cells.

Although we are making significant strides in our understanding of the multi-faceted roles of B cells in humoral and cellular immune responses, there is much that we still do not

understand. The data convincing show that B cells modulate T cell responses by presenting antigen, by providing co-stimulation and by secreting cytokines. Furthermore, it is becoming clear that the different effector functions of B cells are tightly controlled and that these tasks are carried out by functionally and phenotypically distinct effector and regulatory B cell subsets. What is less clear, is whether these functionally defined Be-1, Be-2 or B10 cells truly represent distinct lineages or subsets. Furthermore, we do not know whether the antibody-independent effector functions are hard-wired into certain B cells or whether B cells have the plasticity to change their effector functions depending on their local environment. In addition, we do not know whether B cells that develop an effector or regulatory phenotype represent an end fate decision for the B cells or whether they can enter the memory or plasma cell pools while maintaining some or all of their unique effector functions. Finally, we have not yet identified all of the *in vivo* signals that are required to trigger the development and activation of B cells with regulatory or effector functions. Although there are a large number of important questions remaining to be addressed, it is clear that the scope of B cell biology has expanded beyond its initial boundaries of humoral immunity to encompass the wider field of CD4$^+$ T cell-mediated immunity.

Acknowledgments

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Glossary Terms

Follicular B cells	A re-circulating, mature B-cell subset that populates the follicles of the spleen and lymph nodes.
Transitional T2 B cells	Transitional B cells are short-lived immature B cells, typically found in the spleen, that either die or are selected into the peripheral mature B cell repertoire. Transitional B cells can be subdivided into three subsets (T1, T2 and T3 cells) based on differential phenotypic and functional characteristics.

Figure 1. Resetting the effector and regulatory B and T cell networks after B cell depletion
The mechanistic basis for the effectiveness of B cell depletion in treating autoimmune disease is not understood. Based on the data, we propose a model in which autoreactive effector B and T cells establish a feed-forward loop that destroys the interconnected effector and regulatory B and T cell networks. B cell depletion allows for re-establishment of the regulatory network and favors development of tolerance upon B cell repopulation. In this model, a break in tolerance of either the B or T cell occurs (panel a), allowing for the initiation of cognate interactions between autoreactive T and B cells and the establishment of a B and T cell dependent feed-forward loop (panel b). This antigen-driven feed-forward loop eventually dominates the response and is no longer susceptible to suppression by regulatory B and T cells. When B cells are depleted (panel c), the T/B feed-forward loop is broken and the number of responding T cells decreases. This results in decreased inflammatory cytokine production by the autoreactive T and B cells favors the generation, expansion and function of regulatory T cells (panel d) that can suppress remaining autoreactive effector T cells. Finally, the transitional and naïve B cells that initially repopulate the patient (panel e) are likely to have regulatory or tolerance inducing capacity, similar to the B cells found in neonates. These B cells can drive the induction or expansion of new cohorts of regulatory T cells, resetting the balance of regulatory and effector T cells to favor tolerance rather than responsiveness.
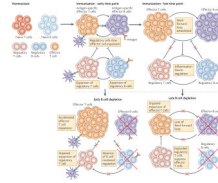
Figure 2. B cells both suppress and enhance T cell mediated immunity
Depletion of B cells at different times following immunization can have drastically different consequences for T cells (ref 57), suggesting that B cells perform functionally and temporally distinct roles during immune responses. In unmanipulated animals, naïve and regulatory B and T cells are present but few effector cells are found. At early timepoints following immunization, antigen-specific effector T and B cells expand, however regulatory T and B cells are also expanding and can slow the proliferation of the effector T cells. Once the ratio of regulatory to effector cells is biased toward the effector response and the cytokine milieu is dominated by pro-inflammatory cytokines, then the regulatory loop is repressed and the B/T cell antigen-driven feedforward loop is established. When B cells are depleted early, before the immune response is initiated, the regulatory B and T cell loop is not established, leading to an unchecked effector T cell response. By contrast, when effector and regulatory B cells are depleted later in the immune response, then effector T cell expansion and inflammatory cytokine production are curtailed due to interruption of the effector T/B cell feed-forward loop. Regulatory B cells are also eliminated by the depletion therapy, but the remaining regulatory T cells which are no longer exposed to the high level inflammatory cytokines can expand and more efficiently suppress the remaining autoreactive effector T cells.
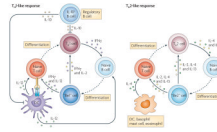
Figure 3. Effector and Regulatory B cells play opposing roles in immune responses
Effector and regulatory B cells can present antigen to CD4$^+$ T cells and can provide co-stimulation and cytokines. Effector B cells (Be-1 and Be-2) can secrete cytokines such as IFNγ, IL-12, IL-4 and IL-2 that reinforce and stabilize the cytokine profile of effector T_H1 and T_H2 cells. In addition, the effector B cells can recruit additional naïve T cells into the inflammatory response. In contrast, the regulatory B cells produce IL-10 which suppresses the inflammatory potential of effector T cells, alters the activity of antigen-presenting DCs and promotes regulatory T cell development and expansion. Thus, effector B cells act as accelerators of CD4$^+$ T cell responses while regulatory B cells act as brakes on the response. In autoimmune disease, the ratio between effector B and T cells and regulatory B and T cells likely favors the effector B cells. Drugs that selectively deplete the effector B cells may be highly effective in resetting this balance in favor of the regulatory populations.

Table 1

Impact of B cell depletion therapy on Effector and Regulatory T cells (humans)

Disease (Human or Mouse)	Treatment protocol	AutoAb titres	T cell activation	T cell prolifera-tion	T cell and systemic cytokines	Regulatory T cells	Effect on disease	Reference
pemphigus vulgaris (human)	Rituximab	Decreased in all patients	ND	ND	Decreased in responder patients	ND	8/11 patients complete remission	28
idiopathic thrombocy-topenic purpura (human)	Rituximab	ND	ND	Altered T cell repertoire	Altered Th1/Th2 ratio	ND	Reduced in ~50% of patients	29
mixed cryoglobuli-nemia vasculitis (human)	Rituximab	Decreased	DecreasedHLA-DR + CD8+ T cells	ND	Decreased systemic IL–12 and IFNγ	Increased FOXP3+ CD25+ CD4+ T cells	Complete remission in 14/21 of patients	36
SLE (human)	Rituximab	No effect on anti-dsDNA	Decreased CD40L + CD4+ T cells	ND	ND	Increased FOXP3, GITR, CTLA4, CD25 and TGFβ1 mRNA in PBL	80% with complete or partial remission	31, 32
SLE (human)	Rituximab	anti-dsDNA and anti-ANA titres decreased in some patients	Decreased CD69+ CD4 T cells	ND	Altered Th1/Th2 ratio	ND	Remission in 8/9 patients	96
SLE (human)	Rituximab	decreased anti-dsDNA no change in anti-Ro or anti-La	Increased CD25+ CD4+ T cells	ND	ND	Increased CD25bright Foxp3+ CD4+ T cells	Improvement in 100% of patients	34
SLE (human)	Rituximab	decreased anti-dsDNA, anti-ANA and anti-phospholipid Abs in subset of patients	ND	Increased apoptosis	ND	Increased CD25bright and TGFβ1+ CD4+ T cells Increased suppressor activity in vitro	Disease remission in 90% of patients	33

Table 2

Impact of B cell depletion therapy on Effector and Regulatory T cells (mice)

Disease (Human or Mouse)	Treatment protocol	AutoAb titres	T cell activation	T cell proliferation	T cell and systemic cytokines	Regulatory T cells	Effect on clinical disease	Reference
SLE (Human CD20 trangenic mice)	Rituximab	Decreased	Decreased memory T cells	ND	ND	ND	In animals with good B depletion see reduced disease severity	27
Diabetes (human CD20 transgenic mice)	Rituximab Before or after development of insulitis. after establishment of clinical disease	Decreased	ND	ND	Decreased IFNγ and IL-17	Increased CD25+Foxp3+ CD4+ splenic T cells	Prevents or delays onset when given early, ameliorates disease in ~40% of animals when given after disease onset	37
Diabetes (mice)	Anti-mouse CD20 Before development of insulitis	ND	No effect	Decreased following anti-CD3 + CD28	ND	No effect	Prevents or delays onset depending on timing of anti-CD20 administration	97
Diabetes (mice)	BCMA-Fc at the time of insulitis	ND	Decreased CD40+ CD4+ and CD8+ T cells		Decreased systemic IL-7, IL-15 and IL-17	Increased CD25+Foxp3+ CD4+ T cells	Prevents development of disease	38
Diabetes (mice)	Anti-CD22 At the onset of insulitis After onset of clinical disease	ND	ND	ND	Decreased IFNγ producing T cells and reduced systemic inflammatory cytokines	Increased CD25+Foxp3+ CD4+ T cells	Prevents onset when given early and reverses disease when given after onset	39
EAE (mice)	Anti-mCD20 Deplete before disease	No AutoAb	Increased CNS infiltrating MOG-specific CD4+ T cells	Increased	Increased IFNγ and IL-17 producing CD4+ T cells	No effect	Disease is worse	57
EAE (mice)	Anti-mCD20 deplete after initiation of disease	Decreased	Decreased CNS infiltrating MOG-specific CD4+ T cells	Decreased	Decreased IFNγ and IL-17 producing CD4+ T cells	No effect	Disease improved	57
proteoglycan induced arthritis (mice)	Anti-mCD20 deplete after disease onset (after 2 immunizations with autoantigens)	Decreased	ND	Decreased	Decreased IFNγ and IL-17 producing CD4+ T cells	ND	Suppression of histopathology and T cells unable to transfer disease	73
Collagen induced arthritis (mice)	Anti-mCD20 deplete before priming	No AutoAb	ND	Decreased	ND	ND	significant delay and suppression of disease	40 98

Disease (Human or Mouse)	Treatment protocol	AutoAb titres	T cell activation	T cell proliferation	T cell and systemic cytokines	Regulatory T cells	Effect on clinical disease	Reference
Collagen induced arthritis (mice)	Anti-mCD20 deplete after prime + challenge with Collagen	Variable effects depending on isotype	Decreased	ND	Decreased (data not shown)	ND	No suppression of clinical disease	98
Autoimmune thyroditis (mice)	Anti-CD20 Deplete before initiation of disease	ND	ND	ND	Reduced # of T cells in target organ	ND	In animals with good B depletion see reduced disease severity	99

ND- not determined