IL-10-independent regulatory B-cell subsets and mechanisms of action

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Abstract

Although classically B cells are known to play important roles in immune protection via humoral immunity, recently their regulatory mechanisms have been best appreciated in the context of autoimmunity. Several studies have identified different subsets of regulatory B cells that vary not only in their phenotype but also in their mechanism of action. Although the best-studied mechanism of B-cell immune regulation is IL-10 production, other IL-10-independent mechanisms have been proposed. These include maintenance of CD4⁰Foxp³ regulatory T cells; production of transforming growth factor-β, IL-35, IgM or adenosine or expression of PD-L1 (programmed death 1 ligand 1) or FasL (Fas ligand). Given that B-cell-targeted therapy is being increasingly used in the clinic, a complete understanding of the mechanisms whereby B cells regulate inflammation associated with specific diseases is required for designing safe and effective immunotherapies targeting B cells.

Keywords: autoimmunity, CIA, colitis, diabetes, EAE, GVHD, regulatory B cell, SLE

Introduction

A regulatory role for B cells was first observed in the 1970s in the context of delayed hypersensitivity reactions, whereby B cells suppressed disease as measured by skin swelling (1). Decades after this initial observation, the first definitive evidence for a regulatory role for B cells in resolving autoimmune disease was demonstrated in part by us (2). In this study, experimental autoimmune encephalomyelitis (EAE), a mouse model of the human central nervous system (CNS) autoimmune disease multiple sclerosis (MS), was used to study the regulatory potential of B cells. The authors observed that unlike wild-type mice, which presented with a monophasic disease accompanied by spontaneous recovery, mice rendered genetically deficient in B cells (µMT) exhibited chronic disease (2).

This novel result demonstrated for the first time in autoimmunity that B cells play a role in attenuation of inflammation. In the ensuing decades, studies on the mechanisms of immune regulation by B cells gained momentum leading to the discovery of varied populations of regulatory B cells in the context of different autoimmune diseases, as reviewed elsewhere (3–6).

The best-studied mechanism of regulatory B-cell function is their ability to produce the immune-suppressive cytokine IL-10 (4). IL-10 plays an important role in dampening an immune response through inhibition of pro-inflammatory cytokine production, co-stimulatory molecule expression and antigen presentation (7). Although all B cells have the capacity to produce IL-10, the signals driving its production in vivo are not well understood. In this context, toll-like receptor (TLR) ligands are potent stimulators of B-cell IL-10 production (8).

Although B-cell stimulation by microbial molecules via TLRs might occur in some autoimmune diseases, in many others, it is debatable. Moreover, IL-10 can also affect B-cell differentiation, activation and function (9, 10). Thus, it is possible that apart from having a direct regulatory effect, IL-10 might modulate other mechanisms of regulatory B-cell function. For example, in the absence of IL-10, a CD73-mediated regulatory function of B cells, via production of adenosine, is diminished due to reduced surface expression of CD73 (11). Hence, a complete understanding of the IL-10-independent mechanisms of regulatory B-cell function is crucial for harnessing the therapeutic potential of this newly identified, potent immune suppressor.

Although IL-10 production is thought to be a hallmark of regulatory B-cell function, in recent years, IL-10-independent mechanisms have been reported in various models of autoimmune disease. From these studies, it is apparent that varied mechanisms exist whereby B cells suppress autoimmune responses (Table 1). This diversity in functional capacity is
likely attributable to differential signals received by B cells during an ongoing immune response in the context of specific autoimmune diseases.

IL-10-independent regulatory B-cell functions in disease

Experimental autoimmune encephalomyelitis

EAE in the mouse is thought to be a CD4+ T-cell-mediated inflammatory disease of the CNS that mimics certain clinical aspects of MS. As mentioned above, Wolf et al. first showed that B cells are important for recovery from EAE (2). Several groups subsequently confirmed this finding, demonstrating a role for B-cell production of IL-10 for their immune suppression (37–39). However, numerous studies also documented mechanisms of regulatory B-cell function independent of IL-10. The first evidence came from an elegant study by Wilson et al., using B cells from helminth-infected mice (12). It was found that CD19+ mesenteric lymph node (MLN) B cells from helminth-infected mice could confer protection upon transfer to uninfected mice and alleviate EAE in the recipients. Importantly, the same protective effect was observed when IL-10-deficient donor B cells were used. Although this study indicated an IL-10-independent function of regulatory B cells, the mechanism was not elucidated.

Extrapolating from previous studies documenting an expansion of regulatory T cells (Treg) following parasitic infection (40, 41), it is tempting to speculate that helminth-induced regulatory B cells might be involved in increasing the number and/or function of Treg. While this hypothesis is yet to be tested, in a recent study, we have documented involvement of B cells in the homeostatic maintenance of CD4+Foxp3+ peripheral Treg (13). Genetic or antibody-mediated ablation of B cells resulted in a significant decrease in the absolute numbers of splenic Treg accompanied with chronic EAE (13).

Mechanistically, we found that B cells induced the proliferation of Treg. Interestingly, this regulatory property of B cells did not require IL-10, as shown using IL-10-deficient B cells, but was dependent on B-cell expression of glucocorticoid-induced tumor necrosis factor-related receptor (GITR)-ligand (GITRL) (13). Blocking GITRL on B cells abrogated their ability to maintain peripheral Treg and thus their ability to resolve EAE disease (13). Treg exhibit high surface expression of GITR (42, 43), which when ligated by Fc-GITRL leads to their expansion (44). In agreement, transgenic mice with a B-cell-specific overexpression of GITRL harbored increased numbers of Treg (45).

Apart from GITR–GITRL, other co-stimulatory molecules, like programmed death 1 (PD-1) and its ligand PD-L1 are also implicated in regulatory B-cell function. Previously, it was found that estrogen treatment had a protective effect in EAE (46). Investigating the mechanism, it was found that this hormone-mediated protection required B cells (14), which correlated with an overexpression of PD-1 on Treg (15). PD-1 plays a crucial role in the development and function of Treg (47). A later study showed that the up-regulation of PD-1 on Treg in the presence of estrogen required PD-L1, but not PD-L2, expression on B cells (16). Although this suggests a regulatory mechanism whereby B cells via PD-L1 provide activating signals to Treg through PD-1 resulting in suppression of immune responses, an estrogen-mediated suppressive effect of other cells cannot be ruled out. Also, it is not known whether estrogen up-regulates PD-L1 expression on B cells.

A recent study provided more direct evidence of a PD-L1-mediated regulatory function of B cells (17). It was found that PD-L1 is differentially expressed on B cells and that PD-L1+ B cells could suppress EAE upon adoptive transfer (17). Interestingly, these PD-L1+ regulatory B cells expressed high
amounts of B cell-activating factor receptor (BAFF-R) and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and negatively regulated T-cell differentiation (17). These studies highlight the importance of co-stimulatory molecules in B–T-cell interactions for the regulation of immune responses. Furthermore, PD-1 is expressed on B cells and recruited to the B-cell receptor upon stimulation, and negatively regulates B-cell activation (48). It will be beneficial to determine when and how the above co-stimulatory molecules are expressed and/or up-regulated on B cells, which could be therapeutically utilized for suppressing an unwanted immune response.

B-cell-secreted cytokines, other than IL-10, have been implicated in regulatory B-cell function. Recently, Fillatreau and colleagues identified an IL-35-mediated regulatory function of B cells in EAE (18). IL-35 belongs to the IL-12 family of cytokines and possesses immunosuppressive properties (49). It is thought that IL-35 inhibits effector T-cell function, thereby limiting immune responses. Although IL-35-mediated immune suppression by Treg has been reported (50), it was not known whether regulatory B cells used the same mechanism. Interestingly, IL-35-mediated immune regulation was not independent of IL-10 (18). Rather, B cells required both cytokines to attenuate EAE (18). When B cells were deficient in either IL-35 or IL-10, they lost their suppressive ability (18).

It is possible that IL-35 is required for the development and/or maintenance of IL-10-producing B cells or that it facilitates IL-10 production by B cells. In a previous study, Tedder and colleagues demonstrated that IL-10-producing B cells required IL-21 for their expansion and function (51). The source of the IL-21 was from T cells, not B cells (51). Similarly, administration of IL-33, thought to have both pro- and anti-inflammatory properties, resulted in the induction of an IL-10-producing B-cell subset (52). It is also possible that some of the above cytokines might affect the aforementioned regulatory B-cell mechanisms involving co-stimulatory molecules. Indeed, apart from expanding IL-10-producing B cells, IL-21 has also been shown to promote granzyme B-expressing regulatory B cells in a tumor setting (53). Thus, comprehensive studies of the interdependence of different regulatory B-cell mechanisms are required to fully understand how to harness their clinical potential.

Diabetes

Type 1 diabetes (T1D) is an autoimmune disease where immune cells attack and destroy insulin-producing β cells in the pancreas. Non-obese diabetic (NOD) mice are the most prevalent model to study the etiology of T1D. Although self-antigen presentation by B cells is thought to initiate autoimmunity in NOD mice, a regulatory role for B cells has also been reported. Lipopolysaccharide-activated B cells upon transfer into prediabetic NOD mice prevented the onset of autoimmune diabetes (19). These B cells expressed Fas ligand (FasL) and secreted transforming growth factor-β (TGF-β) (19). Similarly, CpG-activated B cells with increased expression of FasL upon adoptive transfer protected NOD mice from T1D by induction of effector T-cell apoptosis (20). Since TLR ligands are potent stimulators of B-cell IL-10 production (8), the possibility exists for IL-10-mediated regulation of T1D by TLR-activated B cells. However, it was observed that TLR-stimulated B cells had no significant increase in IL-10 production (19, 20) and blockade of IL-10 receptor did not rescue effector T cells from suppression by CpG-activated B cells (20). These studies suggest that TLR-activated B cells could regulate autoimmunity by inducing apoptosis of pathogenic T cells via FasL and inhibiting the function of antigen-presenting cells via TGF-β secretion.

Transplant tolerance

Organ transplantation is the only hope for patients suffering from terminal organ failure. Although a life-saving process, the success of allogeneic transplantation is marred by graft versus host disease (GVHD). Engraftment is possible with the use of immunosuppressive drugs, but preventing chronic GVHD is a major clinical challenge. GVHD occurs when the recipient immune system recognizes the graft as foreign and mounts an immune response. It has been suggested to use regulatory B cells to promote transplant tolerance. In this regard, anti-CD45RB monoclonal antibody therapy has been shown to induce transplant tolerance with a suggestive role for Treg that likely by suppressing the host anti-graft T-cell response facilitates engraftment (54, 55). Interestingly, it was found that B cells were required for anti-CD45RB-mediated tolerance in a cardiac allograft model (21). B-cell deficiency in graft recipients resulted in loss of tolerance with anti-CD45RB therapy, which was restored upon adoptive transfer of B cells (21). Anti-CD45RB-treated B cells had increased expression of ICAM-1, and blocking ICAM-1–LFA-1 interactions abolished the tolerogenic effect of the B cells (22). Further investigation revealed that this mechanism of B-cell-mediated tolerance was independent of IL-10 (23). This suggests a regulatory mechanism involving B–T-cell interactions resulting in tolerance induction. A recent study explored this possibility and found that regulatory B cells, generated upon anti-CD45RB therapy, were involved in increasing the number of Treg likely through TGF-β (24).

A hallmark of B-cell function is antibody production. Although antibodies are implicated in graft rejection, they might also promote tolerance and thus be protective. IgG molecules with catalytic activity are thought to be immunosuppressive (56). Increased catalytic activity of circulating IgG is associated with a reduced risk of chronic allograft nephropathy in patients (25). It will be worthwhile to determine the factors that might promote catalytic IgG production by B cells in a transplant setting which could be utilized for tolerance induction.

Another important observation was the accumulation of B cells with an inhibitory phenotype in a long-term cardiac allograft model (26). These B cells highly expressed the inhibitory receptor FcγRIIB. Upon adoptive transfer, these FcγRIIB<sup>+</sup> B cells prevented cardiac allograft rejection in the host. Although it is known that FcγRIIB inhibits B-cell activation and restrains its ability to present antigen to T cells (57, 58), it was not clear whether FcγRIIB<sup>+</sup> B cells regulated the pathogenic B cells, or T cells, or both (26). It will be interesting to further elucidate how these regulatory FcγRIIB<sup>+</sup> B cells are generated and their detailed mechanism of immune regulation.
FasL is an important molecule involved in immune suppression and thought to be utilized by tissues at immune-privileged sites for the induction of apoptosis of infiltrating immune cells that express Fas (59). Interestingly, it was found that FasL− B cells could mediate immune tolerance in a male-to-female skin graft model (27). Splenic B cells from male mice, upon transfer, tolerized female recipients resulting in permanent acceptance of male skin grafts. But, transfer of B cells from male FasL-defective (gld/gld) mice failed to induce tolerance, resulting in graft rejection (27).

Collagen-induced arthritis
Collagen-induced arthritis (CIA), an animal model of rheumatoid arthritis, is a chronic inflammatory disease of the synovium. Several studies have identified B-cell-mediated IL-10 immune suppression of CIA (3). Other than IL-10, FasL has been implicated in regulatory B-cell function in CIA. In a T-cell receptor (TCR) transgenic model of CIA, reduced numbers of splenic FasL−CD5+ B cells correlated with increased arthritis severity (28). A reduced presence of these FasL− B cells was also associated with decreased T-cell death, suggesting a regulatory mechanism whereby B cells mediate tolerance by inducing apoptosis of pathogenic T cells via Fas–FasL.

Allergic diseases
Dysregulated inflammatory responses to allergens due to altered immune regulatory mechanisms are thought to contribute to allergic diseases (60). B cells play an important role in immune regulation in the control of allergy. The first evidence of a regulatory role for B cells in allergy was observed in the context of the ovalbumin (OVA)-induced skin hypersensitivity reaction in guinea pigs (1). Although evidence of B-cell-mediated suppression of skin thickness in response to OVA was reported, the precise mechanism was not understood at the time (1). Recent studies provide mechanistic insight into the function of regulatory B cells in allergy. Upon immunization with cockroach allergen, FasL expression was up-regulated on CD5+ B1 cells, which regulated cockroach allergen-induced airway inflammation by inducing apoptosis of effector CD4+ T cells (29). Interestingly, further characterization revealed that only 4% of these killer B cells produced IL-10 (61).

TGF-β is also implicated in regulatory B-cell-mediated control of allergic reactions. Chronic exposure to OVA resulted in oral tolerance with selective expansion of TGF-β-expressing CD5+ B cells in the draining lymph nodes (30). These B cells were capable of inducing Foxp3 expression in CD4+ cells, and upon adoptive transfer, suppressed OVA-induced lung inflammation possibly by increasing the number of Tregs (30). Similarly, a population of CD5+ B cells from the intestinal mucosa, that also expressed TGF-β, suppressed food allergy-induced inflammation in mice (31). In an OVA-induced food allergy model, upon sensitization with OVA, CD35+ B cells were increased in the lamina propria and expressed thrombospondin (TSP) 1 (32). TSP-1-secreting CD35+ B cells induced Treg differentiation via TGF-β, but not IL-10, and down-regulated co-stimulatory molecule expression on dendritic cells (32). Adoptive transfer of these CD35+ B cells suppressed allergic inflammation in the intestine (32). These studies highlight the different mechanisms, other than IL-10 secretion, utilized by B cells in regulating allergic diseases.

Colitis
B cells have also been shown to play a regulatory role in mouse models of colitis. The first such evidence was in TCRα− mice, which develop spontaneous enterocolitis (62). Colitis severity was exacerbated when TCRα− mice were also deficient in B cells (TCRα− × μMT) as indicated by increased disease severity during the early and late phases of colitis as compared with TCRα− mice (62). This same group subsequently determined that when TCRα− mice were housed in a conventional versus a specific pathogen free (SPF) facility, colitis severity was reduced after five generations because of increased production of natural IgM by B1 cells, which were protective upon transfer into TCRα− × μMT mice (33). Moreover, the level of IL-10 production by B cells from TCRα− mice housed in conventional versus SPF conditions was comparable (33).

In dextran sulfate sodium (DSS)-induced colitis, a similar role for IgM production in protection from colon damage was also reported (34). Antibiotic treatment indicated a role for the microbiome in protection. MyD88 signaling in B cells was shown to be critical for the production of IgM that controlled intestinal bacteria via a complement-mediated mechanism (34). In addition, CD73 expression by B1 cells (B220+CD23−) and/or B10 cells (B220+CD23+AA4.1+) was suggested to attenuate DSS colitis via their conversion of adenosine monophosphate to adenosine, which is anti-inflammatory (11, 63). Interestingly, CD73-deficient B cells exhibited no alteration in IL-10 production, but IL-10−/− B cells produced less adenosine (11). These data indicate that at least for some disease models, immune suppression by B10 cells may be due to reduced adenosine production. However, it is unclear whether IL-10 directly regulates the expression of CD73.

In colitis induced by the adoptive transfer of Gα2−/− CD4+ T cells, protection required co-transfer of both MLN B cells and CD8α− T cells (35). The transferred B cells homed to secondary lymphoid sites, and protection was associated with the expansion of CD3+NK1.1+ T cells at that site and an increase in CD4+CD8α− T cells in the intra-epithelial intestinal compartment (35). Furthermore, B cells from Gα2−/− mice lost their protective capacity indicating a role for G-protein-coupled receptors either in the development of regulatory B cells or in their function (35).

Splenic B cells isolated from Hymenolepis diminuta-infected mice were shown to be protective in a number of colitis models, including DSS colitis, via a mechanism requiring macrophages and possibly their production of TGF-β (36). Cumulatively, these studies in colitis demonstrate that B cells can regulate the severity of disease by multiple IL-10-independent mechanisms.

Systemic lupus erythematosus
IL-10-producing B-cell subsets with various origins have been identified as regulators in mouse systemic lupus erythematosus (64). However, Teichmann et al., using MRL. Fas(lpr) mice that spontaneously develop lupus, showed that
IL-10 production by B cells failed to attenuate disease (65). This was demonstrated by the generation of Lpr mice with a conditional deletion of IL-10 in B cells, which exhibited similar disease severity and mortality as controls (65). The IL-10-independent B-cell regulatory mechanisms have not yet been described in lupus.

Conclusions

Although the existing literature highlights different mechanisms utilized by regulatory B cells, it is not known whether specific subsets of B cells are endowed with these regulatory properties or whether certain signals received by B cells during an ongoing immune response lead to their generation. Furthermore, it is likely that different mechanisms utilized by regulatory B cells might cross-talk for effective suppression of an immune response.

In EAE, regulatory B cells have been shown to up-regulate PD-1 on Treg (15), whereas in colitis, B cells controlled disease by adenosine production through CD73 expressed on their surface (11). These two apparently independent mechanisms might have a common link. For instance, one of the known immunosuppressive functions of adenosine is the up-regulation of PD-1 cell surface expression on Treg via the adenosine 2A receptor (66).

Although not well studied, the possibility exists for a cooperative multi-mechanism regulation of immune responses by B cells. Indeed, expression of FasL, utilized by B cells to mediate apoptosis of autoreactive T cells (12), is up-regulated by IL-10 (17). Interestingly, both Fasl+ B cells and IL-10-producing B10 cells share some phenotypic similarities in that they both express CD5. There exists a possibility for a feed-forward regulatory mechanism regulated by adenosine production through CD73 expressed on regulatory B cells might cross-talk for effective suppression of an ongoing immune response.

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