A Case for Regulatory B Cells
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A Case for Regulatory B Cells

Atsushi Mizoguchi and Atul K. Bhan

B cells are typically characterized by their ability to produce Abs, including autoantibodies. However, B cells possess additional immune functions, including the production of cytokines and the ability to function as a secondary APC. As with T cells, the B cell population contains functionally distinct subsets capable of performing both pathogenic and regulatory functions. Recent studies indicate that regulatory B cells develop in several murine models of chronic inflammation, including inflammatory bowel disease, rheumatoid arthritis, and experimental autoimmune encephalomyelitis. The regulatory function may be directly accomplished by the production of regulatory cytokines IL-10 and TGF-β and/or by the ability of B cells to interact with pathogenic T cells to dampen harmful immune responses. In this review, we make a case for the existence of regulatory B cells and discuss the possible developmental pathways and functional mechanisms of these B cells. The Journal of Immunology, 2006, 176: 705–710.

Once generated, immune responses need to be regulated to prevent the responding effector cells from causing harmful effects. The identification of functionally distinct regulatory T cell (Treg) subsets, such as CD4+CD25+ Tregs, IL-10-producing T regulatory 1 (Tr1) cells, and TGF-β1-producing Th3 cells (1–7) among the effector CD4+ T cell population, has revealed one pathway by which immune responses are regulated. It is likely that regulatory pathways involving other immune cells also exist. B cells, a major immune cell population, can play a pathogenic role in acquired immune responses by producing autoantibodies that contribute to the development of autoimmune diseases (8–11). In addition to such pathogenic B cells, recent studies indicate coexistence of distinct B cell subsets that suppress the progression of and/or enhance the recovery from acquired immune-mediated inflammations by mechanisms that include IL-10 and TGF-1 production, secondary Ag presentation, and interaction with other immune cells either directly or through secreted Abs (Fig. 1).

The existence of an immunoregulatory B cell subset that plays a role in immune regulation resulting in complete recovery from acute experimental autoimmune encephalomyelitis (EAE) was first reported by Janeway and colleagues (12) in a murine model of EAE. A regulatory B cell subset capable of enhancing the recovery from this EAE by the production of IL-10 has also been identified (13). Recently, further evidence for the existence of regulatory B cells has come from other experimental models of chronic inflammation (Table I). These studies indicate that, like their T cell counterparts, B cells can be divided into functionally distinct regulatory subsets capable of inhibiting inflammatory responses and inducing immune tolerance. During the last decade, the development of experimental models of inflammatory bowel disease (IBD) has provided an unexpected opportunity to dissect immune networks and regulatory pathways in chronic inflammation (14–16). In most of these models, the chronic inflammation is mediated by the Th1 pathway and resembles Crohn’s disease, a major subgroup of IBD. The inflammation in these models appears to be regulated primarily by Tregs (5). Studies performed by our lab and others in TCRα knockout (KO) mice indicate that the spontaneous colitis in these mice is mediated by Th2 pathway and the disease resembles ulcerative colitis (UC), the other major subgroup of IBD (14, 16). Since the colitis in these mice, as with UC, is associated with presence of autoantibodies (17–20), a pathogenic role of B cells in this disease was initially postulated (18, 20). However, the development of much more severe intestinal inflammation in B cell-deficient TCRα double KO (αβDKO) mice indicated that B cells more likely contribute to the suppression of this UC-like disease (21). Cell transfer studies confirmed this possibility (22, 23). Recent studies by several groups, using different kinds of genetically engineered models, have provided further proof that B cells can regulate UC-like intestinal inflammation (6, 14, 24–28). Moreover, the presence of regulatory B cells has also been identified in some Crohn’s disease-like disease models (29–31). Neonatal thymectomized BALB/c mice that lack Tregs and nu/nu mice, when reconstituted with Treg-depleted T cells, spontaneously develop gastriitis, thyroiditis, oophoritis, and orchitis but not colitis (7, 32, 33). In contrast to these two mouse groups, which have B cells, colitis is reproducibly induced by the transfer of Treg-depleted...
FIGURE 1. Regulatory mechanisms of B cells in immune responses. These include: 1) the production of IL-10 that restores Th1/Th2 balance (1a) and directly inhibits inflammatory cascades (1b); 2) the production of TGF-β1 that induces apoptosis of effector T cells; 3) the ability to dampen activated CD4\(^+\) T cells directly or by acting as a secondary APC; 4) the recruitment of Treg subsets (CD8\(^+\) T cells and NKT cells) in a β2-microglobulin-dependent fashion (MHC class I and CD1d); 5) the production of IgG and IgA that neutralize harmful soluble factors (5a), dampen DC/macrophage activation through the IgG/FcγRIIB interaction (5b), and enhance the clearance of apoptotic cells that are potential source of self-Ags for activating self-reactive T cells (5c).

CD4\(^+\) T cells in the recipient SCID mice that lack B cells (7, 34). Interestingly, regulatory B cells (Bregs) may also be present in diseases such as lupus and experimental rheumatoid arthritis (RA) (10, 35–38), where B cell-producing autoantibodies have a pathogenic role (9, 10, 39) (Table 1).

Tregs are considered to be a major player in immune regulation (3, 5, 7). A recent study performed in EAE indicates that both B cells and Tregs are involved in the regulation of CNS autoimmune disease; B cells may limit the continued expansion of fresh pathogenic T cells from lymph nodes, whereas Tregs directly control this disease at the site of inflammation (40). In this brief review, we will focus on the direct role of B cells in the regulation of inflammatory reactions.

**Cytokine-producing regulatory B cells**

As compared with T cells, B cells have not typically been considered to be a major source of cytokines in immune reactions. However, studies by Harris et al. (41, 42) have elegantly demonstrated that, like CD4\(^+\) T cells, B cells can also produce a wide spectrum of cytokines under inflammatory conditions induced by Toxoplasma gondii or Heligmosomoides polygyrus infection. Notably, B cells produce IL-10, the regulatory cytokine that can suppress harmful immune responses by regulating Th1/Th2 balance and directly dampening innate cell-mediated inflammatory responses (43–46). Activated murine B cells capable of producing large amounts of IL-10 have now been detected in vivo under a variety of experimental inflammatory conditions, including IBD (22, 29), EAE (13, 47), arthritis (36), lupus (37, 38), UV irradiation, and infection with *Schistosoma mansoni* (48) and *Brugia pahangi* (49). B cells also produce IL-10 following in vitro activation with LPS (29), CpG (37, 38), or heat shock protein 60 (50). These inflammation-induced IL-10-producing Bregs inhibit the progression of inflammation and/or hasten the recovery from the experimental inflammatory conditions such as IBD (23, 29), EAE (13), arthritis (36), and lupus (37, 38). Such IL-10-producing B cells have also been identified in humans (51).

The mechanisms by which Bregs suppress inflammation are likely to vary depending on the type of inflammatory response. In IBD where both innate and acquired immune responses are involved in the dysregulated host/microbial interactions (14, 15, 52), Bregs suppress exacerbation/perpetuation of inflammation by directly dampening proinflammatory networks (IL-1 and TNF-α production by macrophages) (23). In EAE and RA, both of which are mainly mediated by T cell-mediated acquired responses, Bregs suppress inflammation by restoring the Th1/Th2 balance (13, 36). Bregs also dampen immune responses that are mediated by dendritic cells (DC) in lupus mice (37, 53).

In addition to IL-10-producing Bregs, a Breg subset that is capable of producing TGF-β1 after in vitro stimulation with LPS has been identified (47, 54, 55). This TGF-β1-producing regulatory B cell subset participates in the induction of low-dose oral tolerance (47, 55). Studies performed in B cell-deficient NOD mice demonstrate that B cells are required for the development of this disease by acting as critical APC for the presentation of pathogenic Ags to effector T cells (56, 57). Interestingly, the transfer of in vitro generated TGF-β1-producing B cell subset following in vitro LPS stimulation suppresses diabetes in the recipient mice by inducing apoptosis of the effector T cells (54). It is also conceivable that, unlike IL-10-producing Bregs, the TGF-β1-producing B cells subset do not develop in vivo. Nevertheless, these findings indicate that in vitro-generated regulatory B cells may provide a new therapeutic strategy to suppress B or T cell-mediated organ-specific autoimmune diseases.

**Activation/differentiation pathway of Bregs**

IL-10-producing Bregs appear under inflammatory conditions (Fig. 2) and are not detected in normal states (23). Although IL-10 production is hardly detectable in the splenic B cells from wild-type mice, these cells produce IL-10 upon transfer into B

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cell-deficient TCRα KO mice with, but not without, intestinal inflammation (23). Therefore, inflammatory microenvironment appears to be required for the differentiation and/or activation of Bregs.

IL-10 production is a well-known feature of peritoneal CD5+ B1a subset (58–60). However, several studies indicate that the phenotype of IL-10-producing Bregs (CD11b−CD5−IgD+) is similar to that of B2 (conventional B cells) but not B1a (CD11b+CD5+IgD−) cells. Furthermore, the Ig secretion pattern (class switching into IgG and IgA) in Bregs vs production of natural IgM in B1 (13, 23, 28, 29, 48) also suggests that Bregs are derived from B2 lineage. This conclusion is supported by our recent study showing that IL-10-producing Bregs are undetectable in the absence of IL-7-signaling cascade (Y. Shimomura, unpublished observation); IL-7 signaling is required for the development of B2, but not B1, lineage cells (61). A potential progenitor of Bregs has been identified in the spleen (62). This B cell subset, which produces IL-10 and acts as a secondary APC using the CD40 and CD86 pathways, is characterized by low cell density and by a unique phenotype (62); this subset expresses CD43, which is generally expressed by B1 but not B2 subset (63), and high levels of CD1d, a feature of splenic transitional type 2 (T2) and marginal zone (MZ) B cells (28, 64, 65). Since CD43 Abs are widely used to enrich B cells but not B2 subset (63), and high levels of CD1d, a feature of splenic transitional type 2 (T2) and marginal zone (MZ) B cells (28, 64, 65). Since CD43 Abs are widely used to enrich B cells by negative selection (removal of CD43+ cells), this separation method may lead to loss of Bregs in the isolated B cell (purified/enriched) population.

Depending upon the type of inflammation present, there are at least two possible pathways by which splenic B cells may differentiate into Bregs (Fig. 2). Breg activation in acquired immune-mediated diseases such as EAE and RA may occur through BCR ligation with self-Ag and/or CD40/CD40L interaction (13, 36). This Breg represents the "acquired type" subset that originates from activated follicular B cell population. Since the pathogenesis of IBD involves both acquired and innate immune responses in the gut-associated lymphoid tissues (GALT) to enteric bacterial products (52, 66), Bregs are most likely to develop in the mesenteric lymph nodes (MLN, a component of GALT) in IBD (23, 30). Interestingly, the MLN Breg shares phenotypic and functional features with the splenic MZ B cell subset (23, 28–30); like splenic MZ B cells (28, 64, 65), the MLN Breg is characterized by a high level of CD1d expression and responsiveness to LPS. Because Breg production in IBD requires polyclonal but not Ag-restricted monoclonal activation of B cells (26), MLN Bregs may represent an "innate type" Breg subset that presumably originates from the splenic MZ B cell population (28). Spontaneous colitis develops in G protein α inhibitory subunit (Gain2) KO (67) and p110δ PI3K KO (24) mice, both of which are characterized by the absence of MZ B cells (24, 29), supporting the MZ origin of Bregs. Interestingly, these genes are located in IBD susceptibility loci; Gain2 on chromosome 3p21 and p110δ PI3K on chromosome 1p36 (28, 68, 69). In addition, the presence of decreased numbers of memory B cells as a result of impaired splenic function is associated with the development of IBD in humans (70). Cell transfer studies indicate that although both MLN and splenic MZ B cells suppress intestinal inflammation observed in Gain2 KO mice, the inflammation is much more efficiently suppressed by MLN B cells as compared with splenic MZ B cells (30). During their migration through the MLN, further activation by enteric bacterial products may be necessary for MZ B cells to acquire full Breg function. Alternatively, the differentiation pathway of MLN Bregs may be different from that of splenic B cells as the BCR-signaling cascade required for splenic B cell activation is not involved in the activation of GALT B cells (71).

B cell development into autoreactive cells capable of inducing certain autoimmune diseases is regulated in the splenic MZ (72–74). Depletion of MZ B cells producing IgM anti-DNA autoantibodies by the administration of anti-CD1d mAb results in the inhibition of lupus (75); in contrast, absence of MZ B cells due to the mutation Y-linked autoimmune acceleration (Yaa) leads to the development of this disease (76). Whether the MZ cells become predominantly pathogenic or regulatory may depend on the presence of additional factors. As MZ B cells
produce large amounts of IL-10 in response to LPS or CpG (29, 37, 38) and can regulate intestinal inflammation (30), “terminal” or “super” activation of MZ B cells by bacterial products may be required for MZ B cells to fully mature into Bregs. This is consistent with recent observations that enteric bacterial products such as LPS and CpG are required for the suppression, rather than induction, of intestinal inflammation (77–80).

Mechanisms other than cytokine production by which B cells control immune responses

B cells may also play an important role in the immune regulation by interacting with other cells or through secreted Abs (Fig. 1). As indicated above, B cells can enhance recovery from EAE by modulating immune response (12). B cells by functioning as second line APC (secondary APC) may either support or dampen ongoing T cell responses initiated by DC (81–83). Bregs with secondary APC activity may be involved in the induction of respiratory and systemic immune tolerances (82, 83). Furthermore, B cells suppress spontaneous chronic colonic inflammation by inhibiting proliferation of effector T cells via the CD40/CD40L interactions (22). Similarly, a B cell subset has been shown to suppress systemic autoimmune reaction (skin inflammation, wasting, and death) by down-regulating the TCR expression on effector T cells (82). B cells also dampen the activation and influence the migration of professional APC such as DC and macrophages by the production of IL-10 or by the expression of CXCL 13 (23, 37, 53, 84, 85).

An attractive new mechanism by which MLN Bregs may suppress intestinal inflammation is suggested by the work of Braun and colleagues (30) in Gα2 KO mice. The MLN Breg subset characterized by CD19hi expression is involved in intestinal immunoregulation by recruiting novel Treg subsets, CD8+ T cells, and NKT cells (30), both of which have recently been shown to inhibit intestinal inflammation (86–88). The presence of such a regulatory mechanism is supported by a recent finding that both impairment of B cell activation and decrease of such a regulatory mechanism is supported by a recent observation that enteric bacterial products such as LPS and CpG are required for the suppression, rather than induction, of intestinal inflammation observed in Gα2 KO mice (89). Furthermore, B cells with regulatory functions in addition to playing a pathogenic role in inflammatory conditions and are capable of suppressing the exacerbation of inflammation and/or enhancing the recovery process. There are a number of issues relevant to regulatory B cells, which need to be addressed in future studies. What are the conditions that favor the development of regulatory vs pathogenic B cells? Can regulatory B cells be pathogenic in some inflammatory conditions or represent a terminally differentiated B cell population capable of performing only regulatory function? Are the regulatory functions (production of IL-10 and TGF-β and secondary APC activity) conducted by the same Breg population or by distinct Breg subsets? What are the signaling pathways involved in the activation of Bregs? The existence of Bregs supports the notion that the immune system has developed many different mechanisms to regulate immune responses.

Conclusions

In this brief review, we have made a case for B cells possessing regulatory functions in addition to playing a pathogenic role in inflammatory conditions. The regulatory functions may be conducted either through secreted Abs or more directly by cellular interactions and/or cytokines (Fig. 1). We suggest that B cells with regulatory functions independent of secreted Igs be called Bregs. Bregs are specifically induced under inflammatory conditions and are capable of suppressing the exacerbation of inflammation and/or enhancing the recovery process. There are a number of issues relevant to regulatory B cells, which need to be addressed in future studies. What are the conditions that favor the development of regulatory vs pathogenic B cells? Can regulatory B cells be pathogenic in some inflammatory conditions or represent a terminally differentiated B cell population capable of performing only regulatory function? Are the regulatory functions (production of IL-10 and TGF-β and secondary APC activity) conducted by the same Breg population or by distinct Breg subsets? What are the signaling pathways involved in the activation of Bregs? The existence of Bregs supports the notion that the immune system has developed many different mechanisms to regulate immune responses.

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BRIEF REVIEW: A CASE FOR REGULATORY B CELLS


