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Cytokine-producing B lymphocytes – key regulators of immunity

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Summary

The successful use of B cell depletion therapy for the treatment of autoimmune disease has led to a resurgent appreciation of B cells as powerful regulators of immunity. However, to the surprise of many, B cells appear to regulate autoimmune conditions independently of their ability to produce autoantibodies. Indeed, disturbances in the ability of B cell subsets to present antigen, produce cytokines and regulate the activities of T cells is emerging as a key feature in many inflammatory diseases. Here we review the recent literature describing cytokine-producing regulatory and effector B cell subsets in health and disease and discuss how future B cell-directed therapies might target the pathologic cytokine-producing effector B cell subsets without impacting the protective regulatory subsets.

Introduction

Rituximab, a humanized anti-CD20 antibody, is now being used clinically to deplete B cells in the treatment of multiple autoimmune diseases, including Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA) [1]. Although it would seem reasonable that B cell depletion therapy would ameliorate symptoms of disease by eliminating autoantibody-secreting B cells, many Rituximab-treated patients enter extended periods of clinical remission without reductions in serum autoantibody titers [1]. These data suggest that B cells contribute to disease pathogenesis in an antibody-independent fashion, perhaps by modulating T cell responses.

Although it is not well-appreciated, there is substantial evidence that B cells both amplify and suppress immune responses by mechanisms that do not involve antibody [1]. For example, B cells respond to Toll-like receptor (TLR) ligands and present antigen. B cells also organize the structure of lymphoid tissues and regulate lymphangiogenesis. In addition, B cells produce cytokines and can be subdivided into discrete cytokine-producing “regulatory” and “effector” B subsets [2,3]. Regulatory B cells (Breg) are distinguished by their ability to secrete IL-10 or TGF β -1, while effector B cell populations produce cytokines such as IL-2, IL-4, TNF α , IL-6 (Be-2 cells) or IFN γ , IL-12 and TNF α (Be-1 cells). Here, we examine new data regarding the origin and functional properties of the effector and regulatory B cells subsets. We also review data showing that these cytokine-producing B cells can be either protective or pathologic and discuss the evidence that alterations in the types and amounts of cytokines made by B cells can affect autoimmune responses in both mice and humans.

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Identification of effector and regulatory B cell subsets

Although B cells produce a variety of cytokines [2,4], the first hint that cytokine-producing B cells regulate *in vivo* immune responses comes from studies examining the role of lymphotoxin (LT) and TNF α in lymphoid tissue organogenesis. These studies show that B cell-derived LT α and TNF α control the development of follicular dendritic cells [5,6], the formation of B cell follicles [7] and the development of specialized stromal cell subsets in the spleen [8]. Furthermore, LT α -expressing B cells regulate the formation of ectopic lymphoid tissue in autoimmune target organs [9], suggesting that cytokine-producing B cells contribute to pathology in autoimmune disease. More recent studies show that B cell-derived TNF α also regulates T cell dependent antibody responses (Wojciechowski and Lund, submitted) as well as T cell responses to various pathogens ([10] and Lund *et al.*, unpublished). Thus, the ability of B cells to produce cytokines in the TNF family is clearly important for multiple aspects of immunity.

Like T cells, B cells are not homogenous with respect to cytokine production. B cells primed by Th1 cells and antigen (Be-1 cells) make cytokines associated with type 1 immune responses, such as IFN γ and IL-12, while B cells primed by Th2 cells and antigen (Be-2 cells) make IL-2, IL-13 and IL-4; cytokines often associated with allergic responses [2,11]. Interestingly, IFN γ and IL-12 producing B cells can be identified in mice infected with pathogens that induce Th1 immunity [11,12], while IL-4 producing B cells are found in mice infected with parasites that induce Th2 immunity [11,13]. IFN γ and IL-12 producing Be-1 like cells are also found in human peripheral blood and tonsil [14–17] as well as ectopic lymphoid tissues [18], while IL-13 [19] and IL-4 producing [20] Be-2 cells are found in nasal polyps and germinal centers. Most excitingly, recent experiments clearly demonstrate that both mouse and human cytokine-producing effector B cells amplify effector T cell responses in a cytokine-dependent manner [11,14–16].

While some cytokines made by B cells amplify immune responses, IL-10- and TGF β -producing B cells actively suppress immune responses [3]. For example, IL-10 producing B cells are protective in models of colitis [21–23], promote remission in an EAE model [24,25] and prevent induction of collagen-induced arthritis [26,27]. The activity of IL-10-producing regulatory B cells is now documented in experimental models of tolerance [28–30], tumor rejection [31], infectious disease [32,33] and autoimmunity [21–24,26,27,34,35]. Likewise, there are also several papers describing the regulatory function of TGF β -1-producing B cells [36–38]. Together, these publications demonstrate that there are distinct cytokine-producing effector and regulatory B cell subsets that can independently alter T cell mediated immune responses (Figures 1–2).

Origins of regulatory and effector B cells

B cells are subdivided into two major lineages; B1 cells, which arise from fetal liver precursors and are enriched in mucosal tissues and the pleural and peritoneal cavities, and B2 cells, which arise from bone marrow-derived precursors and are enriched in secondary lymphoid organs [39]. B1 cells can be further subdivided into B1a cells (CD11b⁺CD5⁺) and B1b cells (CD11b⁺CD5[−]), while B2 cells can be subdivided into immature transitional cells (T1, T2 and T3) and mature follicular B cells (FO) or marginal zone (MZ) B cells. MZ B cells and B1 lineage B cells have the ability to respond very rapidly to inflammatory stimuli and antigen and can terminally differentiate into plasma cells in just one to two days. Thus, these B cells can play key roles in the early innate immune response to pathogens. In contrast, FO B cells represent an important component of the adaptive immune response. FO B cells can differentiate into short-lived plasma cells in three to five days after encounter with antigen or can enter a T cell dependent germinal center reaction where the B cells may undergo class

switch recombination and affinity maturation. FO B cells that exit the germinal center seed the long-lived plasma cell or memory B cell pool.

Although little is known about the origins of effector B cells, the current data suggests that effector B cells are derived from FO B cells (Figures 1–2). First, both Be-1 and Be-2 cells can be generated from splenic B cells isolated from BCR transgenic mice that have few marginal zone B cells [11]. Second, experiments examining cytokine production by purified MZ and FO B cells indicate that only FO B cells produce IFN γ after TLR stimulation [40]. Finally, a recently identified long-lived pool of recirculating follicular B cells (FO II B cells) appears to have a greater propensity to make B effector-associated cytokines like IFN γ , IL-12, IL-4 and IL-2 [41], suggesting that there may be specific subsets of FO B cells that are poised to develop into cytokine-producing Be-1 or Be-2 cells.

Many B cell subpopulations, including B1a, transitional, FO and MZ B cells, produce IL-10 and have the potential to function as Bregs [3]. Using adoptive transfer models, functional Bregs have been identified in the B1a B and MZ B lineages [21,22,27,29,30,42]. Since these B cells express TLRs and have the ability to respond rapidly to pathogen-derived products, they have been referred to as innate Bregs and are postulated to down-modulate inflammation associated with infection or disease [3]. Consistent with this hypothesis, TLR ligands elicit poor inflammatory responses in neonatal mice due to a high frequency of IL-10-producing B1a cells [29,42]. The IL-10 made by TLR-activated neonatal B1a cells blocks IL-12 production by TLR9-activated DCs resulting in depressed Th1 priming and a predominant Th2 response [42] (Figure 3). Likewise, neonatal IL-10-producing B1a cells also suppress Th1 responses to alloantigens [30], perhaps explaining the long-standing observation that neonates are more tolerant to allografts than adult mice.

In neonatal mice, the IL-10 producing Bregs block TLR induced inflammation and death [29]. However, B1a cells are not able to suppress lethal inflammatory responses in adults [29], suggesting that they are not a major source of adult Bregs. Instead, B2 cells from secondary lymphoid tissues play key roles in immune suppression in adults. For example, the transfer of normal B cells from mesenteric LNs (mLNs) into hosts with ulcerative colitis greatly diminishes local inflammation [21,22]. Importantly, IL-10 production by the transferred B cells is critical for blocking the inflammatory response [21,22]. While it is not yet known whether these IL-10 producing Bregs are derived from the FO or MZ lineage, the protective mLN B cells express CD1d [21] and high levels of CD19 [22] and thus phenotypically resemble splenic MZ B cells (Figure 2).

In addition to MZ B cells, MZ B cell precursors also suppress autoimmune disease in an IL-10 dependent manner. Immature B cells emerging from the bone marrow enter the spleen and move through additional rounds of selection and maturation (referred to as transitional T1, T2 and T3 cells) [43]. T2 B cells are phenotypically similar to MZ B cells and are reported to be enriched in MZ precursors [44,45]. Transfer of T2 cells from arthritis-recovered mice to susceptible hosts prevented the recipients from developing Th1-mediated arthritis [27] (Figure 1). The T2 cells isolated from convalescent animals produce minimal amounts of IL-12 and IFN γ in response to antigen (collagen), but make larger quantities of IL-10 than either mature MZ or FO B cells [27], suggesting that the cytokine repertoire of these T2 B cells is biased toward anti-inflammatory cytokines. Importantly, IL-10 production by T2 B cells is critical for their suppressive function, since IL-10-deficient T2 cells are unable to transfer protection to arthritis susceptible hosts [27]. Taken altogether, these data support the model that the Bregs and B effectors are separate subpopulations and that the best-studied IL-10-producing Bregs originate from the B lineages that contribute to innate immune responses, while the effector B cells originate from the FO B cell pool.

Molecular control of effector and regulatory B cell development

While there are still many questions that remain regarding the developmental origins of effector and regulatory B cells, progress has been made identifying the signals that regulate the differentiation of these subsets. Not surprisingly, cytokines play a key role in the “commitment” of naïve B cells to the Be-1 or Be-2 lineages. Be-2 differentiation is dependent on the engagement of IL-4R α on B cells [46] (Figure 2), while Be-1 cell development is dependent on the activation of the transcription factor T-bet and the IFN γ R on B cells [47] (Figure 1). Interestingly, both Be-1 and Be-2 cell differentiation can proceed in vitro without BCR ligation, as long as peptide is provided to elicit cognate interactions between B cells and either Th1 or Th2 effectors [46,47]. Indeed, Be-2 development is dependent on T cells that produce IL-4 and engage CD40 as well as CD80/CD86 [46]. In contrast, blocking the CD40/CD154 interactions between B cells and Th1 cells has little impact on Be-1 expansion or IFN γ production [2]. Despite the differences in the signals required to induce Be-1 or Be-2 development, both B cell subsets are competent to secrete antibody. However, the frequency of antibody secreting cells is increased in the in vitro generated Be-1 cultures [46], suggesting that the Be-1 culture conditions facilitate plasma cell differentiation.

Interestingly, unlike Be-2 differentiation, which is absolutely dependent on Th2 cells, Be-1 differentiation can proceed via a T cell independent mechanism [47] (Figure 1). For example, stimulation of naïve FO B cells with a single TLR ligand does not induce IFN γ production or Be-1 development [40]. However, stimulation of naïve B cells with multiple TLR ligands [40] or with a single TLR ligand and IL-12 and IL-18 [47,48] promotes Be1 differentiation and IFN γ production. As described earlier, IL-10 producing Bregs can also be activated by T cell independent stimuli, particularly TLR ligands [3]. However, T cell dependent stimuli can also drive IL-10 production. In fact, both Be-1 and Be-2 cells make IL-10 and human peripheral blood B cells make IL-10 in response to many stimuli, including CD40 ligation [49,50]. Furthermore, since T2 B cells from convalescent arthritic mice transfer protection much more efficiently than T2 cells from naïve mice [27], antigen priming may be required to activate these Bregs. Alternatively, the inflammatory environment may promote the differentiation of Bregs in the absence of antigen. In fact, type 1 interferons produced by TLR activated DCs enhance IL-10 production by neonatal B1a cells activated with TLR2, TLR4, TLR7 and TLR9 ligands [29] (Figure 3). However, type 1 interferons do not potentiate IL-10 production by adult B cells [29] and instead appear to facilitate effector cell cytokine production (Lund and A. Marshak-Rothstein, unpublished observation). Thus, the local cytokine milieu plays a critical role in regulating the types and quantities of cytokines produced by B cells.

Dichotomy in the cytokine repertoire of naïve and memory B cells – impacts on autoimmunity

Both proinflammatory (TNF α , LT α , IL-12, IL-6) and suppressive (IL-10) cytokines are produced by cultures of total peripheral blood B cell that contain a mixture of naïve and memory B cells [14,15,17,50]. This balance of pro- and anti-inflammatory cytokines is altered in B cells from patients with multiple sclerosis (MS), as B cells from MS patients make significantly less IL-10 than B cells from healthy individuals [51]. Interestingly, this alteration in the pro- to anti-inflammatory cytokine ratio is reversed in MS patients treated with either Mitoxantrone or Rituximab [51]. This change in B cell cytokine production correlates with a significant reduction in the number of memory B cells in the treated patients [51]. Consistent with this result, CD27⁺ memory B cells produce inflammatory cytokines like IL-12, LT α and TNF α [17,51], while CD27^{neg} naïve B cells make IL-10 [51]. Thus, one of the major impacts of B cell depletion therapy is that the ratio of memory “effector” B cells to naïve “regulatory” B cells is reversed in favor of the naïve B cells, leading to a more suppressive or tolerogenic cytokine profile.

Likewise, changes in other B cell subsets also lead to alterations in the B cell cytokine repertoire. For example, IL-10 production by B cells is greatly increased in several mouse models of SLE [34,52] - probably due to the high frequency of MZ B cells in these mice. In another example, stimulation of autoreactive, Rheumatoid Factor (RF)-specific murine B cells with immune complexes that simultaneously engage the BCR and TLR9 induces much higher levels of IL-2 than stimulation with either anti-IgM or CpG ODNs alone [53]. Furthermore, RF-specific B cells consistently produce more IL-2 in response to anti-IgM plus CpG ODNs than comparably stimulated normal B cells [53]. These data collectively suggest that the pattern of cytokines expressed by particular B cells is not fixed and that the types and amounts of cytokines produced by B cells can be influenced by the integration of signals from multiple receptors. Moreover, cytokine production by the overall B cell population in an individual can be altered by intrinsic changes in the activity of individual B cells or by changing the composition of the various B cell compartments.

Conclusions

New and accumulating evidence clearly demonstrates that B cells are not merely antibody-producing factories, but actively regulate immune responses by producing cytokines. IL-10 producing Bregs promote tolerance and suppress inflammatory responses, while effector B cells amplify humoral and cellular immune responses. The current evidence suggests that Bregs and effector B cells likely originate from different precursors - with the IL-10 producing B cells arising in large part from B1a cells and MZ-like B cells and the effector B cells originating from FO B cells. The cytokine profile of B cells is influenced by the cytokine microenvironment, T cell help, and the presence of pathogen-derived TLR ligands. The cytokine profile of B cells can also be altered by disease, leading to an imbalance of proinflammatory and anti-inflammatory cytokines. However, this proinflammatory bias can be reversed in at least some patients treated with Rituximab. This leaves open the possibility that therapeutics specifically targeting the effector B cell pool without impacting the Bregs or their precursors would be highly effective. Future studies dissecting the cytokine-producing effector and regulatory B cell subsets will undoubtedly lead to new insights regarding the functions of these cells in both health and disease and may ultimately influence the design of the next generation of B cell-targeted therapies.

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Figure 1. Regulation of type 1 immune responses by effector and regulatory B cells

DCs activated by TLR ligands initiate type 1 immune responses (see green DC). The activated DCs produce IL-12 and present antigen to naïve T cells, facilitating their differentiation into Th1 effectors. Naïve FO B cells also respond to the TLR ligands and the DC-derived IL-12 and differentiate into IFN γ and IL-12 producing Be-1 cells. The IL-12 and IFN γ producing Be-1 cells can also present antigen to the naïve T cells and promote Th1 differentiation. Th1 effectors form cognate interactions with new cohorts of naïve FO B cells and provide co-stimulatory signals and IFN γ which promote the differentiation of the naïve FO B cells into IFN γ and IL-12 producing Be-1 cells. These cytokine producing and antigen presenting Be-1 cells can subsequently prime new naïve T cells and facilitate their differentiation into Th1 cells. Thus, Be-1 cells can initiate a positive feedback loop that amplifies and maintains Th1 responses. However, naïve MZ and T2 B cells (and potentially FO B) can also respond to signals delivered by TLR ligands, CD40 crosslinking or antigen and differentiate into IL-10 producing regulatory B cells. The IL-10 producing Bregs can block IFN γ production by Th1 effectors and reduce the inflammatory response. It is also possible that the IL-10 producing Bregs block the Th1 dependent differentiation of naïve B cells into Be-1 cells and short circuit the Be-1/Th1 amplification network.

Figure 2. Regulation of type 2 immune responses by effector and regulatory B cells

DCs activated by allergens or antigens derived from helminths initiate type 2 immune responses (see green DC). The activated DCs present antigen to naïve T cells, facilitating their differentiation into Th2 effectors. Th2 effectors form cognate interactions with naïve FO B cells and provide CD154 and IL-4, promoting the differentiation of the naïve FO B cells into IL-4, IL-13 and IL-2 producing Be-2 cells. These cytokine-producing and antigen-presenting Be-2 cells can subsequently prime new naïve T cells and facilitate their differentiation into Th2 cells. Thus, Be-2 cells can initiate a positive feedback loop that amplifies and maintains Th2 responses. However, naïve MZ B cells (and potentially FO B) can also respond to signals delivered by TLR ligands, CD40 crosslinking or antigen and differentiate into IL-10 producing regulatory B cells. The IL-10 producing Bregs can block IL-4 production by Th2 effectors and reduce the inflammatory response. It is also possible that the IL-10 producing Bregs block the Th2 dependent differentiation of naïve B cells into Be-2 cells and short circuit the Be-2/Th2 amplification network.

Figure 3. Neonatal IL-10 producing Bregs attenuate Th1 priming by DCs

TLR ligands initiate immune responses in neonates by activating B1a B cells and DCs. IL-10 produced by the TLR-activated neonatal Bregs blocks production of IL-12 by the TLR-activated DCs. This reduction in IL-12 production by the DCs facilitates the priming of Th2 cells rather than Th1 cells. Type I interferons produced by the TLR activated DCs (particularly plasmacytoid DCs) facilitates IL-10 production by the B cells and further enhances the suppression of the Th1 response to the TLR ligands. B cells from secondary lymphoid organs of adult animals, which are primarily composed of B2 FO B cells, make only small quantities of IL-10 in response to TLR ligands and cannot block TLR-induced Th1 responses.