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Unraveling Effector Functions of B Cells During Infection: The Hidden World Beyond Antibody Production

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Abstract

Antibodies made by B cells are critically important for immune protection to a variety of infectious agents. However, it is becoming increasingly clear that B cells do more than make antibodies and that B cells can both enhance and suppress immune responses. Furthermore, there is growing evidence that B cells modulate cellular immune responses by antibody dependent and independent mechanisms. Although we have a good understanding of the roles played by antibody-secreting effector B cells during immune responses, we know very little about the Ab independent “effector” functions of B cells in either health or disease. Given the recent data suggesting that B cells may contribute to autoimmune disease pathogenesis via an antibody independent mechanism and the increasing use of B cell depletion therapy in autoimmune patients, investigators are beginning to reassess the multiple roles for B cells during immune responses. In this article, we review data describing how B cells mediate protection to pathogens independently of antibody production. In particular, we will focus on the role that B cells play in facilitating dendritic cell and T cell interactions in lymph nodes, the importance of antigen-presenting B cells in sustaining effector T cell and T follicular helper responses to pathogens and the relevance of cytokine-producing effector and regulatory B cells in immune responses.

Keywords

B lymphocytes; antigen presentation; infectious disease; cytokines; antibody

INTRODUCTION

Antibody (Ab)-producing B cells are required for the clearance of most pathogens and are responsible for the long-term protection from infection conferred by essentially all vaccines [1]. However, B cells and the Abs they produce also contribute to immunopathology associated with infection, autoimmune disease and allergic responses [2, 3]. These opposing protective and pathologic roles of B cells were first revealed using mice genetically deficient in B cells (μ MT or JHD mice) which are susceptible to a variety of infections [4–10] yet are

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CONFLICT OF INTEREST

None declared.

protected from multiple autoimmune diseases including Type 1 Diabetes, Systemic Lupus Erythematosus, Sjogren's Syndrome and thyroiditis [11–15]. More recent data from autoimmune patients who have undergone B cell depletion therapy using Rituximab (anti-CD20) or Belimumab (Anti-BLyS) demonstrate that B cells contribute to disease pathogenesis [16, 17]. Interestingly, although B cell depletion in autoimmune patients can lead to decreased systemic autoAb titers, data from patients treated with Rituximab show that the clinical efficacy of anti-CD20 therapy does not directly correlate with a reduction in circulating autoAb titers [18–21]. In fact, some patients without pre-existing autoAbs enter clinical remission after B cell depletion, while other B cell depleted patients enter long periods of remission without any significant reduction in their autoAb titers.

These data suggest that B cells can cause pathology by Ab independent mechanisms and predict that B cells also provide protection from infectious agents via Ab independent mechanisms. Years of effort have greatly increased our understanding of the cues that control B cell differentiation into Ab secreting effector cells [22] and of the ways in which Abs facilitate pathogen clearance [23]. However, our understanding of the Ab independent “effector” functions of B cells is still rudimentary. Given the potential importance of these non-classical effector functions of B cells in autoimmune disease pathogenesis, there is now re-invigorated interest in delving into the complex roles that B cells play during immune responses. In this article, we review data describing how B cells mediate protection to pathogens independently of pathogen-specific Ab. In particular, we will focus on the role that B cells play in facilitating interactions between dendritic cells (DCs) and T cells in lymph nodes (LNs), the importance of antigen (Ag)-presenting B cells in sustaining effector T cell and T follicular helper (T_{FH}) responses to pathogens and the relevance of cytokine-producing effector and regulatory B cells in immune responses.

B CELLS REGULATE CELLULAR IMMUNE RESPONSES TO PATHOGENS VIA ANTIBODY INDEPENDENT MECHANISMS

Several reports using B cell deficient μ MT mice show that B cells are not required for the generation of primary CD4 and CD8 T cell responses following vaccination and some infections [24–31]. Nonetheless, there are also many reports showing alterations in CD4 and CD8 T cell responses in B cell deficient μ MT and JHD mice [32]. As one example, CD4 T cells from the spleens of *Chlamydia trachomatis*-infected μ MT mice secrete lower levels of IFN γ and IL-6 following *in vitro* restimulation with pathogen extracts [33]. By contrast, the frequency of IFN γ -producing T cells is equivalent in B6 and μ MT mice at early timepoints following *Plasmodium chabaudi* infection, however the frequency of IL-4 producing Th2 cells, which dominate the T cell response at the later timepoints following infection, are significantly decreased in the μ MT mice [34]. In another example, B cell deficient mice on the BALB/c genetic background generate a Th1 response to *Leishmania major* and are resistant to infection while control BALB/c mice make a non-protective Th2 response and are susceptible to *Leishmania* [35].

In other mouse models of infection, B cells are not obligate for the development of primary CD4 or CD8 T cell responses, but instead play an important role in CD4 and CD8 memory responses. For example, μ MT and wild-type (WT) mice infected with lymphocytic

choriomeningitis virus (LCMV) make roughly equivalent numbers of LCMV-specific effector CD4 T cells during the primary response [36]. However, the LCMV-specific memory CD4 T cell response is severely compromised in the B cell deficient mice [36]. Similarly, primary CD8 T cell responses to LCMV [29, 36], influenza virus [31] and *Listeria monocytogenes* [30] are normal in μ MT mice. However, the contraction of the effector CD8 T cell response is enhanced in μ MT mice infected with either *Listeria* [30] or LCMV [29], resulting in decreased numbers of memory Ag-specific memory CD8 T cells that persist following virus clearance [36].

The studies described above, as well as many others [32], provide suggestive evidence that B cells modulate T cell responses to pathogens. However, there are caveats with these experiments. First, many of these experiments are performed with mice that lack B cells throughout ontogeny and, as a result, these animals exhibit alterations in immune homeostasis. Indeed, animals that lack B cells during development have reduced numbers of splenic T cells [37], an altered T cell repertoire [38], an absence of Peyer's patches [39], defects in splenic microarchitecture [37, 40–42] and changes in DC subsets [37, 43, 44]. Second, because B cell deficient mice cannot generate a pathogen-specific Ab response, pathogen burden and Ag load are often higher in infected B cell deficient mice compared to infected control animals (see for example [10, 33]). Finally, the activation and function of FcR-expressing cells, including DCs, can be affected by the loss of Ab [23]. Given that any one of these changes can potentially account for the altered T cell responses observed in B deficient mice, it has been difficult to conclude that B cells actively regulate T cell responses to pathogens via Ab independent mechanisms.

Our laboratory has been using the *Heligmosomoides polygyrus* infection model system to address many of these issues. The advantage of this model system is that animals are infected with non-replicating *H. polygyrus* larvae that mature into adult worms within the small intestine [45]. The adult worms mate, but do not replicate, and establish a chronic infection in all immunocompetent and immunodeficient mouse strains [45]. Protective Th2-dependent immunity is established in immunocompetent mice and, following drug treatment to eliminate the adult worms, challenge infections with larvae are rapidly controlled in immunocompetent mice [45]. Since parasite burden following the initial *H. polygyrus* infection is equivalent between μ MT and control mice [46], it is possible to assess the impact of B cell deficiency on the development of primary and memory CD4 T cell responses without the confounding variable of Ag load. Using this experimental system, we found that both primary and memory CD4 Th2 responses are attenuated in *H. polygyrus*-infected μ MT mice compared to control animals [46]. Based on these results, we conclude that the impaired T cell responses observed in *H. polygyrus*-infected μ MT mice are not simply due to changes in pathogen burden.

To address whether the changes in immune homeostasis observed in μ MT mice are responsible for the defective Th2 cell response following *H. polygyrus* infection, we monitored primary and memory CD4 T cell responses in BCR transgenic mice (MD4 μ MT mice). These mice make B cells throughout ontogeny, allowing for normal development of the immune system. However, these mice express a monoclonal repertoire of B cells and cannot respond to *H. polygyrus*. We found that *H. polygyrus*-specific CD4 T cell responses

were defective in these animals [46]. Likewise, we recently showed that CD4 Th2 responses are impaired in mice that are transiently depleted of B cells at the time of infection (León, Ballesteros-Tato and Lund, submitted for publication). Together, these data indicate that the presence of Ag-specific B cells at the time of infection is required for the development of an optimal Th2 response to *H. polygyrus*.

One difficulty with interpreting the data from B cell deficient or B cell depleted animals is that these mice lack B cells as well as Ab. To separate the role of B cells as Ab secreting cells from their other effector functions, we use *Aicda*^{-/-} *μs*^{-/-} mice which have normal repertoire of B cells but are unable to secrete Ab [46]. We find that *Aicda*^{-/-} *μs*^{-/-} mice, which generate *H. polygyrus*-specific B cells but are unable to secrete *H. polygyrus*-specific Ab, make normal *H. polygyrus*-specific primary and memory CD4 Th2 responses [46]. Similarly, mice that can make LCMV-specific B cell responses, but cannot secrete LCMV-specific Ab, generate normal numbers of IFN γ -producing CD4⁺ T cells at both early and memory timepoints following LCMV infection [36]. However, mice that lack both B cells and Ab make very poor CD4 memory responses to LCMV [36]. Collectively, these results strongly suggest that B cells can actively influence pathogen-specific cellular immune responses independently of Ab production. In the following sections we describe some of the Ab-independent mechanisms employed by B cells to modulate immune responses.

B CELLS ORCHESTRATE ENCOUNTERS BETWEEN T CELLS AND DCS

Following immunization or infection, the draining LN undergoes intense remodeling, which is accompanied by increased vascularization and lymphangiogenesis [47]. These changes support increased recruitment of circulating T and B cells through the expanded high endothelial venules (HEV) [48] and enhanced recruitment of migratory DCs that enter the LN through *de novo* generated lymphatic vessels (LV) [47]. Recent studies show that B cells play a key role in LN remodeling following immunization [49, 50]. For example, Liao and co-workers showed that lymphangiogenesis in the skin and draining LN, as well as proper LN vascularization by HEV, are impaired in B cell-deficient mice [49]. Likewise, the expansion of LYVE-1⁺ LVs within the LN following immunization is highly dependent on the presence of B cells [50]. Strikingly, whereas migration of DCs from the site of inflammation to the LN is increased in immunized WT mice, accumulation of DCs in the draining LNs is significantly reduced in the B cell deficient mice [50]. Thus, B cells play an important role in facilitating encounters between newly arriving Ag-specific T cells and mature Ag-bearing DCs,

The experiments described above use mice that lack B cells throughout life which results in improper development of some lymphoid tissues and compromised splenic architecture [37, 39]. Thus, it is possible that the absence of B cells during fetal life alters LN development and impairs the ability of LNs to remodel following infection or inflammation. However, recent experiments from the Stein lab show that transient depletion of B cells also results in a significant reduction in the number of PNA⁺ HEVs in the draining LN of mice infected with LCMV [51]. These results bypass any developmental problems associated with B cell deficient mice and directly show that the acute absence of B cells leads to changes in LN architecture. Similarly, we find that transient depletion of B cells in *H. polygyrus*-infected

mice ablates the expansion of the LYVE-1⁺ LVs in the LN and greatly suppresses the accumulation of DCs in the mesenteric LN (León, Ballesteros-Tato and Lund, submitted for publication). Together, these data suggest that B cells play a direct role in remodeling the LN during the earliest phases of infection and therefore actively facilitate encounters between the naïve T cells entering the LN from the blood and Ag-bearing DCs arriving from the tissue. Interestingly, remodeling of the LN is dependent on VEGF-A [50] which facilitates lymphangiogenesis and lymphotoxin (LT) which promotes expansion of the HEV network [49, 51]. Activated B cells produce VEGF-A [50] as well as LT [52] and LT produced by B cells is required for LN remodeling following LCMV infection [51]. Thus, the data suggest that the immune microenvironment can be modulated by cytokine and growth factor-producing “effector” B cells.

ANTIGEN-PRESENTING B CELLS MODULATE EFFECTOR T CELL AND T_{FH} RESPONSES

In addition to the role that B cells play in facilitating encounters between Ag-presenting DCs and naïve T cells in the LN, activated Ag-specific B cells also interact in a cognate fashion with CD4 T cells and thereby influence the expansion, lifespan and differentiation potential of the T cells. Although CD4 T cells are initially primed by Ag-presenting DCs [53], Ag-activated B cells are also effective Ag-presenting cells (APCs) at later times when Ag becomes limiting [54, 55]. To address relative importance of B cell Ag presentation, we use mixed bone marrow chimeric mice. In these mice all of the B cells are selectively unable to express Major Histocompatibility Complex Class II (MHCII), however the majority of all other APCs, including the DCs, are competent to express MHCII [46]. Using these mice, we find that Ag presentation by B cells is required for the generation or reactivation of memory CD4 Th2 cell responses to *H. polygyrus* [46]. Likewise, we find that optimal expansion of IL-4 producing T cell effectors during the primary infection with *H. polygyrus* is also dependent on Ag-presenting B cells (León, Ballesteros-Tato and Lund, submitted for publication). Similarly, we find that the number of activated CD4 T cells in the lungs of *Pneumocystis carinii*-infected mice is significantly reduced when the B cells are MHCII deficient and unable to present Ag to CD4 T cells [56]. Similar experiments show that Ag-presenting B cells are required for the optimal development of IFN γ -producing CD4 effectors following *Salmonella enterica* infection [57]. Thus, the data from mice infected with fungal, parasitic and bacterial agents all support the conclusion that antigen presentation is an important effector function of B cells during infection.

In addition to modulating the expansion of CD4 effectors, Ag-presenting B cells also play a key role in T_{FH} cell generation and maintenance. Although the early entry of CD4⁺ T cells into B cell follicles occurs independently of interactions with B cells, and may be mediated by DCs [58], once CD4 T cells access the follicles, full differentiation into T_{FH} cells requires interactions with B cells [59]. The key role for B cells in T_{FH} cell development is supported by several studies showing that T_{FH} cell numbers are significantly diminished in B cell deficient mice [60–62] and in mice that have been transiently B cell depleted before infection (León, Ballesteros-Tato and Lund, submitted for publication). In addition, T_{FH} cell development following vaccination [63] or infection (León, Ballesteros-Tato and Lund,

submitted for publication) is severely compromised when B cells are unable to present specific Ag to the responding CD4⁺ T cells. Although Ag presentation by B cells appears to be an important component of T_{FH} cell development [64], neither B cell-mediated Ag presentation nor CD40-dependent T cell-mediated B cell activation is required when Ag is present in excess [63]. In fact, when Ag is present in excess, DCs are sufficient to drive T_{FH} development and maintenance [63]. Thus, T_{FH} development may not be dependent on a unique B cell-derived signal. Instead, it appears that maintenance of T_{FH} cells require continuous or sustained encounters with APCs and that B cells become the primary APCs as the amount of available Ag wanes [63]. Interestingly, Ag presentation by B cells may also curtail T_{FH} cell responses, as recent data from the McHeyzer-Williams lab indicate that MHCII-expressing plasma cells negatively regulate Ag-specific T_{FH} function [65]. Thus, Ag-presenting B lineage cells appear to regulate the expansion and maintenance of T_{FH} responses in the initial phase of the immune response and regulate the contraction of the T_{FH} response late in the immune response.

B CELLS MAKE CYTOKINES IN RESPONSE TO PATHOGEN-DEPENDENT SIGNALS

B cells, like most other hematopoietic cells, produce a variety of cytokines [32]. Experiments using mouse and human B cells reveal that B cells produce both inflammatory and suppressive cytokines in response to TLR ligands [66, 67]. For example, TLR4 signals induce marginal zone B cells as well as the regulatory CD1d^{hi}CD5⁺CD19^{hi} “B10” cells described by the Tedder laboratory [68] to produce IL-10 [67, 69]. By contrast, TLR1/2 activated memory B cells preferentially produce inflammatory cytokines like TNF α and IL-6 [66]. Interestingly, mouse B cells activated with a TLR4 or a TLR9 ligand secrete IL-6 and IL-10, but do not produce IFN γ [67]. However, when purified B cells are activated with combinations of TLR signals (TLR4, TLR9, TLR5 and TLR2) they secrete large quantities of IFN γ [67]. Collectively, the data suggest that cytokine profiles of B cells can be modulated by access to different TLR ligands and by the differentiation status of the B cells.

Given that B cells can respond directly to different components of pathogens by producing cytokines, it is reasonable to expect that B cells will also produce cytokines in response to infection. In support of this hypothesis, B cells from mice infected with *Borrelia burgdorferi* secrete IFN γ and IL-10 following Ag restimulation [70]. Likewise, B cells from *Salmonella enterica*-infected mice produce IL-6, IFN γ and IL-10 in a TLR-dependent fashion following *ex vivo* stimulation with heat-killed *Salmonella* [57]. Interestingly, B cells from *Salmonella*-infected mice produce significantly larger quantities of cytokines upon stimulation with *Salmonella* extracts than equivalently stimulated naïve B cells [57], suggesting that B cells that have been activated *in vivo* in response to *Salmonella* differentiate into more efficient cytokine-producing cells.

In fact, the types of cytokines made by activated B cells can be dramatically influenced by the pathogen. For example, B cells from *T. gondii*-infected mice produced IFN γ and IL-12p40 [71], type 1 cytokines that are required for pathogen clearance [72], while B cells from *H. polygyrus*-infected mice make IL-4 [71], a type 2 cytokine that is required for the development of protective memory Th2 cells [45]. Thus, B cells not only make cytokines

following exposure to pathogen-derived products, but B cells can be directed to make different cytokines depending on the type of pathogen that the B cell encounters.

MICROENVIRONMENT SIGNALS CONTROL THE DEVELOPMENT OF CYTOKINE-PRODUCING EFFECTOR AND REGULATORY B CELLS

An *in vitro* T and B cell co-culture system allows us to manipulate and define the signals that control the development or maintenance of cytokine-producing B cells [71]. Using this system, we co-cultured Th1 and Th2 effectors with highly purified naïve B cells in the presence of cognate peptide to stimulate the T cells and Ag or anti-Ig to crosslink the BCR. We find that B cells from these co-cultures produce large quantities of cytokines, but that the types of cytokines made by the B cells are distinct depending on whether B cells were co-cultured with Th1 or Th2 cells [71]. Experiments with these types of cultures demonstrate that cognate interactions between the B cells and T cells are required for the development of at least two “effector” B cell (Beff) subsets [73]. However, BCR signaling, while important for the expansion of the B cells, is not required for their development into cytokine-producing effector cells [71, 73]. Likewise, co-stimulatory signals between B and T cells play only a modest role in regulating cytokine production by the B cells [74]. Instead, we find that cytokines made by Th cells in the culture dictate the cytokine repertoire of the developing Beff cells. For example, B cells cultured with Th1 cells (Be-1 B cells) produce IFN γ , IL-2, IL-10 and IL-12p40 upon re-stimulation, but do not produce significant quantities of IL-4, IL-13 or IL-2 [71]. If the B cells in the Th1:B co-cultures lack IFN γ R or T-bet or the Th1 cells are unable to produce IFN γ , then the Beff cells that develop are unable to produce IFN γ following restimulation and instead produce IL-2 [73]. Conversely, B cells cultured with Th2 cells (Be-2 B cells) produce IL-2, IL-4, IL-5, IL-6, IL-10, IL-13 but do not make significant quantities of IFN γ or IL-12p40 [71]. However, if B cells in the Th2:B cultures are IL-4R deficient or the Th2 cells are incapable of making IL-4, then the Beff cells that develop lose the ability to produce IL-4 and IL-13 and instead secrete small quantities of IFN γ [74]. These experiments therefore predict that signaling through cytokine receptors on B cells may be sufficient to initiate cytokine programming in the B cells. In support of this idea, human B cells activated in the presence of IL-12 or IL-12 + IL-18 differentiate into IFN γ -producing cells [75, 76]. Likewise, we find that B cells stimulated through the BCR in the presence of IL-12 and BAFF differentiate into IFN γ -producing Beff cells [73]. Together, these data strongly suggest that the development of Beff subsets is influenced by the cytokine milieu.

Both Be-1 and Be-2 cells make IL-10 following restimulation [71], however these B cells are not the same as the IL-10 producing B cell subset referred to as regulatory B cells (Breg) or B10 cells [77, 78]. For example, regulatory B cells, which produce IL-10 but not proinflammatory cytokines, can suppress T cell responses [77, 78]. By contrast, Beff cells make both proinflammatory cytokines and IL-10 and, rather than suppress T cell responses, Beff cells promote T cell proliferation and differentiation into effectors [71]. The mouse B10 cells, which express high levels of CD1d and are CD5⁺ [68], appear to develop from transitional B cells [79] or marginal zone B cells [80] while the Beff subsets can develop from follicular B cells [71]. The mouse B10 cells do not depend on the presence of T cells

for their development *in vivo* [69] while T cells are required for the development of Be-2 cells *in vivo* [74]. Finally, signaling through the Ag receptor is required for B10 development [69] but is not necessary for Be-1 development [73]. Collectively, these data suggest that Be-1 and Be-2 effector B cells and B10 regulatory B cells are developmentally distinct from one another, likely arise from different precursor populations and perform different functional roles in immune responses.

CYTOKINE-PRODUCING EFFECTOR AND REGULATORY B CELLS MODULATE IMMUNE RESPONSES TO PATHOGENS

To address whether cytokine-producing effector or regulatory B cells play important roles in immune responses to pathogens, we and others use mixed bone marrow chimeric mice in which B cells are unable to produce a particular cytokine, while the majority of cells in other hematopoietic lineages are competent to produce the cytokine. Using this approach, we show that IL-2 and TNF α are both required for the development of protective memory responses in mice that were rechallenged with *H. polygyrus* [46]. However, TNF α and IL-2 producing B cells play distinct roles in the immune response to this pathogen. For example, mice lacking IL-2-producing effector B cells make poor class-switched *H. polygyrus*-specific serum Ab responses and have reduced numbers of protective Th2 memory cells [46]. By contrast, mice that lack TNF α -producing effector B cells generate a normal Th2 response upon challenge infection, but have significantly lower titers of *H. polygyrus*-specific Ab that wane rapidly after the primary and challenge infections [46]. Based on these data, we conclude that B cell-derived IL-2 likely influences T:B cell crosstalk, while B cell-derived TNF α is likely important for the generation or maintenance of *H. polygyrus*-specific long-lived plasma cells. Interestingly, Menard and colleagues showed that TNF α -producing B cells control the expansion of IFN γ -producing Th1 cells following *T. gondii* infection [81]. Finally, similar experiments from the Gray lab revealed that IL-17 production by CD4 T cells from *Salmonella*-infected mice is significantly diminished if B cells are unable to produce IL-6 or IFN γ [57]. Taken together, these results indicate that cytokine-producing B cells can modulate both cellular and humoral immune responses to multiple types of pathogens. Furthermore, cytokine-producing B cells are necessary for the development of protective and enduring immunity, at least in response to *H. polygyrus*.

As might be expected, B cell-derived IL-10 is not required for the development of *Salmonella*-specific Th1 or Th17 responses [57] or for the development of *H. polygyrus*-specific Th2 responses [46]. However, given that IL-10 producing B cells typically suppress T cell responses in models of autoimmunity [77, 78], it seems more likely that IL-10 producing B cells function to suppress immune responses or prevent T cell mediated immunopathogenesis following infection. In support of this idea, B cells from mice infected with either *Schistosoma mansoni* or *H. polygyrus* suppress T cell-dependent allergic responses [82, 83]. Incubation of purified B cells with *S. mansoni* parasites *in vitro* induces IL-10 production by B cells and IL-10 producing CD1d^{hi}CD5⁺ B cells were greatly expanded *in vivo* following infection [82]. When the CD1d^{hi} B cells from the *S. mansoni*-infected mice or B cells activated by the parasite *in vitro* are transferred to allergen-sensitized recipient animals, these B cells attenuate pulmonary allergic responses in the

recipients following allergen challenge [82]. Importantly, the transferred B cells suppress the allergic response by an IL-10 dependent mechanism [82]. Similarly, transfer of purified B cells from *H. polygyrus*-infected to exposed recipients prevented eosinophilia [83]. However, adoptive transfer of B cells from IL-10 deficient *H. polygyrus*-infected mice also suppressed the DerP1-dependent allergic responses [83]. Taken together, the data indicate that B cells with the capacity to suppress allergic responses can be induced and expanded following infection with either *S. mansoni* or *H. polygyrus*. Importantly, these suppressive B cells mediate their regulatory function via IL-10-dependent and independent mechanisms.

IL-10 producing B cells can also modulate T cells responses to pathogens by shifting the balance between Th1 and Th2 development. As reported several years ago by the Moser lab, IL-10 producing B cells modulate the balance between Th1 and Th2 immune responses following immunization [84]. They showed that IL-10 produced by B cells decreases IL-12 production by Ag-bearing DCs and suppresses Th1 induction [84]. Thus, Th1 development is favored over the development of Th2 or non-polarized T cell responses in B cell deficient mice. Similar results have been observed following *Leishmania major* infection. Resistance to infection with *L. major* is dependent on the generation of a protective Th1 response [85]. BALB/c mice, which make a predominant Th2 response following *L. major* infection, are highly susceptible to infection [85]. Interestingly, BALB/c mice lacking either B cells [35] or IL-10 [86] no longer make a Th2 dominant response and are resistant to *L. major* infection. IL-10 producing B cells, expressing the B10 phenotypic markers (CD1d^{hi}CD5⁺), can be identified after *in vitro* or *in vivo* exposure to *L. major* [87]. These IL-10 producing B cells suppress IL-12 production by *L. major*-stimulated DCs *in vitro* [87]. Most importantly, while transfer of normal B cells to *L. major*-infected BALB/c µMT mice restores Th2 development and susceptibility to infection, transfer of IL-10 deficient B cells does not induce Th2 development and the mice retain resistance to *L. major* infection [87]. Thus, in this setting, IL-10 producing regulatory B cells do not globally suppress the T cell-mediated immune response to *L. major*. Instead, they cause immune deviation and alter susceptibility to disease.

CYTOKINE-PRODUCING HUMAN B CELLS IN HEALTH AND DISEASE

Human B cells, like their mouse counterparts, can produce a wide array of cytokines [88]. However, our understanding of the factors and/or signals that drive human B cells to secrete inflammatory versus regulatory cytokines is still quite limited. *In vitro* culture experiments indicate that the amount and type of cytokines produced by human B cells depends on the stimulus used to activate the cells. For example, CD40-activated peripheral blood B cells secrete IL-10 but do not make LT, while B cells activated first by Ag (anti-Ig) and then with Ag and CD40L make significantly less IL-10 and elevated levels of LT, TNFα and IL-6 [89, 90]. Likewise, the cytokine profile of TLR-activated human B cells is dependent on which TLR(s) are engaged. For example, IL-13 is made by B cells only in response to TLR1/2 stimulation while IL-10 is made by B cells in response to multiple different (TLR1/2, TLR7 or TLR9) TLR ligands [66].

The developmental state of B cells also influences their cytokine-producing potential. As one example, both naïve (CD27⁻) and memory (CD27⁺) B cells respond to TLR1/2 ligands

by producing IL-13 [66]. However, only CD27⁺ memory B cells produce IL-6 and TNF α following TLR1/2 activation [66]. In a second example, B cells with a naïve phenotype are more efficient producers of IL-10 than memory B cells following co-culture with CD40L-expressing cells [89]. By contrast, memory B cells, but not naïve B cells, secrete pro-inflammatory cytokines such as LT and TNF α following dual stimulation with anti-Ig and CD40L-expressing cells [89]. Finally, only naïve B cells (and not germinal center or memory B cells) secrete IFN γ when stimulated with IL-12 and IL-18 [91]. However, both naïve and memory B cells isolated from tonsils secrete IFN γ when stimulated for 3 days with anti-CD40, anti-BCR and IL-12 [92]. Thus, while the developmental state of the B cells appears to influence its cytokine-producing repertoire, the cytokine potential of B cells is not fixed and can be re-molded by signals from the microenvironment.

One of the most interesting observations from the studies of human B cells is that the cytokine repertoire of B cells is often altered in disease settings. One study showed that B cells from healthy individuals are essentially unresponsive to TLR4 ligands and make only small quantities of inflammatory cytokines in response to TLR2 ligands [93]. By contrast, B cells from patients with chronic *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* infections produce significantly larger quantities IL-8 and TNF α following stimulation with TLR2 or TLR4 ligands [93]. Similar observations have been made in autoimmune and allergic patients. For example, B cells isolated from MS patients secrete more LT and less IL-10 than B cells from healthy controls following engagement of the BCR, CD40 and TLR9 [94]. Likewise, B cells isolated from nasal biopsies of atopic individuals produce elevated levels of IL-13 when stimulated with anti-CD40 and IL-4 relative to comparably stimulated tonsil or peripheral blood B cells from healthy individuals [95].

It is not yet known whether the observed changes in the cytokine repertoire of the patient B cells are due to (i) quantitative changes in the number of naïve, memory, germinal center or transitional B cell subsets present in the sampled peripheral blood or tissues, (ii) differences in the development or maintenance of effector or regulatory B cell populations, (iii) qualitative changes in particular B cell subsets which affect their ability to produce particular cytokines or (iv) changes in the microenvironment which alter the cytokine potential of the B cells. Although each of these issues needs to be addressed experimentally, the data are increasingly clear that cytokine responses of human B cells are often dysregulated in the setting of chronic inflammatory disease. Furthermore, given the potent effects of B cell-derived cytokines on LN remodeling and T cell responses following pathogen exposure, it is possible that abnormal cytokine responses by B cells from autoimmune or allergic patients will contribute to disease pathogenesis. Indeed, recent exciting experiments from the Bar-Or lab suggest that B cells from MS patients exacerbate inflammatory T cell responses in a TNF α and LT-dependent manner and that B cell depletion therapy not only decreases the proinflammatory responses of CD4 and CD8 T cells but also attenuates clinical symptoms of disease [94]. Thus, it is tempting to speculate that future therapies that specifically deplete or inactivate the proinflammatory cytokine-producing effector B cells will eliminate the potentially pathogenic B cells while sparing the regulatory B cells. This

targeted approach may facilitate the re-establishment of a more balanced cytokine microenvironment.

CONCLUDING REMARKS

The unique role that B cells play as Ab-producing cells is well appreciated, however the other functions of B cells as Ag-presenting cells, lymphoid tissue organizers and cytokine-producing effector and regulatory cells are much less understood. The need to better understand the biology and function of B cells is now apparent in light of the fact that B cell depletion therapy is being used to treat an increasing number of chronic inflammatory diseases. In the future, we hope to identify how these “non-classical” Ab-independent effector and regulatory functions of B cells influence protective immunity to pathogens and exacerbate autoimmune and allergic disease. In order to do so, it will be important to identify the precursors of the different effector and regulatory B cells and to determine the specific signals and transcriptional machinery that induce the activation or differentiation of these cells. In addition, it will be helpful to identify phenotypic markers that can be used to isolate and study B cells with particular effector functions. Finally, as we learn more about the biology of the different effector and regulatory populations of B cells, we expect that we will be able to design strategies that will allow us to either selectively deplete particular effector B cell subsets or will permit us to functionally inactivate particular effector functions of B cells that contribute to chronic inflammatory disease.

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