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B10 cell regulation of health and disease

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Summary

While B cells are traditionally regarded as promoters of the immune response via antibody secretion and pro-inflammatory cytokine production, recent studies have also confirmed an important role for B-cell-mediated negative regulation of immunity. Tremendous advances in the characterization of the mechanisms by which regulatory B cells function has led to the identification of a novel subset of regulatory B cells known as B10 cells, which regulate immune responses through the production of the anti-inflammatory cytokine interleukin-10 (IL-10). B10 cells are best defined by their functional ability to produce IL-10, as they are not confined to any particular phenotypic subset. B10 cells function in an antigen-specific manner that requires cognate interactions with T cells *in vivo* to regulate immune responses and have been demonstrated to be potent regulators of allergic and autoimmune disease, cancer, infection, and transplant rejection. Importantly, the recent discovery of human B10 cells has accelerated this field to the forefront of clinical research where the possibility of harnessing the regulatory potential of B10 cells for treatment of aberrant immune responses and diseases may become feasible.

Keywords

B10 cells; IL-10; B cells; immunosuppression; autoimmunity

Introduction

B cells are defined by their humoral effector function through the secretion of antibodies and are also known to play prominent roles in the activation of CD4⁺ T cells and the development of lymphoid tissue architecture (1). However, it has long been suggested that B cells are also critical negative regulators of both normal and aberrant immune responses. Nearly 40 years ago, Katz, Parker, and Turk (2) observed an increase in the severity and duration of contact hypersensitivity responses in guinea pigs following selective B-cell depletion and concluded that B cells were capable of inhibiting T-cell activation. Further studies characterized a similar effect of B-cell suppression on anti-tumor T-cell responses and suggested that in addition to regulatory T cells (Tregs), immunosuppressive B cells are

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also important for maintaining immune homeostasis (3). Despite these findings, the identification of *bona fide* regulatory B cells and the mechanisms by which these cells function remained elusive in the years to follow.

The past decade has seen tremendous advances in our understanding of B-cell immunoregulation. Mizoguchi *et al.* (4, 5) first used the term ‘regulatory B cells’ to describe a subset of gut-associated CD1d⁺ B cells that suppressed inflammatory cytokine production during colitis progression in mice by secreting the anti-inflammatory cytokine IL-10. Shortly thereafter, Fillatreau *et al.* (6) observed that recovery from experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), was dependent on the presence of splenic IL-10-producing B cells. Since these seminal observations, multiple additional studies have conclusively demonstrated the significance of IL-10-producing regulatory B cells in divergent models of autoimmunity, infection, and hematologic malignancy, as summarized in numerous reviews of regulatory B-cell function (7–16). Despite a number of studies that focused on identifying the numerous cell surface markers expressed by regulatory B cells, the specific identification of the individual B cells that were producing IL-10 remained confusing and difficult to untangle. To transcend this complexity, we focused specifically on the characterization of individual B cells uniformly defined by their characteristic production of IL-10. We functionally define these cells as ‘B10 cells’ for simplicity. While it is appreciated that other subsets of regulatory B cells function in an IL-10-independent manner, this review focuses on our contributions to the field of regulatory B10 cell biology in mice and humans.

Mouse B10 cells

Characterization and identification of mouse B10 cells

B10 cells were first characterized in a mouse model of contact hypersensitivity (CHS) in which dendritic cells serve as the predominant if not exclusive antigen-presenting cells and T cells mediate inflammation upon rechallenge with a sensitizing antigen (17). In this model, mice are immunized with oxazolone and resultant inflammation is observed following secondary exposure. Relative to wildtype mice, CD19^{−/−} mice, which have reduced B-cell activation due to the lack of this key B-cell signaling molecule, experienced significantly greater levels of ear swelling up to 96 h post-oxazolone challenge. By contrast, mice overexpressing the human CD19 transgene (hCD19Tg) exhibited far less inflammation than did their wildtype counterparts. This inverse relationship between B-cell function and oxazolone-induced T-cell inflammation was due to a subset of IL-10-producing spleen B cells that were absent in CD19^{−/−} mice, yet represented 1–2% of spleen B cells in wildtype mice and approximately 10% of spleen B cells in hCD19Tg mice. Adoptive transfer experiments of these cells demonstrated that their immunosuppressive effects were selectively dependent on IL-10 secretion, and thus the term B10 cell was introduced to functionally define this unique regulatory subset on the basis of their production of the potent anti-inflammatory cytokine IL-10.

Following the initial characterization of B10 cells in wildtype and hCD19Tg mice, further studies were undertaken to define the activity and understand the *in vivo* development of this unique regulatory population. However, the identification of IL-10-producing immune cells

in vivo is hardly a straightforward task and remains challenging in the field of regulatory B-cell biology (18). This is because individual spleen B cells isolated from naive wildtype mice do not constitutively express or secrete measurable IL-10 protein without *ex vivo* activation. Given the inability to observe B10 cells directly *ex vivo*, well-described *in vitro* assays to detect cytokine production in T cells were modified to identify B cells that were 'competent' to produce IL-10 *ex vivo* (17, 19). Stimulation of purified B cells using the protein kinase C activator phorbol 12-myristate 13-acetate (PMA) and the calcium ionophore ionomycin along with monensin added to block protein secretion (together, PIM) resulted in accumulation of cytoplasmic IL-10 at sufficient levels to allow detection of rare IL-10-competent spleen B cells by immunofluorescence staining. The addition of lipopolysaccharide (LPS) to these cultures along with PIM (L+PIM) results in marginally greater frequencies of spleen B10 cells among total B cells (1–3%), thus making a short-term 5-h culture with L+PIM the ideal assay to identify mouse B cells capable of producing IL-10 directly *ex vivo*. Additionally, incubation of splenic B cells with LPS, CpG, or apoptotic cells induces significantly elevated IL-10 secretion versus PMA and ionomycin stimulation alone, indicating that B10 cells are primed to respond to mitogenic stimuli and are therefore likely antigen-experienced cells (20–22).

Although short-term stimulation of spleen B cells resulted in the identification of 1–3% IL-10-competent B cells within the spleen, 48 h stimulation of B cells by agonistic CD40 monoclonal antibody (mAb) with L+PIM added during the final 5 h results in approximately 12% IL-10⁺ B cells within the total splenic B-cell pool of wildtype C57BL/6 mice (20). This significant increase in IL-10⁺ B cells relative to those obtained from the 5 h L+PIM assay revealed that some splenic B cells required additional stimulation to acquire IL-10 competence, which is in accordance with studies showing the expansion of regulatory B10 cells *in vivo* following chronic CD40 signaling (23). These cells were termed 'B10 progenitor' (B10pro) cells and are considered to be a functionally immature precursor population relative to B10 cells. While CD40 signals mature B10pro cells to B10 cells, BCR cross-linking inhibits this process (20). Thus, although visualization of immune cells actively producing IL-10 *in vivo* remains a difficult task, these *ex vivo* assays to characterize IL-10 competence have shed light on the small subset of B cells that have turned on the IL-10 functional program and are capable of producing this potent regulatory cytokine.

B10 cell distribution *in vivo*

The spleen is the predominant reservoir of B10 cells in mice (20). However, mouse B10 cells have also been identified in the gut-associated lymphoid tissues, such as the mesenteric lymph nodes and peritoneal cavity, as well as in the peripheral blood and lymph nodes (4, 20, 24). The peritoneal cavity also contains substantial frequencies of B10 and B10pro cells. The greatest frequencies of B10 cells in the peritoneal cavity were identified within the CD5⁺ CD11b⁺ B1a B cell subset (38%), followed by the CD5⁺ CD11b⁺ B1b (18%) and the CD5⁺ CD11b⁺ B2 (4%) subsets. Peritoneal cavity B cells also contain substantial proportions of B10pro cells, as 48 h CD40 mAb with 5 h L+PIM stimulation increases IL-10⁺ B-cell frequencies within the B1a, B1b, and B2 subsets to 41%, 22%, and 6%, respectively. Although the overall peritoneal cavity B-cell compartment contains the greatest frequency of B10 cells, the significantly reduced number of total B cells in the peritoneal

cavity as compared to the spleen results in a greater overall number of B10 cells in the spleen. Within other mucosal tissues, B10 cells represent approximately 1% of mesenteric lymph nodes, 4% of *lamina propria*, and 3% of Peyer's patch B cells. Additionally, few, if any, B10 cells are present in the peripheral blood and lymph nodes, though 3–8% of peripheral blood and lymph node B cells are B10pro cells (20). Thus, B10 and B10pro cells are rare within the blood, peripheral lymph nodes, and gut-associated lymphoid tissues, with the exception of the peritoneal cavity, where IL-10⁺ cells represent a substantial portion of the local B-cell pool.

B10 cell phenotype

L+PIM stimulation for 5 h does not change the expression of most cell surface molecules, particularly in the presence of monensin, which allows for the characterization of the *in vivo* B10 cell phenotype (20). An extensive cell surface phenotyping study revealed that mouse spleen B10 cells are IgM^{hi} IgD^{lo} CD19^{hi} MHC-II^{hi} CD21^{int/high} CD23^{lo} CD24^{hi} CD43^{+/-} CD93⁻. Additionally, spleen B10 cells are predominantly enriched (15–20%) within the CD1d^{hi} CD5⁺ subset, as are B10pro cells. However, this designation should not be interpreted as a definitive phenotype for B10 cells, but rather as a feasible means to enrich these cells for functional studies without having to stimulate the cells with L+PIM to induce IL-10 expression (17).

Spleen and peritoneal cavity B10 cells have similar phenotypic profiles with notable exceptions. As in the spleen, peritoneal cavity B10 cells express high levels of IgM, CD5, CD19, CD24, CD43, and MHC class II (MHC-II) and low levels of IgD and CD23 relative to their non-B10 cell counterparts (24). However, the CD1d^{hi} CD5⁺ phenotype cannot be used to enrich peritoneal cavity B cells for B10 cells because high-level CD1d expression is not induced within the peritoneal cavity (17). Furthermore, CD5 expression in this compartment is typically associated with the delineation of B1 and B2 cells, both of which are known to contain B10 cells as discussed above. Thus, B10 cells are present within multiple phenotypically defined B cell subsets in both the spleen and peritoneal cavity, demonstrating that cell surface phenotype does not necessarily delineate B-cell functional homogeneity. The demonstrated capacity to produce IL-10 thereby remains the best way to identify pure B10 cell populations for study.

B10 cell development

The identification of B10pro cells after *in vitro* stimulation led to the hypothesis that some B cells are selected *in vivo* for the unique capacity to produce IL-10 but nonetheless require additional signals to become IL-10 competent. The current developmental scheme for B10 cells posits that this *in vivo* selection is mediated by appropriate BCR-derived signals, which are essential for B10 cell development and function (Fig. 1). Evidence for the role of BCR signaling in B10 cell development is provided by hCD19Tg and CD19^{-/-} mice, which have amplified and reduced BCR signaling and greater and lesser frequencies of B10 cells, respectively, than wildtype mice (17). This positive correlation between BCR signaling and the ability to produce IL-10 indicate that the BCR drives the acquisition of IL-10 competence in a given B cell following antigen receptor selection and the binding of antigens *in vivo*. Further, transgenic mice with fixed BCR specificities have a definitive lack

of IL-10-competent spleen B cells (20), thus proving that appropriate BCR specificities are required for B10 cell development *in vivo*. Lastly, BCR signals also play a role in B10 cell effector function, as evidenced by the fact that B10 cells isolated from mice with prior antigen exposure are more effective at suppressing inflammation or disease in adoptive transfer experiments than their counterparts isolated from naive littermates (25). For example, B10 cell negative regulation of oxazolone-induced CHS is more pronounced when the cells are isolated from mice sensitized with the eliciting hapten. Whether this is due solely to enhanced BCR signaling in the appropriate antigen-specific B10 cells or to the clonal expansion of antigen-specific B10 cells in donor mice is unknown. Nonetheless, these observations demonstrate that BCR-derived signals play a role in early B10 cell development *in vivo* and may confer enhanced B10 cell immunosuppressive activity upon adoptive transfer.

BCR signals select B cells into the B10 cell compartment, but are not the only molecular events required for IL-10 secretion. B10pro cells likely reflect the subpopulation of B cells *in vivo* that have already received appropriate BCR signals to begin opening the *il10* locus, but require additional molecular events to make the locus fully accessible to *il10* gene transcription and/or protein translation. B10 cells thereby represent a further stage of functional maturation following the B10pro phase and have received the correct combination of signals *in vivo* to remodel the *il10* locus such that B10 cells remain poised for IL-10 production (Fig. 1). As such, B10 cells are readily identified after appropriate short-term *in vitro* stimulation, as the provision of L+PIM acts as a potent stimulus to drive intracellular signaling pathways that result in the transcription and translation of genes in an open configuration.

Although B10 cells are poised for IL-10 production, a final set of T-cell-derived signals is indispensable for the actual secretion of IL-10 and B10 cell regulation of immunity *in vivo* (26). For example, B cells deficient in MHC class II or the IL-21 receptor (IL-21R) are incapable of exerting IL-10-dependent suppression of autoimmunity. These observations, coupled with normal numbers of B10 cells in T-cell-deficient mice as assayed by 5-h L +PIM (20), indicate that while B10 cells do not require T cells to acquire IL-10 competence, they do need cognate interactions with IL-21-producing T cells to become B10 effector cells (B10eff) and secrete IL-10 *in vivo* (Fig. 2). These checkpoints further explain the antigen-specific regulatory function of B10 cells *in vivo*.

Given that B10 cells require additional signals *in vivo* to actually secrete IL-10, the current model for understanding B10 cell development now includes the recently characterized B10eff cells described above (Fig. 1). B10eff cells are those B cells that are actively secreting IL-10 *in vivo* and are modulating immune responses via antigen-specific interactions with T cells. Thus, B10pro cells represent a maturing population of B10 cells, with B10 cells poised to secrete IL-10 but lacking the T-cell-derived signals to do so. B10eff cells, however, are those B cells that have activated the correct molecular program to transcribe *il10* and have received appropriate input *in vivo* to secrete IL-10 protein and suppress immune responses following cognate interactions with antigen-specific T cells. Antigen-specific B10eff cells are thereby controlled by multiple levels of regulation, leading to rare numbers of these cells *in vivo* and partially explaining why it has been so difficult to

identify B cells producing IL-10 *in situ* above the background levels inherent in the assays used for IL-10 detection. Consistent with this, it has been possible to observe rare but measurable numbers of B cells expressing IL-10 reporter transgenes *in vivo* after polyclonal B cell stimulation with LPS, bacteria, or other mitogens (27), but this is facilitated by the more durable half-lives of the reporter molecules in comparison with IL-10 (18).

The discovery of B10eff cells was aided in part by the development of a 9-day *in vitro* culture system that massively expands B10eff cells from mouse spleen B cells (26). In this system, mouse B cells are cultured on highly selected NIH-3T3 cells expressing BLYS and CD154 (CD40L) in the presence of IL-4 for 4 days and IL-21 for an additional 5 days. By the end of the 9-day culture period, B10eff cells are expanded 4 000 000-fold and provide potent IL-10-dependent suppression of autoimmune inflammation before or during the course of EAE. These cells are therefore referred to as 'ex vivo-expanded B10eff cells' to distinguish them from B10eff cells generated *in vivo*. The development of this culture system will allow a more comprehensive investigation of B10 cell biology, as the acquisition of this normally rare population for molecular and biochemical studies is greatly facilitated.

Molecular regulation of IL-10 production and the fate of B10 cells

Although several studies have described crucial *in vivo* signals that confer IL-10 competence to B cells, the transcriptional network and upstream signaling molecules controlling B-cell IL-10 production remain incompletely understood. Although molecular programs regulating IL-10 expression in T cells and macrophages have been described, B-cell IL-10 regulation is largely unexplored, most likely due to the rarity of these cells (28). Previous studies have shown that some transcription factor genes related to plasma cell differentiation are upregulated in B10 cells, which is consistent with IL-21 induction of B10 cells (26, 27). A more complete picture of the regulatory transcription factor landscape in B10 cells is needed to further understand basic B10 cell biology and reveal molecular targets that can be exploited to manipulate B10 cells for therapeutic purposes.

Whether B10 cells retain their capacity to express IL-10 *in vivo* or adopt other fates following the induction of IL-10 expression has been characterized using the following two strains of IL-10 reporter mice: Tiger knockin mice, which contain an IRES-GFP element following the endogenous *il10* locus, and transgenic 10BiT mice that contain an ectopic Thy1.1 gene under control of the IL-10 promoter (27). In both of these IL-10 reporter strains, B-cell reporter protein expression was predominantly observable only after *in vitro* stimulation with L+PIM. Reporter-positive B10eff cells also appear to only produce measurable IL-10 transiently *in vivo* for 24–48 h before some of the cells progress toward plasma cell differentiation and antibody production. This is consistent with transcriptional data indicating an upregulation of known plasma cell genes such as *irf4*, *prdm1*, and *xbp1* in reporter-positive B cells. Neither B10 nor B10eff cells express cell surface markers commonly associated with plasmablasts and plasma cell populations. However, the IL-10 reporter Thy1.1 remains on the cell surface for a period of time after IL-10 expression has ended; thus, this durable reporter allows the tracking of B10eff cells after they have ceased to express measurable IL-10. Remarkably, *in vivo* LPS administration in 10BiT mice

revealed that a significant proportion of IL-10 reporter-positive B cells subsequently adopt a plasmablast phenotype following IL-10 production (27), which also occurs with non-B10 cells following antigen or mitogen encounter and activation.

Whether all or only a fraction of B10 cells become plasma cells following IL-10 induction is unknown, but it is quite clear that the majority of plasmablasts do not derive from B10 cells. The long-term contribution of B10eff cell antibody production to their regulatory function has yet to be determined, but the vast majority of B10 cell function results from their acute IL-10 production. Additional fates of B10eff cells following IL-10 production, such as memory cell commitment or the secretion of other regulatory molecules, also remain active areas of investigation (Fig. 2).

B10 cell BCR specificity and antibody production

As mentioned above, some B10 cells are known to progress toward the plasma cell differentiation program following IL-10 secretion. Terminal plasma cell differentiation and antibody secretion by B10 cells has been confirmed *in vivo* by the adoptive transfer of B10 cells into lymphocyte-deficient RAG2^{-/-} mice (27), whereby LPS-stimulated B10 cells were able to reconstitute serum IgG and IgM antibody levels within 10 d. Subsequent *in vitro* studies of antibodies derived from these recipient mice showed that B10 cell-derived serum contains antigen-specific IgM and IgG, as well as IgM that is significantly enriched for autoreactivity when compared to IgM derived from non-B10 cells. This is consistent with the observation that B10 cells represent a population of B cells with diverse, predominantly germline BCRs, including those with reactivity for self-antigens. Thus, B10 cells secrete both foreign antigen-specific and autoreactive antibodies.

Despite the demonstrated importance of BCR signals in B10 cell activity, B10 cells are not measurably restricted in their usage of BCR genes. Single-cell repertoire analyses in spleen and peritoneal cavity B10 cell populations have revealed diverse heavy and light chain gene usage with a remarkable absence of somatic mutations in B10 cell immunoglobulin genes (24, 27). Even in the peritoneal cavity, where some restricted gene usage has been reported in B1a and B1b cells, IL-10⁺ B cells contain diverse BCR genes associated with both the B1 and the B2 subsets. These studies bolster the hypothesis that B10 cells are a functionally-defined population and are not a restricted lineage with a single cell type of origin.

B10 cell regulation of immune responses

The observation that B-cell-derived IL-10 could suppress T-cell-mediated inflammation in a diverse array of mouse models led us to study the role of B10 cells in autoimmunity, hematologic malignancy, and infection. In the context of autoimmunity and transplantation, B-cell-derived IL-10 acts on a local, antigen-specific level to combat T-cell autoimmune responses and dampen inflammation (26). During infection, however, B10 cells have significant regulatory effects on the innate immune system that can negatively affect the clearance of certain pathogens (29). In a separate mouse model of hematologic malignancy, B10 cells were enriched during chronic lymphocytic leukemia (30). Thus, B10 cell interactions with the immune system are context-dependent, and additional studies have expanded upon those described above to conclusively demonstrate a potent and important

function for B10 cells in immune system homeostasis as well as the suppression of both appropriate and deleterious immune responses (31–36).

B10 cell regulation of autoimmunity

A role for B10 cells in the regulation of autoimmunity was first observed by characterizations of B10 cell numbers in several autoimmune-prone mouse strains. Both CD1d^{hi} CD5⁺ and IL-10⁺ spleen B cells are significantly increased in non-obese diabetic mice as well as in NZB/W F1 and MRL.lpr^{fas} mice, both of which are prone to lupuslike disease (20). Subsequent studies to understand how both endogenous and adoptively transferred B10 cells regulate autoimmunity have revealed that B-cell-derived IL-10 is a potent inhibitor of autoimmune inflammation with great promise for translation into human therapies.

EAE

The role of B10 cells during autoimmunity has been investigated most thoroughly in the antigen-specific model of MS, EAE. In this experimental system, mice are immunized with myelin oligodendrocyte (MOG) peptides, and autoimmune disease development is typically observed over a 28 days time course. Hallmarks of EAE-induced autoimmunity include MOG-specific T-cell infiltration into central nervous system tissues, which is accompanied by increased levels of Th17-derived pro-inflammatory cytokines (37, 38).

B cells exhibit both pro- and anti-inflammatory roles during the course of EAE. B-cell autoantibodies specific for MOG can contribute to increased levels of inflammation and worsened disease (39, 40). For this reason, CD20 mAb-induced depletion of mature B cells once disease is established (at least 14 days post-MOG immunization) can lower disease scores, reduce demyelination of the CNS tissue, and limit MOG-specific T-cell infiltration into the CNS (41). However, B-cell depletion 7 days prior to MOG immunization results in the opposite phenomenon, with B-cell-depleted mice experiencing heightened disease as compared to mice treated with control mAb. Increased inflammation when B cells are depleted before disease initiation implicated a regulatory role for B cells during EAE. This Bcell-mediated regulation was attributed to B10 cells themselves because the adoptive transfer of B10 cell-enriched CD1d^{hi} CD5⁺ splenic B cells normalized disease in B-cell-depleted, MOG-immunized mice (41). Similar results were obtained when μ MT mice that congenitally lack B cells were immunized with MOG peptide and experienced worsened disease and enhanced Th1-derived autoimmunity compared to wildtype mice (6). Further studies of EAE severity in different mouse strains revealed that CD19^{-/-} mice had worse EAE than wildtype controls, most likely due to markedly decreased numbers of endogenous B10 cells. By contrast, hCD19Tg mice had elevated B10 cell numbers and milder EAE disease severity than wildtype mice, thus confirming an inverse relationship between the abundance of B10 cells and EAE severity (25). Thus, B10 cells play a definitive role in the amelioration of antigen-specific T cell-mediated autoimmunity.

The EAE model not only provides a physiologic means for assessing the role of B10 cells during autoimmunity but also allows for the investigation of B10 cell kinetics in the context of other immunoregulatory populations. Interestingly, B-cell depletion late in the EAE

disease course lessened disease, but late ablation of Tregs significantly worsened disease (25). Similarly, B10 cells were evident in the CNS tissue before disease initiation and increased only slightly in the 28 days following MOG immunization. Tregs, however, significantly increased in the CNS during EAE and peaked at 21 days after MOG immunization. Collectively, these studies highlight an early role for B10 cells during EAE wherein B-cell-derived IL-10 prevents disease initiation. At later stages of disease, B-cell antigen presentation and autoantibody production may dominate the antiinflammatory effects of B10 cells.

Although adoptive transfers of B10 cells confirmed the role these cells play in blocking early autoimmune inflammation, B10eff cells are also capable of blunting established disease (26). Adoptive transfer of 1×10^6 *ex vivo*-expanded B10eff cells at days - 1, 7, or 14 relative to MOG immunization significantly reduced EAE disease scores in recipient mice when compared to those mice that received an equivalent amount of *ex vivo*-expanded non-B10 cells. These studies demonstrated the *in vivo* potency of B10eff cells, as they were able to significantly reduce disease at a time-point when previous studies showed that the absence of B cells is beneficial for combating autoimmunity. Furthermore, the transfer of 1×10^6 CD1d^{hi} CD5⁺ splenic B cells at 14 days post-MOG immunization was without effect in previous studies (25). This appears to be attributable to the fact that B10eff cells represent a more pure population of B10 cells than any freshly isolated splenic B-cell population as well as the potentially increased anti-inflammatory potency of *ex vivo*-expanded B10eff cells.

Systemic lupus erythematosus

As in many other autoimmune diseases, B cells are able to promote the progression of systemic lupus erythematosus (SLE), a multi-organ autoimmune disease characterized by autoantibody production. The autoimmune-prone mouse strains NZB/W F1 and MRL.lpr^{fas} are the models of choice for investigating SLE due to their spontaneous lupus-like disease that can be accelerated by treatment with pristane. B cells produce anti-nuclear antibodies and other autoantibodies in both of these models and contribute to renal disease caused by IgG deposition (42). As such, mature B-cell depletion at 12–32 weeks of age in NZB/W F1 mice increases overall survival as compared to mice treated with control mAb (43).

As in EAE, however, CD20 mAb-mediated depletion of B cells prior to disease development in the NZB/W F1 SLE model (4 weeks of age) leads to decreased survival, and this effect is attributable to the loss of B10 cells (43). NZB/W F1 mice have approximately fourfold more B10 cells at 10 weeks of age than do age-matched C57BL/6 mice that are not prone to autoimmune disease. The observation that B10 cells play a protective role during lupus-like disease was further highlighted by the generation of a CD19^{-/-} mouse on the NZB/W background. CD19^{-/-} NZB/W F1 mice experienced worse disease than their wildtype counterparts, with increased proteinuria and glomerulonephritis and decreased overall survival (44). As in CD19^{-/-} C57BL/6 mice, CD19^{-/-} NZB/W mice had a distinct lack of B10 cells with a barely detectable CD1d^{hi} CD5⁺ compartment and less *il10* transcription in the overall splenic B-cell population when compared with B cells from wildtype NZB/W F1 mice.

The increased severity of lupus-like disease in CD19^{-/-} NZB/W F1 mice was especially striking considering that CD19 deficiency impaired the production of characteristic autoantibodies thought to drive the lupus-like disease phenotype (44). Therefore, even with the decrease in autoantibodies, CD19 deficiency and the subsequent lack of B10 cells drove more aggressive disease. Furthermore, the adoptive transfer of CD1d^{hi} CD5⁺ spleen B cells from wildtype NZB/W F1 mice ameliorated disease in CD19^{-/-} NZB/W F1 mice and increased Treg frequencies. Thus, as in other models of autoimmunity, B cells play both pathogenic and protective roles during pristane-accelerated lupus.

The notion that B10 cells were capable of blunting autoimmunity during lupus was questioned in a study in MRL.lpr^{fas} mice with a B-cell-specific deficiency in IL-10. In this system, CD19^{Cre} IL-10^{fl/fl} MRL.lpr^{fas} mice did not exhibit worse lupus symptoms as compared to wildtype MRL.lpr^{fas} mice, implying that endogenous B10 cells do not limit spontaneous autoimmunity (45). These observations are difficult to interpret because not all CD19⁺ B cells express sufficient levels of CD19-driven Cre recombinase (46), leading to the possibility that residual B10 cells may remain that inhibit disease severity. Additionally, a congenital defect in B10 cell development may be compensated for by the increased availability of a separate IL-10-producing population. The same compensatory mechanisms are not evident in mice depleted of mature B cells, as discussed above, which may provide a more comprehensive assessment of the role of B10 cell function during lupus.

Inflammatory bowel disease

Early studies of B cell immunoregulation relied on colitis as a model of autoimmune inflammation. A role for IL-10-mediated suppression of colitis was demonstrated by the generation of IL-10^{-/-} mice, which develop spontaneous, chronic colitis by 7–11 weeks of age (47). Mizoguchi *et al.* (4, 48) demonstrated that B cells are capable of suppressing colitis in a separate model using TCR α ^{-/-} mice in which mesenteric lymph node B cells upregulated CD1d and provided the IL-10 necessary for dampening disease.

While studies by Mizoguchi and others have shown that B cells can mitigate gut-associated inflammation, a definitive role for B10 cell-mediated suppression by cells from both the spleen and the peritoneal cavity was demonstrated in three separate inflammatory bowel disease (IBD) models. Dextran sulfate sodium (DSS)-induced colitis, which is achieved by short-term administration of DSS in rodent drinking water and causes acute intestinal injury throughout the length of the colon, is significantly worse in B10 cell-deficient CD19^{-/-} mice than in wildtype mice (49). The adoptive transfer of splenic CD1d^{hi} CD5⁺ cells ameliorates disease by slowing weight loss and intestinal bleeding in an IL-10-dependent manner. Additional studies of DSS-induced colitis in IL-10 reporter Tiger mice have also shown that B10 cells in the spleen, mesenteric lymph nodes, and peritoneal cavity are capable of expressing IL-10 during the acute inflammation caused by DSS treatment (24). Thus, B10 cells play an active role in reducing gut-associated inflammation.

B10 cells also suppress the spontaneous, chronic IBD modeled in IL-10^{-/-} mice. Peritoneal cavity-derived B10 cells significantly delayed the onset of IBD in IL-10^{-/-} recipients when transferred at 10–12 weeks of age and decreased the frequency of activated and cytokine-producing CD4⁺ T cells in the peritoneal cavity, mesenteric lymph nodes, and inguinal

lymph nodes of recipient mice. The anti-inflammatory effects of peritoneal cavity B10 cells were confirmed in a separate colitis model where the transfer of CD25⁻ CD45RB^{hi}CD4⁺ T cells into RAG2^{-/-} mice causes an IBD-like disease (50). As in spontaneous IBD, the transfer of peritoneal cavity B10 cells suppressed T-cell-induced colitis in an IL-10-dependent manner and decreased levels of IFN- γ - and IL-17-producing T cells in the peritoneal cavity, mesenteric lymph nodes, and spleen (24). Therefore, B10 cells from the spleen and peritoneal cavity are capable of inhibiting gut-associated inflammation in both spontaneous and induced models of IBD.

Collagen-induced arthritis

Collagen-induced arthritis (CIA) serves as a model for human rheumatoid arthritis (RA) and is characterized by joint destruction and infiltration of antigen-specific T cells to sites of inflammation following collagen immunization in the DBA/1 strain of mice (51). B-cell depletion prior to collagen immunization results in reduced disease, as pathogenic autoantibodies can no longer drive autoimmune inflammation (52). Despite evidence that B cells are inflammatory mediators of autoimmunity during CIA, some B cells subsets have also been shown to negatively regulate antigen-specific immune responses during arthritic disease. Mauri *et al.* (53) have described that *in vitro* activation of B cells with agonistic CD40 mAb induces production of IL-10 and to a much lesser extent, IFN- γ . Transfer of these *in vitro*-activated B cells into collagen-immunized DBA/1 with transgenic T-cell receptors specific for collagen significantly delayed disease onset and ameliorated established disease. The observed effects were attributed to the ability of the transferred B cells to produce IL-10, as B cells derived from IL-10^{-/-} mice were without effect.

An immunosuppressive role for B cells during CIA was further described in studies where the CD21^{hi} CD23⁺ IgM⁺ transitional 2 marginal zone precursor (T2-MZP) cell population was isolated and transferred to collagen-immunized DBA/1 mice (54). T2-MZP cell adoptive transfers delayed disease onset and treated established disease via inhibition of Th1-type immune responses in an IL-10-dependent manner. Moreover, adoptive transfer of total splenic B cells from apoptotic cell-treated mice also suppressed autoimmune pathogenesis in an IL-10-dependent manner that encouraged CD4⁺ T cell IL-10 production, thus verifying the ability of particular B-cell subsets to instigate tolerance and inhibit arthritic inflammation in CIA (22).

A role for B10 cells specifically during CIA was investigated by Yang *et al.* using B cells cultured *in vitro* with BAFF for 72 h, which is reported to induce IL-10 production in approximately 32% of CD1d^{hi} CD5⁺ B cells (55). The B10 cell-enriched B cells were then transferred to collagen-immunized DBA/1 mice where they delayed the onset of arthritis and specifically downregulated Th17 responses. Taken together, these studies confirmed the role of B cells during CIA and documented that while some pathogenic B-cell populations certainly contribute to the progression of disease, regulatory B-cell subsets, especially B10 cells, are capable of inhibiting effector T-cell responses during the course of CIA.

B10 cell regulation of pathogen clearance

Unlike autoimmune models in which B10 cells ameliorate disease and improve symptoms, the presence of B10 cells during infection prevents efficient pathogen clearance. During *Listeria monocytogenes* infection, antibody-mediated B10 cell depletion resulted in reduced bacterial load and enhanced macrophage activity (29). Although B10 cells inhibited innate cells in this model, their regulatory effects were nonetheless dependent upon cognate interactions with T cells, as B10 cells deficient in MHC-II or IL-21R did not affect *Listeria* responses. These findings clearly document a role for B10 cells in regulating immune system homeostasis, whereby the presence of B10 cells establishes the normal course of pathogen clearance. Remarkably, the depletion of B10 cells thereby enhances immunity and accelerates pathogen clearance.

Regulatory B cells have also been implicated in suppression of immune responses during *Salmonella typhimurium* infection. In this study, B-cell-deficient mice reconstituted with IL-10^{-/-} B cells exhibited enhanced survival following *S. typhimurium* infection, which was the result of amplified humoral immune responses not observed in mice reconstituted with wildtype B cells. In this model, B-cell immunoregulation was dependent on MyD88 and was specific to a suppression of neutrophils, NK cells, and T-cell cytokine responses. *S. typhimurium* infection also causes accumulation of CD138⁺ B cells in the spleen, and some of these cells may produce antibodies in response to infection. Notably, up to 50% of the CD138⁺ B cells in the spleens of B-green IL-10 reporter mice were reporter-positive 1 day following infection, indicating that some regulatory B cells can inhibit immune responses against pathogens and express plasma cell-associated surface markers (56). However, the extent and specificities of antibodies produced by regulatory B cells during infection remain to be determined.

B10 cells in cancer

B10 cells are critical modulators of the anti-tumor immune response. A role for B-cell inhibition of tumor clearance was demonstrated in CD20^{-/-} mice, where the presence of endogenous B cells decreased the therapeutic effect of CD20 mAb treatment (57). B-cell and B10 cell development, frequencies, and numbers are comparable between CD20^{-/-} and C57BL/6 mice, but a single dose of CD20 mAb depletes more than 95% of blood, spleen, and lymph node B cells in wildtype mice, whereas endogenous B cells in CD20^{-/-} mice remain intact. Similarly, CD20^{-/-} mice given B cell lymphomas prior to CD20 mAb treatment had decreased survival and increased tumor volumes compared to tumor-bearing wildtype mice treated in the same fashion.

While the presence of endogenous B cells negatively regulated anti-tumor responses in CD20^{-/-} mice, a specific role for B10 cells in this phenomenon was demonstrated by the adoptive transfer of CD20^{-/-} CD1d^{hi} CD5⁺ B cells into tumor-bearing wildtype mice prior to CD20 mAb treatment. The transfer of this CD20^{-/-} B10 cell-enriched population abrogated the therapeutic benefit of CD20 mAb, whereas the adoptive transfer of CD20^{-/-} CD1d^{lo} CD5⁻ or CD20^{-/-} IL-10^{-/-} CD1d^{hi} CD5⁺ B cells did not affect tumor growth or survival. Further experiments to understand the mechanism underlying B10 cell inhibition of CD20 mAb-dependent tumor clearance revealed a role for B10 cell regulation of

macrophage activity. Macrophages cultured with CD1d^{hi} CD5⁺ B cells produced less nitrous oxide and tumor necrosis factor- α (TNF- α) than did those cultured with CD1d^{lo} CD5⁻ B cells, an effect that was entirely dependent upon B cell IL-10 production (57). Thus, B10 cells specifically inhibit lymphoma depletion by CD20 mAb via the negative regulation of *in vivo* innate immune responses and potentially by additional mechanisms.

As discussed later, patients with CLL commonly have enhanced serum IL-10 levels and some cases of CLL have elevated expression of T-cell leukemia protein 1 (TCL1), a transcription factor known to augment Akt activation and subsequent cell proliferation and survival. These and other observations prompted B10 cell studies in TCL1 transgenic mice that overexpress human TCL1 and typically develop overt CLL-like disease by 12 months of age (58). While B10 cells develop normally and occur at comparable frequencies and numbers in 2-month-old TCL1 transgenic mice relative to wildtype mice, there is an observed age-associated expansion of blood and spleen B10 cells by 6 months of age. Indeed, by 12 months, most TCL1 transgenic mice have 50–90% B10 cell frequencies among spleen B cells, versus 10% for age-matched wildtype mice, leading to an average fourfold expansion of total B10 cell numbers. The age-associated expansion of B10 cells in TCL1 transgenic mice is strongly correlated with the increased acquisition of a CLL-like cell surface phenotype (CD5⁺ B220^{int}) among all B cells. The massive expansion of IL-10-competent B cells always preceded the development of malignant CLL-like cells in TCL1 transgenic mice. As is the case for endogenous B10 cells, activated but not resting CLL-like cells inhibited macrophage TNF- α production, an effect that was entirely dependent on IL-10 secretion. Additionally, low-dose inflammation induced by injections of small quantities of LPS resulted in CLL-like cell production of IL-10 and increased levels of serum IL-10 in TCL1 transgenic mice. These results suggest that infectious or other inflammatory responses may enhance CLL cell IL-10 competence and secretion, which may contribute to the systemic immunosuppression that is observed in patients with CLL and significantly influence disease treatment and progression. Thus, the shared ability of B10 and CLL cells from TCL1 transgenic mice to secrete IL-10, inhibit innate effector cell activation, and express CD5 indicate that either similar molecular processes, comparably self-reactive BCRs, or shared cellular origins dictate the IL-10-dependent regulatory function observed in both B10 and CLL cells.

Human B10 cells

Human B10 cell identification

IL-10-competent B10 cells have been recently characterized in humans (59). Human B10 cells are easily visualized following a 5-h *ex vivo* stimulation with PMA and ionomycin plus brefeldin-A (together, PIB) to block protein secretion. B10 cells are present in low but detectable numbers in human blood ($0.8 \pm 0.1\%$ of total B cells, $1.9 \pm 0.3 \times 10^{-3}$ B10 cells/ml blood) and have also been identified in the spleen ($0.31 \pm 0.06\%$), tonsils ($0.31 \pm 0.11\%$), and newborn cord blood ($0.45 \pm 0.14\%$). Blood B10 cell numbers appear to decrease with advanced age (59). As in mice, the addition of different TLR agonists to these cultures does not significantly affect the overall mean frequencies of blood B10 cells from

healthy individuals. Thereby, B10 cells are present at low but readily identifiable numbers in humans as in mice.

Mouse B10pro cell maturation into B10 cells occurs during *in vitro* culture with agonistic CD40 mAb as discussed earlier. Human B10pro cells are visualized in a similar manner, where the culture of blood B cells with recombinant CD154 and CpG for 48 h induces the maturation of B10pro cells into B10 cells that then express IL-10 in response to PIB stimulation for 5 h (59). These cultures thereby enumerate the numbers of *in vitro* matured B10pro cells as well as *ex vivo* B10 cells, because both subsets express IL-10 after PIB stimulation ($7.0 \pm 1.4\%$ of total B cells, $1.6 \pm 0.3 \times 10^{-4}$ B10 + B10pro cells/ml blood). Signaling pathways similar to those observed for mice induce human B10pro cell maturation and IL-10 expression, although select individuals have increased B10 cell frequencies following TLR9 (CpG) or TLR4 (LPS) stimulation, indicating that B10pro cells from some humans respond preferentially to different TLR stimuli. In contrast to the maturing effect of CD40 signaling, BCR crosslinking with antibody inhibits B10pro cell maturation in these human B-cell cultures (59, 60). Others, however, have reported that human B cell IL-10 production is most optimally induced by concomitant CpG and BCR stimulation and that B10pro cell development is largely independent of CD40 signaling (61) (Table 1).

Human B10 cell phenotypes

Blood B10 cells express levels of canonical B-cell markers comparable to those of non-B10 cells, including CD1d, CD5, CD20, CD21, CD22, CD23, CD25, CD28, and CD40, although CD19 and IgD expression are generally elevated and IgM levels are lower as in mouse (59). Additionally, most B10 cells express high levels of CD27, CD48 and CD148, which are indicative of activation/memory, and do not express CD10, which is associated with immature/transitional B cells (59). Despite extensive phenotypic analysis, blood B10 cells do not uniquely express markers specific to their functional program other than the ability to express IL-10. Likewise, blood B10 cells are not restricted to any of the standard B-cell subsets, such as those traditionally defined by CD38 or CD27 versus IgD expression. However, both B10 and B10pro cells are enriched within the CD24^{hi} CD27⁺ blood B-cell subset, which on average represents approximately 25% of total blood B cells in healthy individuals; B10 cells are 10-fold greater in number in this subpopulation than in the CD24^{lo} CD27⁻ subset (59). As in mice, human B10 cells become activated and proliferate quickly in response to polyclonal mitogen stimulation, which correlates with their memory phenotype. Thus, based on their cell surface marker expression, human B10 cells most likely represent a subset of the *in vivo* antigenexperienced population of B cells.

Ex vivo B10pro cell phenotypes are generally unknown because these cells are functionally identified after 48 h of *in vitro* stimulation, which significantly alters their phenotype along with non-B10 cells and makes them difficult to distinguish from activated non-B10 cells (59). Studies by others have noted an enrichment of blood regulatory B cells specifically in the CD19⁺ CD24^{hi} CD38^{hi} population following coculture for 72 h with Chinese hamster ovary (CHO) cells transfected to express CD154, though IL-10-producing B cells were also observed in other phenotypic groups (62). By contrast, others have reported that the blood CD27⁻ B-cell subset contains the majority of IL-10-producing B cells (60). Thus, although

B10 cells can be modestly enriched in some B-cell subsets, such as the CD24^{hi} CD27⁺ subpopulation, in our hands, their functional ability to produce IL-10 remains the most unambiguous way to define B10 cells.

B10 cells in human disease

The presence of B10 cells in healthy individuals presents the possibility that a reduction or change in B10 cells may affect disease development in humans as occurs in mice. Thus, the frequency of B10 cells in patients with autoimmune disease, including SLE, RA, Sjögren's syndrome (SjS), autoimmune vesiculobullous skin disease, and MS, was examined (59). Although B10 cell frequencies on average were comparable between healthy donors and autoimmune patients receiving a variety of therapies, mean B10pro cell frequencies were significantly elevated in each disease group relative to healthy donors. Even though most patients were receiving immunosuppressive treatments at the time of evaluation, there were no patients that had significantly fewer B10 or B10pro cells than healthy donors, and a subset of patients had significantly elevated B10 and/or B10pro cell frequencies and numbers. The underlying mechanism behind this expansion of B10 and B10pro cells in these patients remains to be elucidated, but the phenomenon may be a result of increased inflammation and/or due to persistent B10 cell stimulation by autoantigens.

One of the most fascinating discoveries from human B10 cell studies was the identification of a strong relationship between the presence of blood B10 cells and the development of malignant lymphocytic disease (30). In a study of B10 cells and chronic CLL, an otherwise healthy individual recruited as a normal donor had 30% blood B10pro cells, which is sixfold and fourfold-greater than frequencies noted for average healthy donors and autoimmune individuals, respectively. More than 90% of the donor's blood B cells were CD5⁺ CD20^{int}, and this patient was eventually diagnosed with monoclonal B-cell lymphocytosis, an asymptomatic condition that may lead to CLL. Additionally, one CLL patient with pemphigus vulgaris had 29% blood B10pro cell frequencies, with the majority being CD5⁺ CD20^{int} clonal cells. This individual was later diagnosed with early-stage CLL. Subsequently, it was determined that approximately 27% of surveyed CLL patients had IL-10 competent CD5⁺ CLL cells after 5-h culture with LPS, where total blood IL-10⁺ B-cell numbers among these individuals were 18–30 times greater than those of healthy controls. A remarkable 86% of CLL patients had IL-10 competent CD5⁺ CLL cells after 48 h culture with CD154 and CpG; this resulted in 98-fold more IL-10⁺ B cells than what is normally present in the blood of healthy donors. Collectively, approximately 90% of CLL cases included malignant cells with the capacity to express IL-10 as defined by B10 or B10pro cell assays. The IL-10-competent CLL cells observed in these patients had cell surface phenotypes similar to that of B10 cells, including high CD5, CD24, and CD27 expression. Some CLL patients also have significantly increased plasma levels of IL-10, although the *in vivo* cellular origins of IL-10 remain unknown. Thereby, it remains possible that CLL cell IL-10 competence contributes to immunosuppression, which is variable among patients but may reduce patient responsiveness to immunotherapies including rituximab (CD20 antibody) (57). Most importantly, these parallel features suggest a common cellular origin between B10 cells and CLL cells or at least a shared functional program between the two cell types.

Human B10 cell function

Given their rarity in blood, human B10 cells have proven to be difficult to study and are virtually impossible to isolate in sufficient numbers for functional studies. Nonetheless, functional studies of B-cell populations enriched for B10 cells have suggested that they are capable of suppressing other immune cells through IL-10-dependent pathways, particularly those of the innate immune system. B10 and B10pro cells within the CD24^{hi} CD27⁺ blood B-cell subset provided IL-10-dependent inhibition of LPS-induced TNF- α production by monocytes during 24 h assays, whereas CD24⁺ CD27⁻ cells were largely without effect (59). In a similar coculture assay using CD4⁺ T cells, activated CD24^{hi} CD27⁺ blood B cells regulate T-cell cytokine production, although this is influenced by factors in addition to IL-10, but do not influence mitogen-induced T-cell activation or proliferation. These observations parallel what has been observed for mouse B10 cells using similar *in vitro* culture assays. Studies by others have demonstrated that B-cell subsets enriched for IL-10 production following culture with CD154-expressing CHO cells can inhibit the proliferation and cytokine production of stimulated CD4⁺ T cells in a manner dependent on IL-10, CD80, and CD86 expression (62, 63). Yet, these B cells also produced significant levels of IL-6, IL-12, and IFN- γ , which confounds the interpretation of such observations. The inability to generate pure human B10 cell populations in sufficient numbers for functional studies as well as the rarity and complexity of human antigen-specific *in vitro* culture systems severely reduced the ability to study B10 cell function in isolation; thus, these studies should be interpreted with great caution and an appreciation for the sophistication of the regulatory system being examined. Nonetheless, it is highly likely that there is a functional relationship between B10 cell numbers and adaptive immune responses as well as innate immune cells whereby human B10 cells inhibit inflammatory responses as clearly demonstrated in mice.

While studies by us and others suggest that human B10 and regulatory cells negatively regulate adaptive immune responses in healthy individuals, a study of IL-10-producing B cells from patients with SLE, but not those from patients with SjS or osteoarthritis, suggests significant impairment in their ability to regulate T-cell cytokine responses (62). This effect was observed despite comparable frequencies of IL-10-producing B cells in healthy and autoimmune individuals and was ascribed to defective CD40 signaling due to a reduction in phosphorylation of signal transducer and activator of transcription 3 (STAT-3) following CD40 stimulation of the cultured regulatory B cells. These studies highlight the need for further characterization of human B10 cells and other regulatory B-cell functions, including the complete range of immunoregulatory molecules that they secrete and their antigen specificities, particularly among patients with autoimmune disease.

Conclusions

Although significant advances in the understanding of B10 cell biology have been made in recent years, many unanswered questions remain. Of particular importance is a clarification for the role of antigen in driving the B10 cell functional program during development. Preliminary studies have indicated that antigen experience and BCR-derived signals are essential for the function of mouse B10 cells, but the exact antigens that drive B10 cell development in humans and mice have yet to be identified. Understanding the complete

mechanisms by which B10 cells are regulated and exert their immunosuppressive effects also requires further study. A related and equally intriguing question is concerned with how B-cell-derived IL-10 mediates suppression in autoimmunity. Are B10 cell effects short-lived or do they induce long-term tolerance to self-antigens? Also, how do B10 cells gain IL-10 competence? Is B cell IL-10 production restricted by specific transcription factor expression, or a sequentially regulated series of changes in locus accessibility and posttranscriptional modifications? The answers to these and other questions concerning B10 cell biology will aid efforts to clinically exploit B10 cells for the treatment of debilitating immune-related disorders, particularly those diseases driven by combinations of factors where targeting diverse molecular pathways is required.

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Fig. 1. Model for mouse B10 cell development

After B cells encounter the correct self or foreign antigen *in vivo*, appropriate levels of B-cell antigen receptor (BCR) signaling induce a small subset of B cells to progress down a developmental program that leads to IL-10 production. B10 progenitor (B10_{pro}) cells do not express IL-10 but can become competent to do so after culture with agonistic CD40 monoclonal antibody (mAb) or lipopolysaccharide (LPS). Once B10_{pro} cells become IL-10 competent, they are functionally defined as B10 cells. B10 cells do not express measurable IL-10 but are primed for transcription of the *il10* locus. Stimulation of B10 cells with either phorbol-12-myristate-13-acetate and ionomycin or LPS induces the acute production of IL-10 transcripts and cytoplasmic IL-10 protein that accumulates to measurable levels within 5 h when monensin is present. Once B10 cells begin to secrete IL-10, they are functionally defined as B10 effector (B10_{eff}) cells, which are rarely visualized *in vivo*. Based on their cell surface phenotypes, B10_{pro} and B10 cells are likely to be chronically stimulated through their BCRs but require additional signals to become B10_{eff} cells that produce IL-10, which can include LPS or CpG. Following transient IL-10 production *in vivo*, some B10_{eff} cells acquire cell surface markers found of plasmablasts and differentiate into plasma cells that secrete polyreactive, autoreactive or self-reactive antibodies depending on their BCR specificity. Alternatively, the culture of spleen or blood B cells with NIH-3T3 cells expressing cell surface CD154 and BLyS in the presence of IL-4 for 4 days can induce B10_{pro} cell expansion and the development of proliferating B10 cells when stimulated IL-21 for 5 days, resulting in the *in vitro* generation of B10_{eff} cells.

Fig. 2. Model for B10 cell regulation of *in vivo* adaptive and innate immune responses following antigen-specific cognate interactions with T cells

To become B10eff cells and secrete IL-10 *in vivo*, B10 progenitor (B10_{pro}) cells must (1) receive appropriate signals through B-cell antigen receptor interactions with antigen. These B cells are presumed to display antigenic peptides through their cell surface MHC class II (MHC-II) molecules (2), which facilitate their cognate interactions with peptide-specific CD4⁺ T cells (3). The antigen-specific T cells become activated and express CD154, which binds CD40 on the cognate B cells and induces B10 cell maturation and IL-10 competence. T-cell receptor (TCR) and CD154 engagement induces T-cell IL-21 production (3), which then acts locally on B10 cell IL-21 receptors (IL-21R) to promote IL-10-secreting B10 effector (B10_{eff}) cell generation (4). IL-10 production by B10_{eff} cells in the local microenvironment negatively regulates adaptive immune responses by inhibiting antigen-specific T cells (5) and inhibiting innate macrophage function (Mac, 6) during immune responses.

Table 1

Human IL-10-producing regulatory B-cell subsets

Effector molecule(s)	Phenotype	Tissue	Frequency of IL-10 ⁺ B cells	Stimulus	Effector function	Diseases	Defect in disease?	References
IL-10	Blood CD19 ⁺ CD24 ^{hi} CD27 ⁺ (enriched)	Blood, cord blood, spleen	0.8 ± 0.1% / 7.0 ± 1.4% (B10/B10pro+B10) of total blood B cells	CpG ± CD40 mAb	Reduce monocyte cytokine production	SLE, RA, SjS, BD, MS, CLL	Expanded in patients with SLE, RA, SjS, BD, MS, CLL	(24, 48)
IL-10, CD80, CD86	Blood CD19 ⁺ CD24 ^{hi} CD38 ^{ghi} immature B cells	Blood	2–4% of total blood B cells	CHO cells expressing CD154	Reduce CD4 ⁺ cytokine production	SLE, RA	Defective in SLE, reduced and defective in RA	(51, 52)
IL-10 and other factor	Blood CD19 ⁺ CD27 ⁺ and CD19 ⁺ CD38 ^{ghi} (enriched)	Blood, spleen	1.8% of total blood B cells	CpG ± anti-Ig	CD4 ⁺ T cell proliferation	None described	None described	(50)
IL-10	Blood CD27 ⁺ naïve B cells	Blood	None described	CD40 mAb	None described	MS	CD27 ⁺ B cells have reduced IL-10 expression in MS	(49)

mAb, monoclonal antibody; SLE, systemic lupus erythematosus; B10pro, B10 progenitor cells; RA, rheumatoid arthritis; SjS, Sjögren's syndrome; BD, blistering skin disease; MS, multiple sclerosis; CLL, chronic lymphocytic leukemia; CHO, Chinese hamster ovary cells.