Editorial: **Are regulatory B10 cells a viable target for autoimmune diseases?**

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B cell abnormalities have been identified in a number of autoimmune diseases and are generally thought to contribute to disease progression through their production of autoantibodies and their activation of $CD4^+$ T cells. However, more recent data in a number of murine models of autoimmune disease reveal that other critical functions of B cells, including their ability to regulate cytokine production, may be altered and thus, contribute to disease progression in this manner. For instance, the ability of B cells to negatively regulate cellular immune responses and inflammation has been discovered recently with the identification of a population of regulatory B cells. The absence or loss of these regulatory B cells exacerbates disease symptoms in murine models of contact hypersensitivity, EAE, chronic colitis, collagen-induced arthritis, and lupus-like models of autoimmunity [1–7] (B10 cell data from these models are summarized in **Table 1**). Significant findings from each of these models and their relevance to human disease have been summarized recently in a review from Kalampokis et al. [9]. In many cases, a specific B cell subset was identified that regulates inflammation and T cell-mediated autoimmunity through their production of IL-10, which is a pleiotropic cytokine with numerous anti-inflammatory properties, including suppression of Th1 and Th2 polarization, inhibition of antigen presentation, and inhibition of proinflammatory cytokine production (**Fig. 1**). Whereas many cells of the adaptive and innate immune system, such as Th1, Th2, Th17, and regulatory T cell subsets, myeloid DCs, macrophages, monocytes, mast cells, NK cells, eosinophils, and neutrophils, express and secrete IL-10, a subset of IL-10-competent regulatory B cells has been functionally identified by its ability to express cytoplasmic IL-10. B10 cells, like other IL-10-producing cells, also secrete IL-10 yet were functionally identified and characterized by their cytoplasmic staining of IL-10, using the protein transport inhibitor brefeldin A to block secretion of IL-10 and enable detection of the specific subset producing IL-10 [10]. Importantly, B cell expression of cytoplasmic IL-10 protein parallels their expression of IL-10 transcripts and secretion of IL-10 [1, 3]. Given the low predominance of B10 cells in the human blood [10] (see below for further discussion), however, treatment of B cells with brefeldin A is necessary for the detection of IL-10 but not for the expression of IL-10 [10]. These IL-10-competent B cells have been labeled as B10 cells to identify them as the predominant, if not exclusive, source of B cell IL-10 production and to distinguish them from other regulatory B cell subsets that may also exist. Although multiple cell types produce/secrete IL-10, no study thus far has identified a distinct function for IL-10 secreted by B10 cells compared with monocytes or DCs. Instead, the differences in IL-10 expression between B10 cells and other IL-10-producing cells are a result of (1) the location of the particular cell subset when IL-10 is induced; (2) the mechanism(s) by which IL-10 expression is induced; for instance, CD40L + CpG stimulation induces IL-10 expression in B10 cells but not in monocytes or T cells; and (3) the kinetics of IL-10 expression, dependent on the cell type and stimuli used to induce expression.

B10 cells were identified originally in mice [1, 3], and only recently has a comparable IL-10-competent B10 cell subset been characterized in human blood [10]. In mice, the B10 cell subset represents 1–3% of total splenic B cells. In humans, similar findings were made. Two subsets of IL-10-competent B cells were identified, termed B10 and B10pro. These were identified in the blood, based on the ability of each subset to express cytoplasmic IL-10 after 5 h ex vivo stimulation with PIB, yielding mature, IL-10-competent B10 cells, or 48 h stimulation with PIB and CD40L and TLR ligands that induce B10pro cells to become IL-10-competent B10 cells. These two subsets, B10 and B10pro, represent 0.6% and ~5%, respectively, of human blood B cells and were found predominantly within the $CD24^{hi}CD27^+$ B cell subpopulation [9]; these markers are shared with activated and memory B cell subsets. To begin to put this into the context of autoimmune diseases, mean blood B10 + B10pro cell frequencies were examined in a small group of patients (n=91) with systemic lupus erythematosus, rheumatoid arthritis, primary Sjögren's syndrome, autoimmune vesiculobullous skin disease, or multiple sclerosis, and no patients were found to express significantly lower B10 cell frequencies than age-matched, healthy controls [10] (summarized in **Table 2**). Stimulation of cells with CD40L + LPS or CD40L + CpG ex vivo showed that patients with autoimmune disease had significantly higher mean B10 + B10pro cell frequencies than age-matched, healthy controls [10]. These findings set up an interesting and important para-

Abbreviations: CD40L=CD40 ligand, EAE=experimental autoimmune encephalomyelitis, PIB=PMA, ionomycin, and brefeldin A

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TABLE 1. B10 Cell Numbers in Murine Autoimmune Diseases

Mouse disease model	Splenic B10 cell numbers	Effect of B10 cell reconstitution
T1D in NOD mice versus C57Bl6 mice	Fourfold increase	Reduced disease severity
NZB/W lupus model	Threefold increase	Prolonged survival
MRL/*lpr* lupus model[a]	Threefold increase	ND
Age-related (6- vs. 2-month-old C57Bl6)	Increased	ND
DBA/1 versus C57Bl6	50% Decrease	ND
SJL versus C57Bl6	50% Decrease	Reduced disease severity
T cell antigen receptor [alpha]-chain-deficient (IBD)	ND	Suppressed disease
Collagen-induced arthritis	ND	Reduced disease severity

Reviewed in ref. [8]. T1D, Type 1 diabetes; DBA/1, autoimmune arthritis; ND, not determined. [a]B cell-derived IL-10 did not regulate spontaneous autoimmunity in this model.

digm between murine models of autoimmune disease and human autoimmune disease that needs clarification. In murine models of autoimmune disease, most of the systems examined to date support expansion of regulatory B10 cells for reducing disease severity, whereas expansion of B10 cells already appears to exist in human autoimmune disease [1–7, 9, 10]. A case in point is the recent paper by Yanaba et al. [8], using the imiquimod-induced, psoriasis-like model of skin inflammation. They found that the percentage of splenic B10 cells was reduced significantly in the spleens of imiquimod-treated WT mice, whereas they were increased in the blood and LNs, suggesting either migration of these cells from the spleen to the LNs or maturation of pre-existing B cells to B10 cells [8]. The idea that a further expansion of this regulatory B cell subset would be advantageous for managing the disease came from the identical studies in CD19-deficient mice. Here, they found that adoptive transfer of B10 cells from WT mice to CD19-deficient mice brought the psoriasis index down to the level observed in WT mice, yet WT mice still had measurable psoriasis [8]. Additionally, adoptive transfer only worked to reduce disease severity in CD19-deficient mice if it were performed 2 days before induction with imiquimod [8]. These findings point to multiple, new questions that must be addressed for a clear understanding of whether and how B10 cells can be used for the treatment of such autoimmune diseases in humans. For instance, can the current findings be expanded experimentally to show complete regression of imiquimod-induced psoriasis in WT mice? Is it simply the case that if we expand and transfer more B10 cells to

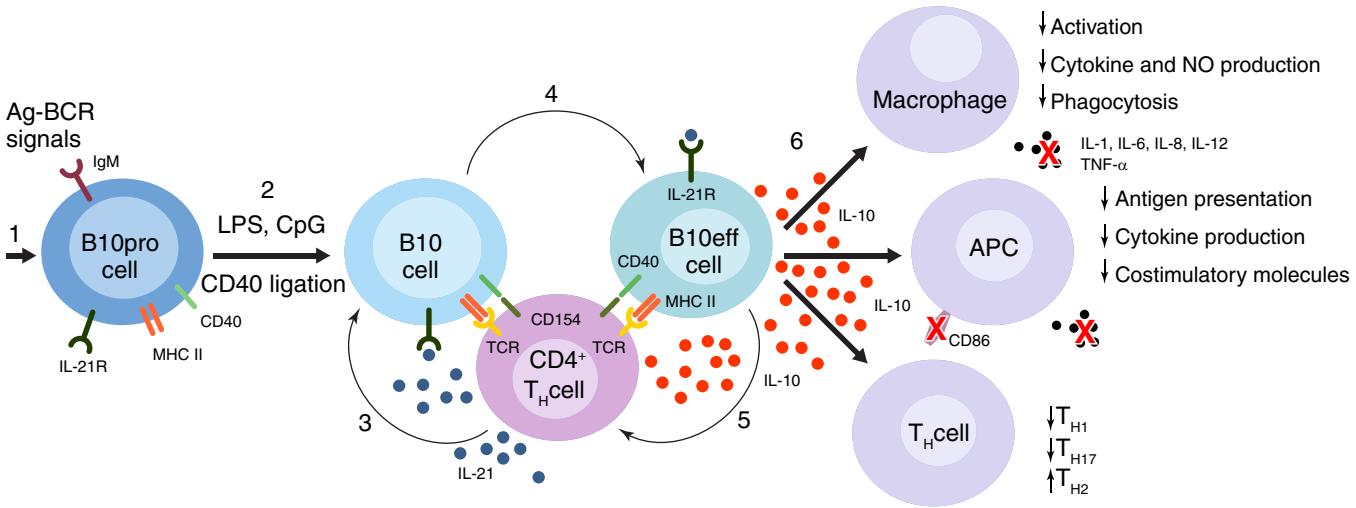

Figure 1. Model for autoantigen (Ag)-specific B10 cell regulatory effects in autoimmune disease. B cells capture unidentified autoantigens that trigger appropriate BCR signals (1) and promote IL-10-competent B10pro cell development. Following exposure to CD40 ligation and/or TLR ligands (2), B10pro cells mature into B10 cells that present peptides to antigen-specific T cells through cognate interactions that induce T cell activation and CD40/CD154 interactions. Activated T cells may produce IL-21 locally, which binds to proximal B10 cell IL-21R (3). IL-21R signals, along with MHC II and CD40 cognate interactions with $CD4^+$ T cells, to induce B10 cell IL-10 production and effector (B10eff) function (4), which may negatively regulate antigen-specific T cell function (5). B10 effector cells have multiple functions (6). They regulate macrophage function by decreasing their activation, phagocytosis, and cytokine and NO production. In APCs, B10 cells negatively regulate antigen presentation, expression of costimulatory molecules, such as CD86, and proinflammatory cytokine production that limits T cell activation. In $CD4^+$ T_H cells, B10 cells skew responses toward a T_{H2} phenotype and away from T_{H1} and T_{H17} responses. The negative regulatory effects of B10 cells thereby limit inflammatory responses and subsequent tissue damage.

TABLE 2. IL-10 Production and B10 Cell Frequency in Blood from Patients with Autoimmune Diseases

Human disease	IL-10 production[a]	Peripheral blood B10 cell frequency
Systemic lupus erythematosus	Increased/decreased[b]	Increased
Rheumatoid arthritis	Increased	Increased
Systemic sclerosis	Increased	ND
Primary Sjögren's syndrome	ND	Increased
Multiple sclerosis	ND	Increased
Autoimmune bullous diseases	ND	Increased

Reviewed in refs. [8, 10]. [a]Not necessarily from B10 cells. [b]Two independent studies gave different findings.

the mice that they will be protected? If so, will this type of treatment be feasible in humans? Given that transfer of B10 cells after induction did not reduce disease severity significantly, how can we envision a treatment strategy for expansion of B10 cells in humans before disease onset? Last, what would be the appropriate time-point for adoptive transfer to target disease initiation rather than disease progression?

It is currently unclear whether B10 cells identified in patients with autoimmune diseases are functioning properly. In some studies, the production of IL-10 by blood B cells was shown to be higher in patients with rheumatoid arthritis, lupus, and systemic sclerosis (reviewed in refs. [10]). Yet, in other studies, patients with multiple sclerosis and lupus have been reported to produce decreased amounts of IL-10 [9]. The recent studies by Iwata et al. [10] suggest that B10 cells are functionally competent to express IL-10 ex vivo in healthy persons and autoimmune patients after stimulation. It is expected that disparities such as these will be clarified in the near future once protocols have been established to identify and enumerate clearly IL-10-competent B10 cell frequencies within patients. It is certainly possible that our current interpretation of results is from mixed populations of B cells.

Additional research will be required to determine exactly how B10 cells behave in humans and whether they have other important functions than production of IL-10, as many cell types have the ability to produce IL-10. In this regard, systemic administration of human rIL-10 to psoriasis patients in a randomized, double-blind, placebo-controlled study demonstrated only a temporary improvement for the treatment of psoriasis (reviewed in ref. [8]). This disappointing outcome may have been a result of the short serum half-life of rIL-10 or the fact that B10 cells, when they are adoptively transferred, provide additional immune benefits, not accounted for by IL-10 alone. This begs the question of what else are regulatory B10 cells doing in the blood of patients with autoimmune diseases. Given the recent findings of Yanaba et al. [8] and others [1–7, 9], determining the functional capacity of B10 cells in human autoimmune diseases is highly warranted and may lead to novel approaches for the treatment of a variety of autoimmune diseases. Challenges in the future will be to narrow the gaps in our understanding of B10 cell function in murine models of autoimmune disease and human autoimmune disease.

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