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Regulatory B Cells That Produce IL-10: a Breath of Fresh Air in Allergic Airway Disease

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Keywords

Asthma; IL-10; regulatory B cell

In this issue of the *Journal of Allergy and Clinical Immunology*, Amu and colleagues demonstrate a significant role for interleukin-10 (IL-10)-producing regulatory B (Breg) cells during mouse models of allergic airway inflammation[1]. The authors identified a Breg cell subpopulation that expands *in vivo* and *in vitro* in response to parasitic *Schistosoma mansoni* worm infection. The adoptive transfer of these Breg cells into allergen-sensitized mice suppresses anaphylaxis and allergen-induced airway hyper-responsiveness through IL-10-dependent mechanisms[1, 2]. These important findings expand the clinical significance of studies showing that IL-10-competent Breg cells dramatically regulate inflammation and autoimmunity in mouse models of contact hypersensitivity[3], experimental autoimmune encephalomyelitis (EAE)[4, 5], collagen induced arthritis[6], and inflammatory bowel disease[7].

These studies focus on a relatively rare IL-10-competent mouse B cell subset that represents only 1–2% of spleen B cells in naïve wild type mice[8]. We call these cells “B10 cells” because IL-10 secretion is universally recognized as their mechanism of regulatory function, they only produce IL-10 transcripts[3, 9], and multiple other B cell subsets with regulatory properties are likely to exist. B10 cells are predominantly contained within a phenotypically unique $CD1d^{hi}CD5^{+}CD19^{hi}$ B cell subpopulation that normally represents only 2–7% of spleen B cells[3]. B10 cells appear to be functionally mature since they can be identified by cytoplasmic IL-10 expression following only 5 hours (h) of *in vitro* stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin. B10 progenitor (B10pro) cells have also been functionally identified within the spleen $CD1d^{hi}CD5^{+}$ B cell subpopulation, but these cells require 48 h of *in vitro* stimulation through CD40 or with LPS before they acquire the ability to express cytoplasmic IL-10 after 5 h PMA and ionomycin stimulation[9, 10]. Thereby, purifying spleen $CD1d^{hi}$ or $CD1d^{hi}CD5^{+}$ B cells enriches for functionally potent B10 and B10pro cells that can be adoptively transferred into recipient mice to shift the normal balance of regulatory networks towards a more immunosuppressive phenotype.

Parasitic infections with *S. mansoni* worms in the current study drives both B cell and B10 cell expansion in mice[1]. Transferring spleen $CD1d^{hi}$ B cells from worm-infected mice into ovalbumin (allergen)-challenged recipients inhibits both acute and established airway

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inflammation. Most likely, B10 cells expand more than other B cells *in vivo* because they proliferate more vigorously in response to polyclonal mitogens when compared with non-B10 cells[9]. Since antigen-specific B10 cells are required to inhibit contact hypersensitivity and autoimmunity[3, 5], it is unlikely that worm antigen-specific B10 cells would inhibit ovalbumin-driven disease. Thus, it will be important to determine whether *S. mansoni* worms are driving polyclonal, antigen-specific or cross-reactive B10 and B10pro cell expansion/maturation. It will also be important to determine whether worm-driven B10 cell expansion can regulate contact hypersensitivity and autoimmunity. Regardless, helminth-driven B10 cell expansion supports the "hygiene hypothesis," whereby a decrease in helminth infections within a population is proposed to increase allergic disease incidence[11]. Thus, B10 cell function and their relative frequencies may also be important factors contributing to human allergic diseases.

The authors propose to have identified a distinct $IL10^{+}CD1d^{hi}CD21^{hi}CD23^{+}IgD^{+}IgM^{hi}CD19^{+}$ spleen Breg cell subpopulation. However, there are currently no cell surface markers that uniquely delineate all IL-10-competent B10 cells or B10pro cells. Rather, the ability of B10 cells to produce IL-10 is the single functional marker that unifies most current studies and identifies a population of cells with a fairly homogenous cell surface phenotype[3]. Isolating B cells based on IL-10 expression alone is technically problematic as this selects for either IL-10 secreting cells or cytoplasmic $IL\text{-}10^{+}$ cells that must be permeabilized, while functionally important B10pro cells are lost using these methods. Moreover, IL-10 competence is most frequently measured after PMA and ionophore stimulation *in vitro*. As shown by Amu and colleagues, single markers such as $CD1d^{hi}$, $CD21^{hi}$, $CD23^{+}$, IgD^{low}, or IgM^{hi} could be used to enrich for B10 cells, but they also exclude a substantial proportion of functionally competent B10 cells that are then diluted within the remaining non-selected B cell population. IL-10 competent B cells found within other mouse tissues also differently express some of these cell surface markers[9]. Despite these technical issues, most studies within the evolving Breg cell field are likely to be examining the same rare and functionally unique B10 and B10pro cell subset that regulates immune responses through the production of IL-10.

B cells contribute to asthma pathogenesis by producing IgE[12]. However, Amu and colleagues also identified B cell subsets that either exacerbated or regulated allergic airway inflammation. While most B cells express CD1d, a spleen $CD1d^{low}$ B cell subset was expanded in helminth-infected mice. Asthma was exacerbated when these $CD1d^{low}$ B cells were adoptively transferred into allergen-sensitized mice. Although it was not determined whether these B cells contributed to IgE production, the future characterization of these cells may reveal a novel B cell subset that preferentially contributes to disease pathogenesis through unknown mechanisms. Distinct B cell subsets with opposing pathogenic and negative regulatory functions have also been observed during EAE pathogenesis in mice[5]. Mature B cell depletion using CD20 monoclonal antibody before EAE induction exacerbates subsequent disease, while B cell depletion during EAE progression dramatically reduces disease symptoms. Exacerbated autoimmune disease results from B10 cell depletion before disease initiation, which is ameliorated by the adoptive transfer of spleen $CD1d^{hi}CD5^{+}$ B cells. B10 and other B cells have also been found to have opposing protective and pathogenic functions during mouse models of systemic lupus erythematosus, respectively[13, 14]. Thereby, different B cell subsets may display opposing protective and pathogenic functions during human asthma, since B cell depletion may improve atopic eczema[15]. Thus, future mouse and patient studies are needed to further uncover the likely complexities of B cell function during different stages of airway immunopathology.

Amu and colleagues demonstrate that IL-10 production by $CD1d^{hi}$ B cells is required to observe their regulatory effects, and they show that *S. mansoni* infected CD1d-deficient mice are highly susceptible to allergic airway inflammation. However, it remains essential to determine whether B cell CD1d expression is required for B10 or B10 pro cell function since CD1d

expression is not required for B10 cell development[9]. Multiple leukocyte lineages and subsets produce IL-10, and the mechanisms by which IL-10 can inhibit or augment immune responses are equally complex. However, Amu and colleagues propose that adoptively transferred $CD1d^{hi}$ B cells suppress airway inflammation by inducing natural $FoxP3^+$ $CD4^+$ regulatory T (Treg) cell recruitment into the lungs, where they suppress lung inflammation. Whether B10 cells must enter the lung to induce these changes or exert these effects distally remains unknown. It is also unknown if B10 cells actually control Treg cell migration or whether enhanced Treg emigration into the lung is an indirect consequence of reduced inflammation. Nonetheless, Treg cell numbers are significantly decreased in CD19-deficient NZB/W mice that have few B10 cells, while wild type $CD1d^{hi}CD5^+$ B cell transferred into $CD19^{-/-}$ NZB/W mice induces Treg cell expansion[14]. These independent studies suggest a potential link between B10 cell function and Treg cell frequencies that needs to be explored.

Multiple laboratories have demonstrated that Breg cells are functionally significant in diverse diseases. It will be important to determine whether other parasites and infectious agents also drive B10 cell expansion as a potential mechanism for reducing host immune responses. This will further open the door for identifying B10 cell-directed therapies. Turning these laboratory observations into therapeutic targets for modulating immune responses and pathology will be a significant, but important challenge for the future.

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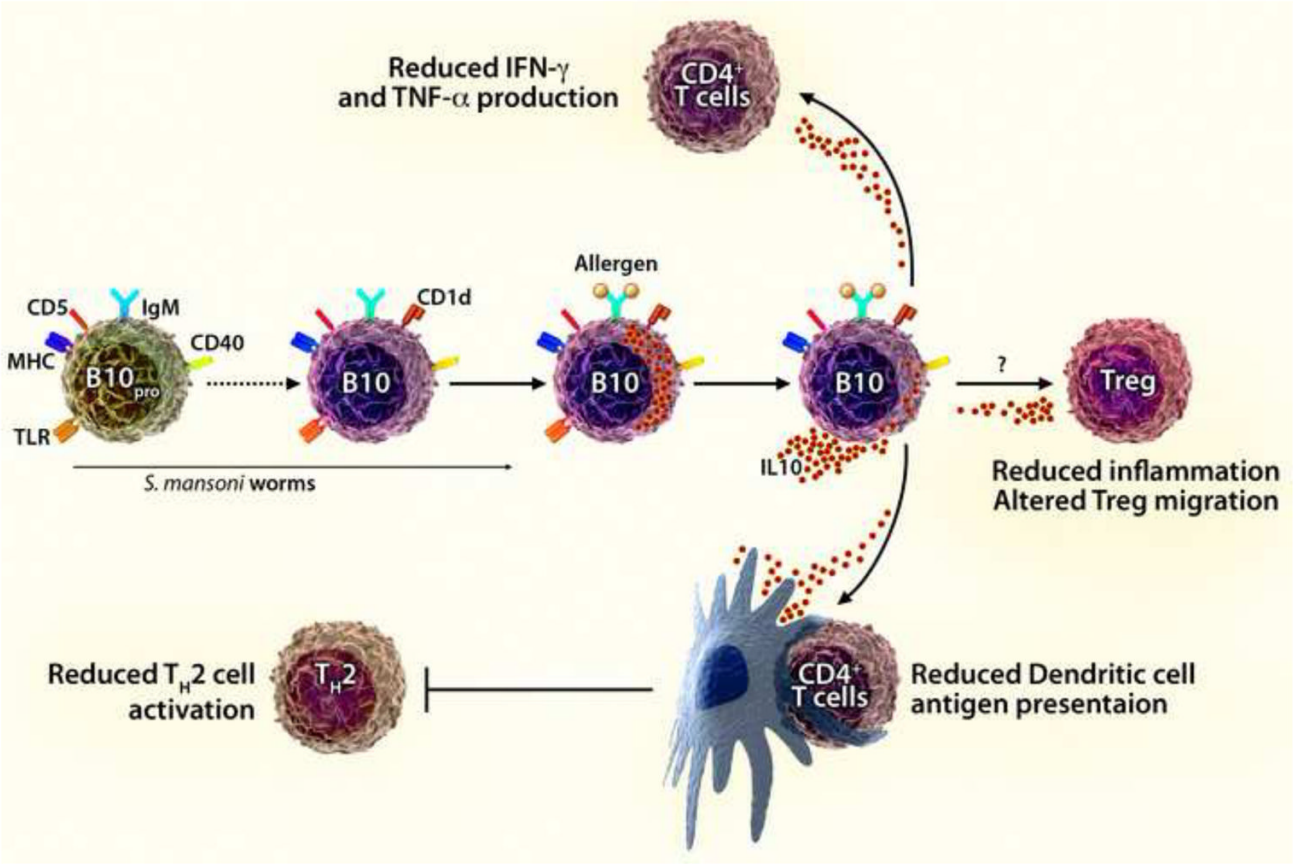

Figure 1.

IL-10-producing regulatory B10 cells inhibit allergic airway disease. Spleen B10pro cells mature into antigen-specific $CD1d^{hi}CD5^{+}$ regulatory B10 cells that are competent to produce and secrete IL-10 in response to allergen (antigen) challenge. Amu and colleagues show that spleen B10pro/B10 cells are induced to mature/expand in response to *S. mansoni* worm infection. The subsequent adoptive transfer of $CD1d^{hi}$ B cells purified from infected mice into allergen-sensitized recipients suppressed the induction of acute and allergic airway inflammation, which is proposed to result from the recruitment of Treg cells into the lungs of challenged mice. From our studies, B10 cells are also known to inhibit $CD4^{+}$ T cell production of IFN-γ and TNF-α, to reduce the antigen-presenting capacity of DCs, and to reduce inflammatory responses through the production of IL-10. Thereby, B10 cells are likely to inhibit lung inflammation through multiple IL-10-dependent mechanisms of negative regulation.