

B regulatory cells in allergy

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Abstract

B cells have classically been recognized for their unique and indispensable role in the production of antibodies. Their potential as immunoregulatory cells with anti-inflammatory functions has received increasing attention during the last two decades. Herein, we highlight pioneering studies in the field of regulatory B cell (Breg) research. We will review the literature on Bregs with a particular focus on their role in the regulation of allergic inflammation.

KEYWORDS

allergen immunotherapy, Breg cells, IL-10, tolerance

1 | INTRODUCTION

Initial reports that indicated immunoregulatory functions exerted by B cells date back to the 1970s. It was demonstrated that B cell-depleted splenocytes isolated from OVA-sensitized guinea pigs exerted a significantly reduced suppressive capacity on delayed-type hypersensitivity reactions in recipient animals compared with non-B cell-depleted splenocytes. The authors concluded that B cells and T cells are important in the homeostasis of T-cell functions and that the mechanisms of suppression could not be attributed to antibodies.¹ Despite this early recognition of the potential immune regulatory function of B cells that appeared to be independent of antibodies, it took more than two decades until several landmark studies were published that shed some more light on the mechanisms of suppression employed by B cells.

In 1996, Wolf et al² published a study that demonstrated an immunosuppressive role for B cells in experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis. B cell-deficient (μ MT) and wildtype (WT) mice immunized with the myelin basic protein (MBP) peptide Ac1-11 in complete Freund's adjuvant (CFA) showed a similar onset and severity of disease symptoms. However, while WT mice spontaneously recovered, μ MT mice failed to do so. The authors concluded that while B cells were not required to induce EAE, they play a role in protection against the autoimmune response in EAE.² Several years later, Fillatreau et al³ provided some mechanistic insight into the immunosuppression exerted by B cells in EAE. They found that in mice with an interleukin-10 (IL-10) deficiency that was restricted to B cells, the pro-inflammatory type I immune response induced in the EAE model persisted and mice did not recover. Adoptive

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transfer of IL-10-proficient B cells isolated from WT mice that recovered from EAE could reduce the severity of EAE symptoms in recipient mice with a B cell contained IL-10 deficiency. These findings demonstrated that IL-10 secreted by B cells plays an important role in controlling autoimmune responses in the EAE mouse model. Around the same time, other studies reported that IL-10 producing B cells also exerted protective effects in other mouse models of chronic inflammation.^{4,5} Mauri et al⁴ found that activation of arthritogenic splenocytes with anti-CD40 and antigen resulted in high levels of IL-10 production by B cells. Adoptive transfer of these B cells into recipient mice in a collagen-induced arthritis (CIA) model prevented the development of arthritis and ameliorated established disease. Adoptive transfer of B cells isolated from IL-10 knockout mice, or WT B cells treated with anti-IL-10 or anti-IL-10R failed to confer protection against CIA. In 2002, Mizoguchi et al⁵ reported that chronic intestinal inflammation in a murine model of colitis led to the induction of an IL-10 producing B cell subset with an upregulated expression of CD1d in gut-associated lymphoid tissue (GALT).

While these early studies were highly important in demonstrating that B cells can suppress inflammation through the production of IL-10, they provide limited insight into the origin and phenotype of these B cells. Moreover, as these studies were conducted solely in animal models, it remained unclear to what extent these findings could be translated to humans. The first indications that B cells may also exert immune regulatory functions in humans came from several case reports of patients treated with the anti-CD20 B cell-depleting antibody rituximab for a variety of indications. B cell depletion in these patients was associated with the development of psoriasis⁶ or exacerbation of ulcerative colitis.⁷ Similar findings have reported by other groups.⁸ Since then, the field of Breg research has taken a significant leap forward. Many studies have been published describing in-depth the phenotypic and functional characteristics of subsets of murine and human B cells with immune regulatory functions, either through IL-10 production or through other suppressive mechanisms (Table 1). While many studies on Bregs primarily focused on their role in autoimmune diseases (reviewed elsewhere⁹), potential immune regulatory roles for B cells have been reported in the context of allergy,¹⁰⁻¹⁵ parasite infections,¹⁶⁻¹⁸ bacterial infections,¹⁹ cancer,²⁰⁻²³ and transplantation.^{24,25} Our work on Bregs has mainly been focused on their potential role in the induction and maintenance of tolerance to allergens. We made the observation that IL-10 expression was increased in B cells in response to bee venom AIT and reported this in 1998.²⁶ It was not until 2013 that we published a more detailed analysis of IL-10 producing B cells in the context of allergen tolerance.²⁷

In this review, we provide an overview of the different types of Bregs that have been reported to date, with a particular focus on their roles in allergic inflammation. We describe the mechanisms involved in Breg-mediated regulatory functions, their phenotypic characteristics, and developmental pathways.

2 | THE ORIGIN AND DIFFERENTIATION OF BREG CELLS

Rodents and humans have different B cell subsets with different surface markers and development pathways. A consolidated model for B cell development supports 2 major groups of B cells in both mice and humans: B-1/fetal and B-2/adults. CD5⁺B-1 cells are defined as B-1a cells, and B-1b cells are those lacking CD5 expression. Follicular and marginal zone (MZ) B cell populations are referred to as B-2 cells. B-1 cells are developed from fetal liver, and B-2 cells are developed from the bone marrow precursors later than B-1 cells in ontogeny.²⁸ Even though the cell surface phenotype can change contingent on the microenvironment, the interactions in the micro-niche might determine the development of the B-1/B-2 axes.²⁸ B-1 and MZ B cells were derived from fetal progenitors in the liver and independent of hematopoietic stem cell (HSC) during development, whereas B-2 progenitors were found in the spleen and bone marrow in adults.²⁹ Moreover, conventional B2 cells constantly recirculate between lymphoid tissues and blood and are the main source of affinity-maturated long-running antibody responses. Innate-like B cell subtypes enclose marginal B cells in the spleen, whereas B1 cells mainly reside in mucosal sites and coelomic cavities.³⁰

Prior to the settling of all types of B cells at their specific locations, the precursor cells need to complete their maturation stage. Subsequent to functional (non-auto reactive) B cell receptor (BCR) expression of precursor cells, these immature B cells that express IgM and IgD on their surface and lack of CD27 translocate to the peripheral blood. Immature B cells, also referred to as transitional B cells, gain CD24, CD38 expression and become mature antigen-naïve B cells. Upon activation by antigen recognition, B cells can either differentiate into plasmablasts/IL-10⁺plasmablasts, plasma cells, memory B cells, or IL-10⁺Breg cells.³¹ Bregs have been identified in CD24^{hi} CD38^{hi} immature B cells; however, the driven factors and mediators for differentiation to Bregs have not been clarified in humans. However, various types of Breg cells have been described in humans: CD19⁺CD24^{hi}CD27⁺³² Breg cells that similar to B10 cells in mice, immature Breg cell type CD19⁺CD24^{hi}CD38^{hi}^{33,34} which also includes CD19⁺CD24^{hi}CD38^{hi}CD1d^{high} immature transitional (it) Breg cells³³ and Br1 (CD19⁺CD25^{hi}CD71^{hi}) subsets which are present only in humans.²⁷ The ontogeny and differentiation pathways of Bregs is an area of active investigation because we currently do not know whether IL-10⁺ B cells have a separate differentiation pathway other than memory B cells. The concept that naïve B cell-derived CD27⁺ human plasmablasts produce more IL-10 compared with memory B cells supports the idea that different Breg origins might be present.³⁵ In addition to humans, the accumulation of Bregs or IL-10 producing B1a (CD5⁺) (Breg-like) B cells has been shown in peritoneal cavity and spleen of rodents.^{36,37} Moreover, numerous subsets of IL-10 producing Breg cells have been identified in mice: transitional 2 marginal zone precursor (T2-MZP) cells,^{38,39} B10 (CD5⁺CD1d^{hi} B) cells,^{40,41}

TABLE 1 Different Breg cell subsets and suppressor molecules

Types of Breg cell	Surface markers		Location	Hallmark production	Mechanisms of action
	Mouse	Human			
B1a cell	CD19 ⁺ CD5 ⁺	—	Spleen and bone marrow	IL-10	Control TLR mediated lethal inflammation in neonatal mice. ⁵³ Increase CD4 ⁺ T-cell death and regulate arthritis. ⁵⁵
B10 cells	CD19 ⁺ CD5 ⁺ CD1d ^{hi}	CD24 ^{hi} CD27 ⁺	Spleen of mice and human blood	IL-10	Suppress inflammation via ex vivo stimulation from LPS, PMA, and ionomycin. ⁵⁷ Suppress TNF- α production by monocytes. ³² Suppress effector CD4 T cells ^{40,41,51,58,59,61} and Th17 cells. ⁶⁰
T2-MZP cells	CD19 ⁺ CD21 ^{hi} CD23 ^{hi} CD24 ^{hi} (IgM ^{hi} IgD ⁺ CD1d ⁺)	—	Spleen	IL-10	Induce Treg cells and suppress effector CD4 ⁺ and CD8 ⁺ T cells. ^{15,38,39,62-65}
MZ cells	CD19 ⁺ CD21 ^{hi} CD23 ⁻	—	Spleen	IL-10	Passive transfer of IL-10 producing MZ B cells from apoptotic cells-treated mice provided protection from arthritis. ⁴² Suppress antigen-specific CD8 ⁺ T-cell responses in the early stages of <i>Leishmania donovani</i> infection. ¹⁸
Plasma cells	CD138 ⁺ MHC-II ^{lo} B220 ⁺	—	Spleen	IL-10 and IL-35	Suppress autoimmune inflammation by inhibition of dendritic cell function in a pathogenic T-cell generation. ⁴⁴
	sIgM ⁺ CD138 ^{hi} TACI ⁺ CXC R4 ⁺ CD1d ^{int} Tim1 ^{int}	—	Spleen	IL-10 and IL-35	Suppress CD4 ⁺ T cells. ^{19,44}
Tim-1 ⁺ B cells	Tim-1 ⁺ CD19 ⁺	—	Spleen and lymph node	IL-10	Enhance IL-10 expression on Treg cells. ^{43,67,68} Control Th1 and Th17 cells during inflammation. ^{66,69}
				IL-4	Promote Th2 cells. ⁴³
Immature cells	—	CD19 ⁺ CD24 ^{hi} CD38 ^{hi}	Blood and the inflammation sites	IL-10	Inhibit naive T cell differentiation into Th1 and Th17 cells and converted CD4 ⁺ CD25 ⁻ T cells into Treg cells via the production of IL-10. ^{33,34}
Br1 cells	—	CD19 ⁺ CD25 ^{hi} CD71 ^{hi} CD73 ⁻	Blood	IL-10 and IgG4	Control inflammation via the release of IL-10, which induce differentiation of regulatory T cells, inhibit pro-inflammatory responses, and enhance IgG4 production. ²⁷
Plasmablasts	CD138 ⁺ CD44 ^{hi}	CD19 ⁺ CD24 ^{hi} CD27 ^{int} CD38 ⁺	dLNs in mice and human blood	IL-10	Suppress effector CD4 ⁺ T cells and DCs. ³⁵

MZ B cells,⁴² T cell Ig and mucin domain (Tim)-1⁺ B cells,⁴³ CD138⁺ plasma cells,^{19,44} and plasmablasts (CD44^{hi} CD138⁺).³⁵ The subtype of Breg cells, characteristics, and their effector functions are shown in Table 1. Nevertheless, numerous subsets of Breg cell are similar in phenotypes, and in function, how Breg cell subsets are developmentally linked is not clear.

Breg cell subsets are sophisticated and often overlap with other B cell populations. With various stimuli, such as IL-1b, CD40L, GM-CSF, or CpG, it has been possible to generate functional human Bregs in vitro. Whether or not these generated Bregs all derived from peripheral blood B cells, the ones with regulating functions indicate different phenotype frequencies.⁴⁵ Recently, it has been showed that immature transitional regulatory B (iTreg) cell development was induced by CD40L-expressing palatine tonsil-derived ILC3s in healthy individuals.¹³ Although this research suggests a role for innate lymphoid cells in iTreg cell development, differentiation into Bregs by ILC3 or other subsets of ILCs requires further research. Additionally, inflammation is also important in the differentiation of Breg cells. Recently, it has been demonstrated that chronic skin inflammation has induced IL-10 production from innate-like B cells in mice. B1 cells assembled in the skin of mice, contributing to IL-10 production. Similar to mice B1 cells, the same study showed the presence of IL-10⁺ innate-like B cells among the cutaneous lymphocytes also in normal human skin which had found with a higher percentage among the skin resident B cells in terms of their blood counterparts.³⁰ This study demonstrates that B cells can relocate from the peritoneal cavity and give rise to IL-10-producing B1 cells. However, the factors apart from inflammation which induces the process for differentiating into Breg-like phenotype have not been revealed yet. Moreover, inflammatory stimulation through IL-1b and/or IL-6 increased the frequency of splenic IL-10⁺B cells and IL-10 expression in mice.³⁹ These data suggest that Breg cell fate might be determined by the presence of inflammatory signals and conditions, similar to T_H17 differentiation.

In addition to stimulations through inflammatory receptors, BCR also displays regulatory mechanisms by involving in pathways for Bregs to detect inflammatory stimuli. In an experimental autoimmune encephalomyelitis (EAE) mouse model, it was demonstrated that Breg activation was also dependent on BCR signal transduction and IL-10 production by B cells in the absence of CD19.⁴⁰ Furthermore, highly proliferative CD138⁺ plasmablasts, which express high amounts of B lymphocyte-induced maturation protein 1 (Blimp1), produces IL-10 in a BCR-dependent manner.³⁵ Taken together, BCR engagement is important for Breg function. However, there is no clear evidence for antigen-specificity of BCR activation which reconsidering the idea for a specific B cell lineage.

In addition to B cell signaling, epigenetic regulations can have a role in inducing Bregs in terms of differentiation and function by modulating somatic hypermutations and class-switch DNA recombination for the B-cell activation and differentiation. Furthermore, the enzymes which are main players in the B-cell differentiation steps, transcription factors can also influence the process. Particularly, genetic modifications of a transcription

factor expression were found to be associated with plasma cell ontogeny. Blimp-1 (encoded by Prdm1 gene) is essential for differentiation into plasma cells, and both expressed in antibody-secreting cells (ASCs) from human and mouse.^{46,47} Integration of GFP reporter gene into the Blimp-1 locus in mice results in green fluorescence protein-expressing ASCs. Thus, they can be followed both in vivo and in vitro.⁴⁶ RNA-sequencing technology with the combination of Blimp-1-GFP reporter mouse strain provides information that contributes to the nature of B cell terminal differentiation. Whole genome microarray expression analysis showed that IL-10-producing B cells express JAK1 and STAT3, STAT5A, and PTPN11.²⁷ Furthermore, overexpression of IL-10 in Bregs was linked with SOCS3 expression.¹³ On the contrary, the activation-induced cytidine deaminase (AID) enzyme which is crucial for class-switch recombination and somatic hypermutation was downregulated in IL-10 overexpressing B cells. Considering the B1 cells immunosuppressive functions which include both IgG4 induction and IL-10 production, interestingly, abundant IL-10 levels do not affect IgG4 expression on transcriptional levels and, however, decreases IgA expression.¹³ Accompanied by data, the AID might have a role for modulating the immunoglobulin class switching in transcriptional levels. Although the suppressive function of stimulated IL-10 expressing B cells on CD4⁺ T cells was reversible,²⁷ this reversible process might be conducted by the niche in vivo conditions.

Acquiring of lymphoid lineage potential and restrict their differentiation to the B cell lineage is a vast network by means of transcription factors (eg, IKAROS, E2A, EBF1, FOXO1, PAX5, and IRF4) which take action to induce molecular mechanisms.⁴⁸ Particularly, upregulation of IRF4 is one of the hallmarks of memory B cells during transition before differentiating to plasma cells.¹³ This transcription factor is essential for IL-10 production in plasmablasts³⁵ and which in turn can lead Bregs more plasma cell-like phenotype.⁴⁹ Furthermore, in the absence of Blimp-1, B cells were able to secrete IL-10 in vitro, which demonstrates that differentiation toward plasma cells may occur.⁴⁷ This might be due to early activation of IL-10 which has already started at the preplasmablastic stage of plasma cell development. In addition to IRF4, IRF8 has been shown to have a role in secretion of IL-10. Co-recruitment of IRF4 and IRF8 to the immune suppressive genetic loci might be needed for the maximum capacity of IL-10 and IL-35 production in Bregs.⁴⁹ Studies have shown Bregs producing IL-35 (i35-Bregs) have an impact in regulating autoimmune diseases and are a major source for IL-35. They can be present in secondary lymphoid organs in mice and capable of crossing the blood-retina barrier in experimental autoimmune uveitis (EAU) mice model.^{49,50} Recently, an additional mechanism for Breg suppression has been proposed through bioactive IL-35 (i35-Exosomes). Subsequent to the stimulation of splenic mice B cells, these cells released i35-exosomes.⁵⁰ Neither the phenotype nor the origin of Bregs capable of releasing IL-35 has been described so far. It remains unknown whether the secretion is restricted by activated or induced Bregs. Moreover, in tissue analysis IL-35-producing B cells also

showed IL-10 production in the EAU mouse model and were described by CD19⁺B220^{low}CD38⁺CD138⁺ immunophenotype under autoimmune conditions.⁴⁹ However, these findings need further investigation.

The studies discussed above, illuminates the differentiation capacity of immature B cells, mature B cells, and plasmablasts into IL-10-producing Breg cells. The initial step for Breg cell differentiation should not be considered as an expression of a Breg-specific lineage factor alone. The environment which a B cell resides in also drives Breg cell differentiation and development. In brief, the identification of stimuli essential to induce B cells to differentiate into regulatory cells must be taken into consideration to evaluate the origin of Breg cells.

3 | DIFFERENT TYPES OF BREG CELL SUBSETS

The unique phenotypes of Breg cells have been intensively characterized in several studies from mice and humans. Although there are numerous regulatory subsets with defined phenotypes and biological functions, IL-10 is still a marker as well as the essential cytokine by which Bregs suppress. However, some Breg cells have overlapped phenotypes with previously described B cell subsets.

3.1 | Subsets of murine breg cells

In a murine model, there are up to 5% of Breg cells compared to the whole B cell population from spleen and lymph nodes.⁵¹ As mentioned above, Breg cells were first identified in mice by Wolf et al² in 1996 and a few years later in 2002 the term "regulatory B cells" was given by Mizoguchi and his team. A pivotal study by Carter et al⁵² confirmed that chimeric mice lacking IL-10-producing B cells established an exacerbated CIA compared with wildtype B cell mice. Collectively, Breg cells are an important key player in the maintenance of tolerance and prevention of several experimental inflammatory and autoimmune diseases in mice. Here, we list the different subsets of murine Breg cells together with their phenotypes and functions (Table 1).

3.1.1 | B-1a cells

B-1a cells are innate immune-like B cells that predominantly inhabit the peritoneal and pleural cavities.²⁸ These cells produce natural antibodies, especially IgM that upregulate the clearance of apoptotic cells. B-1 cells further grouped into functionally different subsets based on the expression of CD5. Murine B1 cells are composed of 2 subsets: CD19⁺CD5⁺B1a and CD19⁺CD5⁺B1b cells. The CD19⁺CD5⁺B1a cells mainly produced a suppressor cytokine, IL-10. In neonatal mice, B-1a cells were able to control Toll-like receptor (TLR)-mediated lethal inflammation by diminishing massive inflammation in an

IL-10-dependent manner.⁵³ In a Th2-mediated colitis murine model, B-1 B cells produce a significantly large amount of natural IgM antibodies in response to microbial flora and suppress colitis.⁵⁴ In a CIA murine model, B-1a cells which expressed Fas ligand (FasL) or known as "killer B cell," increase CD4⁺ T-cell death and could play a role in regulating arthritis.⁵⁵ Furthermore, the tolerogenic murine B cell population was identified as CD5⁺ CD19⁺ CX3CR1⁺ TolBCs by Liu et al⁵⁶ These TolBCs can induce Tregs in the intestine and suppress food allergy-related Th2 inflammation in mice.

3.1.2 | B10 cells

A similar IL-10 production was also observed in murine CD5⁺CD1d^{hi} B10 cells by Yanaba et al⁴⁰ The co-expression of CD1d and CD5 markers in B cells produce mainly IL-10 and suppress inflammation in a variety of immune-induced disorders via ex vivo stimulation from LPS, PMA, and ionomycin.⁵⁷ Matsushita et al⁵⁸ was the first group to demonstrate that depletion of an IL-10-producing CD1d^{hi}CD5⁺ Breg cell subset or B10 cells increased EAE symptom severity and T-cell infiltration into the central nervous system in mice. Several studies on the immune regulatory role of B10 cells have been shown in other disease models including allergic inflammation, intestinal inflammation, arthritis, and lupus.⁵⁹⁻⁶¹

3.1.3 | Transitional 2-marginal zone precursor cells

Another type of murine Breg cells is called "immature" or "transitional" B cells. Depending on the maturation of these B cells, they are denoted as transitional stage 1 or transitional stage 2 B cells.²⁵ Evans et al³⁸ was the first group to describe the suppressive functions of splenic transitional 2-marginal zone precursor (T2-MZP) B cells with a CD19⁺CD21^{hi}CD23^{hi}CD24^{hi}IgM^{hi}IgD^{hi}CD1d^{hi} phenotype. In this experimental arthritis murine model, T2-MZP B cells were the major producers of IL-10 after collagen stimulation and act as immune suppressors both in vivo and in vitro. Furthermore, these T2-MZP B cells display immune-mediated functions in several diseases including allergy, autoimmunity, cancer, and skin allograft.^{15,62-65} In the spleen and bone marrow, a different B cell subset resides called marginal zone (MZ) B cells.²⁴ These MZ B cells express a high level of CD1d and produce IL-10. Passive transfer of IL-10 producing MZ B cells from apoptotic cell-treated mice provided significant protection from collagen-induced arthritis.⁴² In mice with early stages of *Leishmania donovani* infection, MZ B cells were able to suppress antigen-specific CD8⁺ T-cell responses.¹⁸

3.1.4 | CD138⁺ plasma cells

A massive quantity of antibodies is secreted from plasma cells in response to specific antigens. In mice with EAE, IL-10-producing CD138⁺ plasmablasts are decisive in reducing autoimmune inflammation by suppression of dendritic cell function for the generation

of pathogenic T-cells in the draining lymph nodes. The deficiency of plasmablasts also develops an exacerbated EAE.³⁵ In 2014, Shen et al¹⁹ were able to classify IgM⁺CD138^{hi}TACI⁺CXCR4⁺CD1d^{int}Ti m1^{int} plasma cells expressing the transcription factor Blimp1 (also known as Prdm1) in mice with EAE. These plasma cells produce both IL-10 and IL-35 cytokines that suppress the inflammation and immune response to *Salmonella* infection.

3.1.5 | TIM-1⁺ B cells

Tim-1, a type I transmembrane glycoprotein, contains an IgV domain and a mucin domain that controls T-helper (Th) cells Th1 and Th17 during inflammation and autoimmunity.⁶⁶ In 2012, Xiao et al⁶⁷ generated a Tim-1-mutant mouse (Tim-1^{Δmucin}) that genetically mucin domain was deleted. These mutant mice have deep defect in IL-10 production from Breg cells and developed autoimmunity-related hyperactive T cells, with high production of IFN-γ and increased serum levels of immunoglobulins and autoantibodies. In addition, TIM-1^{Δmucin} mice also exhibit accelerated allograft rejection.⁶⁸ Furthermore, Tim-1 expression and signaling are required for the ideal production of IL-10 in Breg cells. Tim-1-defected B cells reduced IL-10 production but increased IL-1 and IL-6 production. Tim-1-deficient B cells elevate Th1 and Th17 responses but suppress the development of regulatory T cells and increase the severity of EAE.⁶⁹

3.2 | Subsets of human Breg cells

In healthy humans, Bregs comprise a small fraction (1%-2%) of the total amount of B cells in the blood.³² Similar to murine models, human Breg cells are characterized by their IL-10-producing capacity. Correale et al⁷⁰ isolated Breg cells from patients with multiple sclerosis and were also infected with helminths. Their results showed an increased frequency of IL-10-producing CD19⁺CD1d^{hi} B cells, reduction of T-cell proliferation, and decreased IFN-γ production. Blair et al³³ identified Breg cells among immature transitional B cells as CD19⁺CD24^{hi}CD38^{hi} in healthy individuals but were functionally impaired in SLE patients. In 2013, van de Veen et al²⁷ initially described a novel Breg subset, CD19⁺CD25⁺CD71⁺CD73⁻ inducible Breg cells (Br1 cells), in the context of allergen-specific immune tolerance studies. Other B cell subsets secreting IL-10 include CD24^{hi}CD27⁺B cells (B10) and CD27^{int}CD38^{hi} plasmablasts also suppress pro-inflammatory responses. The different subsets of human Breg cells and their phenotypes and functions discussed above are summarized in Table 1.

3.2.1 | CD19⁺CD24^{hi}CD38^{hi} Breg cells

Breg cells were first identified in the CD19⁺CD24^{hi}CD38^{hi} immature B-cell fraction in peripheral blood of healthy individuals.³³ These CD19⁺CD24^{hi}CD38^{hi} Breg cells inhibited naive T cell generation into Th1 and Th17 cells and converted CD4⁺CD25⁻T cells into Treg cells via IL-10 production. Moreover, the suppression of the Th1 response by Breg cells

was partly IL-10-dependent and needed CD80-CD86 interaction with T cells.³³ The lack of CD19⁺CD24^{hi}CD38^{hi} Breg cells has been described in autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus (SLE).^{34,71} In SLE patients, B cells present lipid antigen to CD1d-restricted invariant natural killer T (iNKT) cells that maintain tolerance in autoimmunity. A decreased frequency of iNKT cells was positively correlated to dysregulated B cell responses. After αCD20 treatment, CD1d expression was normal in repopulated immature B cells, the iNKT cell number and function were restored.⁷¹ These results showed that healthy B cells are crucial for iNKT cell homeostasis. Furthermore, Breg cells have shown potential as a therapeutic target in organ transplantation.⁷²

3.2.2 | Br1 cells

CD19⁺CD25⁺CD71⁺CD73⁻ inducible Breg cells (Br1 cells) were discovered in 2013 and play an important role in allergen-specific immune tolerance.⁷³ Br1 cells are suppressive B cells that control inflammation by the release of IL-10 and induce generation of regulatory T cells and inhibit inflammatory responses and enhance IgG4 production.^{10,74-77} Br1 cells express CD25 (IL-2 receptor α chain), CD71 (transferrin receptor 1), and very low CD73 on the cell surface, which was shown in bee venom allergic patients and healthy beekeepers.²⁷ Changes in the immune response induced by venom immunotherapy were evident 3 to 4 months after initiating treatment. The frequency of phospholipase A2-specific IL-10-producing Br1 cells increased twofold to fivefold, reaching a level comparable to that of healthy beekeepers during the season.⁷⁸⁻⁸⁰ In addition, PLA-specific B cells increased CCR5 expression after high-dose allergen exposure whereas CXCR4, CXCR5, CCR6, and CCR7 expression did not change.¹¹ IL-10 producing Breg cells are characterized by upregulation of CD25, programmed death-ligand 1 (PDL1), suppressor of cytokine signaling 3 (SOCS3), and glycoprotein A repetitions predominant (GARP) molecules.¹³ Furthermore, the frequency of plasmablasts and IL-10 and/or IL-1R antagonist producing Breg cells was higher in responders in comparison with non-responders after 2 years in a house dust mite AIT-receiving allergic cohort. Improved clinical symptoms showed positive correlation with increased frequency of Der p 1-specific IgG4⁺ B cells, plasmablasts, and IL-10⁺ and dual-positive IL-10⁺IL-1RA⁺ Breg cells throughout immunotherapy.¹² A recent study demonstrated that CD40L⁺ ILC3s and B cells are in a jointly favorable affiliation with each other. ILC3s stimulate IL-15 production in B cells via BAFF-receptor. IL-15 is a potent growth factor for ILC3s, increase CD40L expression on ILC3s. IL-15-activated CD40L⁺ ILC3s promotes B cell survival, proliferation, and differentiation to IL-10-secreting and PD-L1-expressing Breg cells.⁸¹

3.2.3 | CD24^{hi}CD27⁺ B cells (B10)

B10 cells are well known to produce a large amount of IL-10 and suppress TNF-α production by monocytes in peripheral blood. These B10 cells actively respond to stimulation with LPS and CpG.³² The number of B10 cells was described to be higher in some systemic

and organ-specific autoimmune diseases.⁸² B10 and B10pro cells are found within the CD27⁺ memory compartment in humans and can be preprogrammed *in vivo* to express IL-10 after *ex vivo* stimulation.³² Studying the capacity of individual B10 cells to produce IL-10 will provide useful information for precise medical treatments in patients.

3.2.4 | CD27^{int}CD38^{hi} plasmablasts

It was shown that during EAE induction Plasmablasts mainly produce IL-10. Matsumoto et al³⁵ isolated CD27^{int}CD38^{hi} plasmablasts from peripheral blood of healthy individuals, stimulated with CpG (a TLR9 agonist) and/or cytokines IL-2, IL-6, and interferon-alpha (IFN- α), which are the conditions for efficient plasmablast differentiation. The isolated CD27^{int}CD38^{hi} plasmablasts displayed morphological maturation into plasma cells, by their bigger size with rich cytoplasm, eccentric nuclei, and perinuclear haloes. Furthermore, CD27^{int}CD38^{hi} plasmablasts particularly produced IL-10 as measured by ELISA.

4 | IMPORTANT SUPPRESSOR MOLECULES PRODUCED BY BREG CELLS

Recent studies identifying molecules expressed in mouse and human Breg cells have greatly improved the stratification of patients prior to treatment. Breg cells mainly produce the major suppressive cytokines, including IL-10, TGF- β , and IL-35, and other important molecules including programmed death-ligand 1 (PD-L1), granzyme B, CD39, CD73, and Aryl-hydrocarbon receptor (Figure 1).

4.1 | Interleukin-10 (IL-10)

IL-10 is a regulatory cytokine with suppressor functions on several immune cells and inflammatory diseases, including allergy, autoimmunity, organ transplantation, and tumor tolerance.⁸³ The IL-10 cytokine is produced mainly by Breg cells, Treg cells, monocytes, some of natural killer (NK) cells, macrophages, dendritic (DCs) cells, and innate lymphoid cells (ILCs).⁸⁴ The positive regulation of IL-10 has an important effect on B-cell survival, class-switch recombination, and plasma cell differentiation. We have previously discussed the importance of IL-10 in the different types of Breg cell subsets section.

4.2 | TGF- β

Another immune regulation cytokine, namely TGF- β , is also expressed on the surface of Breg cells. This cytokine regulates the conversion of CD4⁺T cells to Treg cells and plays a major role in tissue remodeling and wound healing processes in the body. A study by Mi Lee et al⁸⁵ showed that Bregs produce the TGF- β -associated latency-associated peptide (LAP) and mediated graft prolongation. This effect is abrogated by neutralization of TGF- β activity.

4.3 | IL-35

IL-35 is an immunosuppressive heterodimeric cytokine containing two IL-12 family subunits, p35 and EB13, and it was shown to be essential for Treg and Th17-mediated suppression.⁸⁶ Wang et al⁸⁷ investigated in a murine model that IL-35 gave protection from experimental autoimmune uveitis. They found that mice missing IL-35 (p35 knockout (KO) mice) or defective in IL-35 signaling (IL-12R β 2 KO mice) had less Breg cells endogenously and developed severe uveitis. Adoptive transfer of Breg cells induced by recombinant IL-35 suppressed experimental autoimmune uveitis symptoms by inhibiting pathogenic Th17 and Th1 cells. Their results suggest that IL-35 induce autologous Breg cells and IL-35⁺ Breg cells play an important role in autoimmune and inflammatory diseases. Moreover, Egwuagu et al⁸⁸ showed IL-35 produced by i35-Bregs prevent the activation of pathological immune responses mediated by a pathogenic T-cell subset that promotes the development of autoimmune diseases.

4.4 | Programmed death-ligand 1 (PD-L1)

Programmed death-ligand 1 (PD-L1) has been implicated in B cell-mediated immunosuppression. It is a transmembrane protein that binds to inhibitory molecule PD-1 and activates immune cells such as T cells, B cells, and myeloid cells. Khan et al⁸⁹ demonstrated that Breg cells regulate innate immunity mediated by CD4⁺CXCR5⁺PD-1⁺ follicular T-helper cells and inhibit inflammation in autoimmune diseases via increased expression of PD-L1. Furthermore, a recent study showed that PD-L1⁺ B cells suppressed T cells and this effect significantly reduced in untreated rheumatoid arthritis patients but enhanced in response to successful treatment.⁹⁰

4.5 | Granzyme B

A recent study described granzyme B (GrB⁺)-expressing B cells with a CD19⁺CD38⁺CD1d⁺IgM⁺CD147⁺ phenotype. Granzyme B is a serine protease produced by natural killer cells (NK cells) and cytotoxic T cells. These cells infiltrate tumors and regulate T-cell responses.²¹ IL-21 that secreted from T cells induce GrB⁺ Bregs. These GrB⁺ Bregs cells also expressed IL-10, indoleamine-2,3-dioxygenase (IDO), and CD25. Future studies are needed to understand the role of GrB⁺ Bregs in the improvement of human cancer as well as autoimmune and graft-versus-host pathologies.

4.6 | CD39 and CD73

CD39 and CD73 are important enzymatic molecules in the adenosine (ADO) pathway. Independent of IL-10, circulating CD39⁺CD73⁺ B cells can make a shift from an ATP-driven pro-inflammatory environment to an anti-inflammatory milieu induced by adenosine. Saze et al⁹¹ examined CD39 and CD73 co-expression, the ability to produce

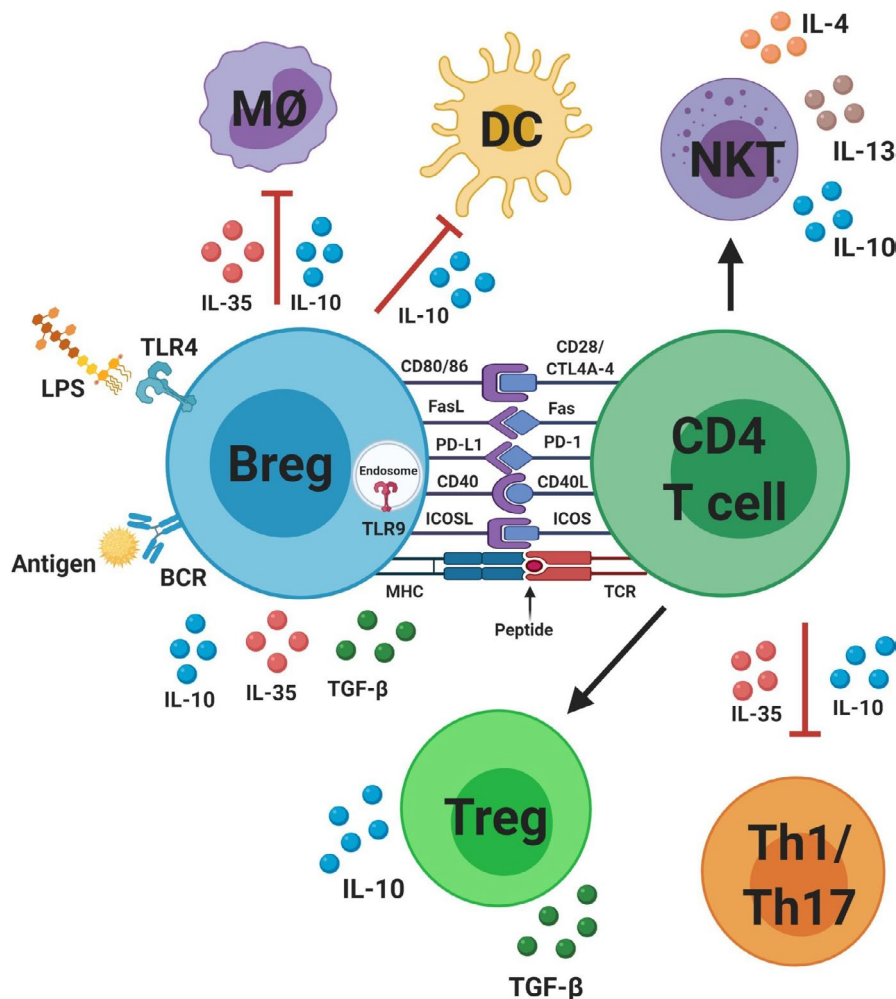


FIGURE 1 Suppressor molecules of B regulatory cells. Breg cells release suppressor mediators via the induction of TLR ligand stimulants such as LPS, CpG, BCR, and CD40. Breg cells inhibit macrophage and DC activation via the production of IL-10 and regulate the T cell responses by inhibiting the effector T-cell in particular TH1 and TH17 and promoting Treg cell expansion via the production of IL-10, TGF- β , and IL-35. The membrane-bound molecules at the interface between B cells and T cells include CD80, CD86, Fas ligand (FasL), CD40, inducible costimulator ligand (ICOS-L), and MHC-II on the B-cell side and T-cell receptor (TCR), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), CD28, Fas, PD-1, CD40 ligand (CD40L), and inducible costimulator (ICOS) on the T-cell side. Furthermore, Breg cells activate natural killer T (NKT) cells to produce IL-4, IL-10, and IL-13 with suppressive function

adenosine 5'-monophosphate (AMP), and adenosine (ADO) in the presence of exogenous adenosine triphosphate (ATP) and adenosine receptor (ADOR) expression. They found that *in vitro*-activated B cells downregulated CD73 expression produced 5'-AMP, suppressed T-cell proliferation and cytokine production. Their results suggest that B cells play a role in the regulation of T cell-B cell interactions, and their dual regulatory activity is mediated by the products of ATP hydrolysis, 5'-AMP, and ADO. Moreover, Figueiro et al⁹² demonstrated that a subpopulation of ADO-producing CD39⁺CD73⁺ B cells has also regulatory properties.

by commensal microbiota. It maintains both the innate and adaptive immune systems and regulates several immune cells including Tregs, Th17, dendritic cells, and stem cells. In 2019, Piper et al⁹³ first revealed that AhR, as an important transcription factor, and plays a direct role in the regulation of IL-10 transcription. A recent study showed that AhR is highly expressed in IL-10⁺CD19⁺CD21^{hi}CD24^{hi} B cells, a Breg cell subset. They demonstrated that AhR-dependent gene transcription in CD19⁺CD21^{hi}CD24^{hi} B cells is activated by the supplementation of short-chain fatty acid, butyrate and later supports Breg function and inhibits germinal center (GC) B cell and plasma cell differentiation.⁹⁴

4.7 | Aryl-hydrocarbon receptor (AhR)

The aryl-hydrocarbon receptor (AhR) is an environmental sensor that binds xenobiotic ligands and compounds derived from the digestion

5 | HOW TO INDUCE BREG CELLS

Although Breg cells are naturally present since birth, the induction of Breg cells throughout life is very important. Neonatal mice have

relatively low numbers of IL-10 producing Breg cells and this increases over time and under inflammatory conditions.⁹⁵ Similarly, in humans the amount of Breg cells also increases gradually during childhood.⁹⁶ So far, there is strong evidence that Breg cells are mainly induced in response to inflammation. In vitro this can be achieved by providing inflammatory stimulants, and in vivo this happens naturally after infections with parasites, bacteria, or viruses (Figure 2).

5.1 | In vitro

There are several ways to induce Bregs cells in vitro. The best-described mechanism is through a combination of CpG and CD40L stimulation. CpG motifs are abundant in microbial genomes and therefore function as pathogen-associated molecular patterns (PAMPs) that are recognized by TLR9. CD40L binds to CD40 on B cells, which causes B cell activation. The combination of these two

factors can induce high IL-10 production in B cells.^{32,97} In addition, it has been shown that IL-21 also plays an important role in the induction of IL-10 producing B cells^{95,98} and that IL-21 is able to induce granzyme B-expressing regulatory B cells (GrB cells).²¹ Komlósi et al⁸¹ demonstrated that Breg cells can also be induced by cells expressing CD40L. Type 3 innate lymphoid cells (ILC3) can induce IL-15 production in B cells, which is a potent growth factor for ILC3s and induces the expression of CD40L on these ILC3s. These IL-15 activated CD40L⁺ ILC3s can induce IL-10 secretion and PD-L1 expression on B cells and thereby can contribute to the maintenance of immune tolerance. However, CD40L can prevent the induction of GrB cells. CD4 T cells isolated from human immunodeficiency virus type-1 (HIV) patients, which secrete IL-21 but hardly express CD40L, are able to directly induce the differentiation of B cells into GrB cells, but the addition of soluble CD40L could prevent this differentiation.⁹⁹

Another molecule that can contribute to IL-10 production in B cells under specific conditions is IFN- γ . Although this cytokine is

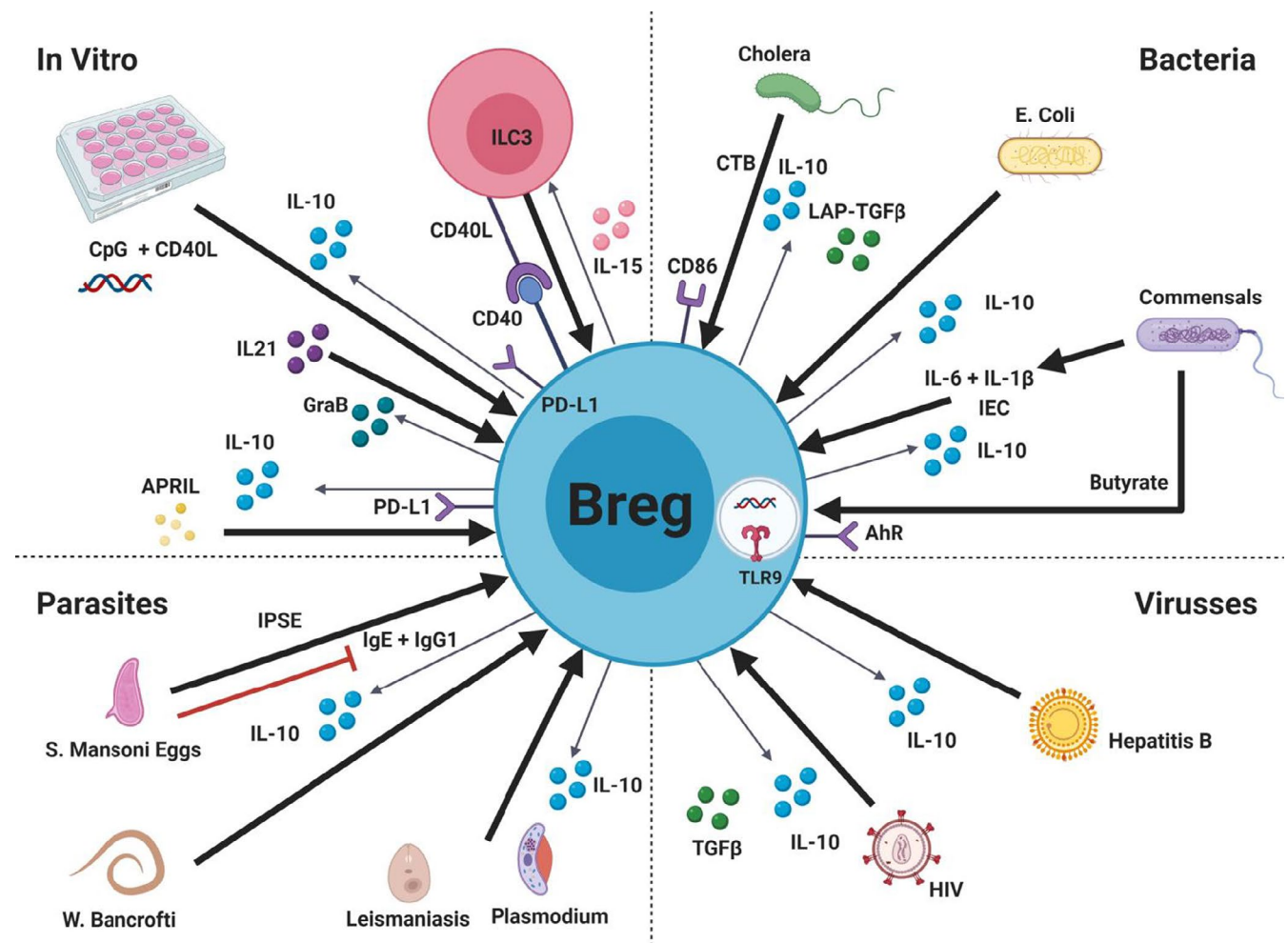


FIGURE 2 Induction of B regulatory cells. Breg cells can be induced by many different stimuli. In vitro CpG, CD40L, IL-21, and APRIL have been shown to be able to induce Breg cells and the production of IL-10, GrB, IL-10, and PD-L1. Parasites, such as *S. Mansoni*, *W. Bancrofti*, *Leishmaniasis*, and *Plasmodium*, *Cholera*, and *E.coli*, all can induce Bregs cells and IL-10 production. Commensal bacteria or pathogenic bacteria such as *Cholera* can also induce IL-10 production in Bregs and TGFβ. Lastly, viruses such as *Hepatitis B* and *HIV* are able to induce Bregs and IL-10 production

known to be able to inhibit IL-10 production in monocytes, it can also have regulatory functions. In combination with CpG stimulation, IFN- γ could spike IL-10 production in B cells, and these B cells were capable of inhibiting T cell proliferation.¹⁰⁰

A proliferation-inducing ligand (APRIL), a molecule that together with B-cell activating factor (BAFF) plays an important role in B cell development, was also shown to be able to induce Bregs. APRIL could promote differentiation of naive human B cells to IL-10-producing IgA⁺ B cells. These APRIL-induced IgA⁺ B cells could suppress T cell and macrophage responses through IL-10 and PD-L1.¹⁰¹

5.2 | Parasites

Parasitic helminths have been reported to induce Breg cells and are used experimentally to identify and explore mechanisms for Breg cell function. *Schistosoma Mansoni* is the best-described helminth that can induce IL-10 producing B cells, both in vitro and in vivo.¹⁰² Especially, the soluble egg antigens (SEA) from *S mansoni* are able to induce IL-10 production in B cells. One of the major antigens in SEA, IPSE/alpha-1, by itself is sufficient to trigger induction of these cells. However, there are also other components in SEA that are able to induce Breg cells, since SEA deprived from IPSE was still capable of this induction.¹⁰³ A proposed mechanism for this IL-10 induction involves type 1 interferons that helminths can induce. Type 1 interferons are able to serve as an additive signal to induce IL-10 production in Bregs cells but are unlikely to participate in the development of Bregs in response to *S mansoni* eggs, since this signal is dispensable for activation.¹⁰⁴ Surprisingly, despite the strong Th2 responses to these helminths, *S mansoni* eggs can protect against the development of allergic airway inflammation by reducing lung eosinophilia and allergen-specific IgG1 and IgE antibodies.¹⁰⁵

Young adults infected with a related species, *Schistosoma haematobium*, have increased numbers of IL-10 producing CD1d^{hi} Breg cells compared with uninfected individuals.¹⁰⁶

Another helminth parasite that also induces Breg cells is *Wuchereria Bancrofti*. *W Bancrofti* can cause lymphatic filariasis, although often infected individuals remain asymptomatic. Ritter et al¹⁰⁷ demonstrated that IL-10-producing CD19⁺CD24^{hi}CD38d^{hi} Breg cells were specifically increased in asymptomatic infected individuals compared with uninfected individuals. In addition, CD19⁺CD24^{hi}CD5⁺CD1d^{hi} and CD19⁺CD5⁺CD1d^{hi}IL-10⁺ Breg cells significantly increased in both latent infected and asymptomatic individuals.

In addition to helminths, other parasites are also able to induce Breg cells. Human B cells exposed to *Leishmaniasis infantum* amastigotes exhibited a regulatory phenotype (CD19⁺CD24⁺CD27⁻) and higher capacity to produce IL-10.¹⁰⁸ Furthermore, during a *Plasmodium chabaudi chabaudi* infection in mice, B cells were the main IL-10-producing cells, most were identified as IgG⁺ B cells but only 20% of these cells were part of the Breg subset CD19⁺CD5⁺CD1d^{hi}.¹⁰⁹

5.3 | Bacterial infections

Bacterial infections can also trigger the induction of Breg cells. An early study demonstrated that B cells that are exposed to cholera toxin B subunit (CTB) can promote the expansion of regulatory T cells in a mouse model. After CTB exposure, the B cells expressed higher levels of IL-10 and CD86. Furthermore, adoptive transfer of these cells in an oral tolerance model led to efficient suppression of proliferation and the induction of apoptotic depletion of Ag-specific effector T cells.¹¹⁰ A similar study demonstrated that CTB can suppress the immune system in an OVA model by inducing the formation of Tregs and IgA production in a B cell-dependent manner.¹¹¹ B cells stimulated with CTB acquired a regulatory phenotype and increased production of IL-10 and LAP/TGF β in a study of EAE,¹¹² and in a model for atherosclerosis.¹¹³

The induction of Breg cells is not exclusive to pathogenic bacteria; also, commensal bacteria play an important role. *Escherichia coli* was shown to be a potent Breg cell stimulator. Stimulation with *E coli* led to increased production of anti-inflammatory cytokines, such as IL-10, and an increased percentage of B10 cells, and they were able to prevent the proliferation and polarization of T-helper (Th)1 and Th17 cells.¹¹⁴

Capsular polysaccharide A (PSA) from the gut commensal *Bacteroides fragilis* also has the capacity of inducing Breg cells. A study showed that PSA-treated mice had significantly reduced brainstem inflammation after experimental infection with herpes simplex virus. The reduced inflammation was due to the induction of T regulatory cells and IL-10 production by PSA-bound B cells.¹¹⁵

A study on enteric bacteria showed that their lysates were able to induce IL-10 production and activate the regulatory capacity of intestinal B cells. They showed that this was related to TLR2, MyD88, and PI3K pathways.¹¹⁶

A study by Rosser et al³⁹ demonstrated that antibiotic treatment and sterile housing conditions reduce the number and function of Breg cells in mice. Mice housed in sterile conditions had lower levels of IL-1 β and IL-6, and both cytokines were able to directly promote Breg cell differentiation and IL-10 production. Microbiota-derived metabolites were essential players for the induction of these Breg cells. They observed that patients with rheumatoid arthritis have reduced levels of microbial-derived short-chain fatty acids and that supplementation with butyrate, a short-chain fatty acid, can reduce arthritis severity. Butyrate supplementation activates the aryl-hydrocarbon receptor (AhR), a newly discovered Breg marker, and thereby supports Breg function while inhibiting the differentiation of plasmablasts.⁹⁴

5.4 | Viral infections

Breg cells can also be induced by different types of viruses. It has been shown that the percentage of IL-10-producing B cells is enriched in patients with chronic hepatitis B virus infection. Phenotypically, these cells were predominantly immature

(CD19⁺CD24^{hi}CD38^{hi}) and these cells were able to suppress hepatitis B virus-specific CD8 T cell responses in an IL-10 dependent manner *ex vivo*.¹¹⁷

Breg cells also seem to be induced upon infection with HIV. In a recent study, a significantly higher proportion of B cells isolated from HIV⁺ individuals expressed IL-10.¹¹⁸ Lopez-Abente et al¹¹⁹ demonstrated that HIV-1 can induce a higher expression of IL-10, TGF- β EBI3, or IL-12(p35), indicating a more regulatory B cell phenotype. Furthermore, these B cells were able to reduce the proliferation of CD4⁺ and CD8⁺ T cells and their TNF α production.

6 | BREG CELLS IN ALLERGIC INFLAMMATIONS

6.1 | How does allergic inflammation occur?

The development of allergic inflammation is a complex interaction between local tissue and inflammatory cells, which can be re-divided into several inflammatory subtypes to improve our understanding of the disease.¹²⁰ The recent concept of precision medicine brings more attention to the realization of the inflammatory endotype of a disease. Inflammation is a type 2 immune response that is considered responsible for most of the allergic symptoms. Generally, both the innate and adaptive immune systems contribute to a type 2 response, involving the participation of epithelial cells, antigen-presenting cells, ILCs, Th2 cells, and inflammatory cells.¹²¹ Diseases such as allergic rhinitis, allergic asthma, and food allergy are mainly mediated by antigen-specific IgE and Th2 cells (Figure 3). In contrast, atopic dermatitis is thought to mainly occur in an IgE-independent manner.¹²¹ Allergic inflammation can be divided into two main phases: the allergen sensitization and the effector phase.¹²²

The sensitization phase is the preface of allergy without clinical symptoms. Allergen sensitization starts from allergen capture by dendritic cells (DCs) resident in the skin, gut, or the airway. DCs internalize and process the antigen into peptides, presenting them to naive CD4⁺ T cells by MHC class II molecules on the cell surface. The generation of allergen-specific CD4⁺ Th2 cells drives IgE production by promoting immunoglobulin class-switch recombination in B cells, induced by IL-4 and IL-13. IgE memory B cells differentiate into plasma cells producing allergen-specific IgE antibodies that bind to basophils and mast cells. Allergen-specific memory Th2 and B cells expand into a given population, which are stimulated upon re-exposure to allergen.

The effector phase is often classified into two stages. The early-phase reaction is a type I hypersensitivity reaction induced within seconds to minutes of allergen exposure. The allergen activates sensitized basophils and mast cells by cross-linking of the IgE, triggering their activation and release of anaphylactogenic products, such as histamine, heparin, proteases, lipid-derived mediators, leukotrienes, and cytokines.^{120,123,124} The release of these mediators causes acute inflammation and allergic symptoms.

Late-phase reactions occur within several hours after exposure to allergen and can be provoked by the activation of mast cells or T cells depending on the target organ.¹²⁵ In IgE-mediated cases, mast cells also release newly synthesized cytokines, chemokines, and growth factors, which are secreted more slowly.¹²⁶ In the skin of atopic subjects, late-phase reactions can be induced in the absence of mast cells in patients with asthma comorbidities.¹²⁷ Actually, these late-phase reactions were not dependent on IgE and were major histocompatibility complex (MHC)-restricted suggests that the activation of T cells alone is adequate to start airway narrowing in patients with allergic asthma.¹²⁵

There are some other important types of cells involved in allergic inflammatory processes. Epithelial cells act as the first line of defense to block allergic antigens and are considered immunoregulatory cells that produce IL-25, IL-31, IL-33, and thymic stromal lymphopoietin (TSLP), activating DCs and stimulating ILC2s to produce cytokines, such as IL-5 and IL-13 that drive allergic responses.⁸⁴ NKT cells may also produce Th2 cytokines and interact with Th2 cells and ILC2s to enhance allergic inflammation.^{128,129}

6.2 | Role of Breg cells in allergic diseases

Tregs and Bregs have many molecules expressed on their cell surface in common, such as IL-10, TGF- β , IL-35, CD39, and CD73. Some of the molecules expressed by Tregs and Bregs can interact with each other, such as in the PD-1/PD-L1 signaling pathway. However, the role of Bregs in allergic diseases is not fully understood. Table 2 summarizes recent studies on some common allergic diseases performed in human and in mice models.

6.3 | Allergic asthma

Allergic asthma is a type 2-dominated chronic inflammatory disease characterized by hyper-responsiveness to inhaled allergens in the lower respiratory tract.¹³⁰

In asthmatic patients, the number of CD24^{hi}CD27⁺ B cells was reported to be downregulated and its IL-10-secreting capacity was significantly lower in response to LPS compared with healthy individuals.¹³¹ In addition, there was no significant difference in CpG-induced IL-10 compared with healthy individuals, consistent with our previous study.⁷³ Braza et al¹³² demonstrated that IL-10-secreting Bregs are enriched in a CD9⁺ B cell subset, but decreased in asthmatic mice. A recent study has identified CD9 as a marker of IL-10-competent Bregs in mice and in humans.¹³³ The CD9⁺ B cell subset was demonstrated to inhibit Th2- and Th17-driven inflammation, increase the Treg/effector T-cell ratio in lung tissue,¹³⁴ and induce CD3⁺CD4⁺CD25⁻ effector T cell apoptosis in mice and humans via the mitogen-activated protein kinase signaling pathway.¹³³

Human respiratory syncytial virus (RSV) is a risk factor for asthma development. A recent study found that in neonates, RSV-infected neonatal-specific Bregs frequencies correlated with increased viral

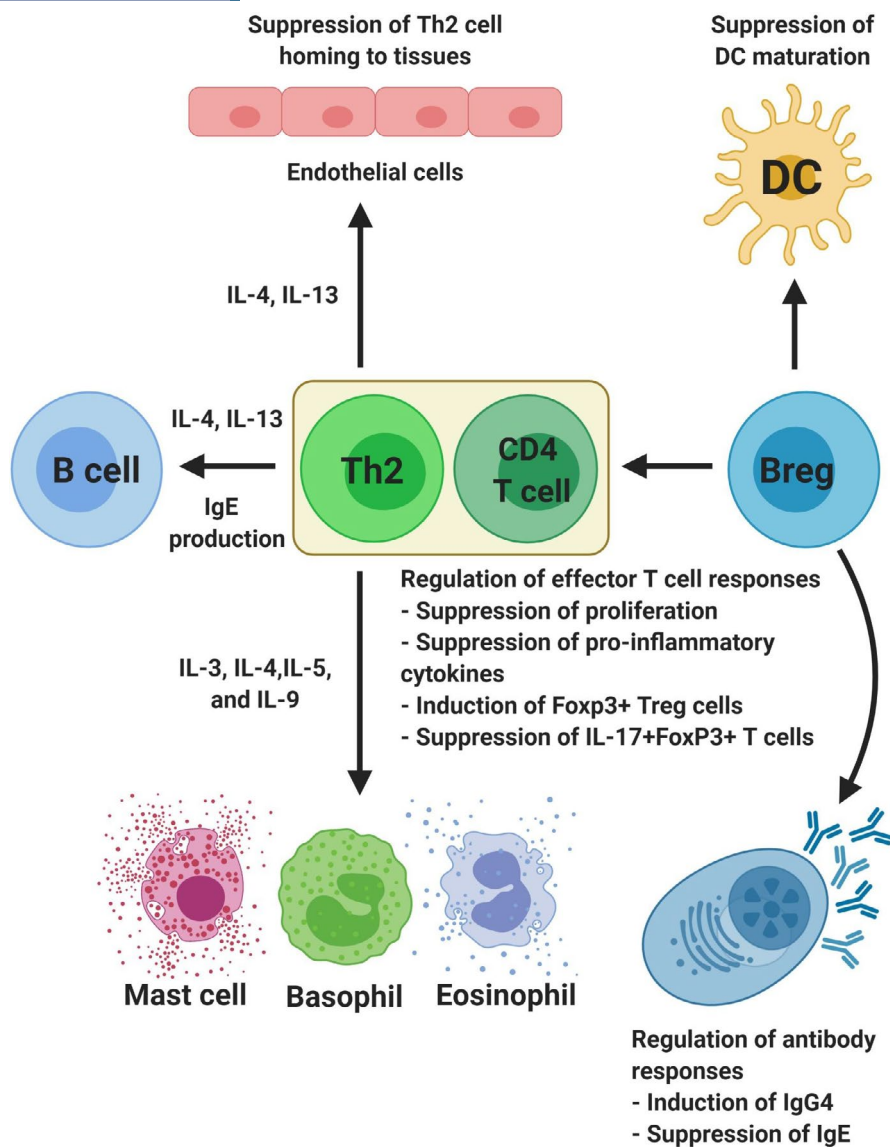


FIGURE 3 Mechanisms of Breg-mediated regulation of immune responses during AIT. Allergic inflammation is driven by allergen-specific Th2 responses that promote IgE-induction, tissue inflammation, and mast cell, basophil, and eosinophil activation. Bregs can suppress these responses through direct suppression of CD4⁺ effector T cell responses, induction of Treg cells, suppression of DC maturation, and enhanced production of IgG4 antibodies while suppressing IgE responses

load and decreased blood memory Th1 cell frequencies, which may constitute an early-life host response favoring microbial pathogenesis later in life.¹³⁵ The preventive role of CD9⁺ Breg cells in the development of asthma and immunological tolerance was reported in an HDM-induced asthma mice model. Lundy et al¹³⁶ used a cockroach-sensitized mouse model to demonstrate the positive regulatory role of CD5⁺CD19⁺ Bregs in reducing pulmonary inflammation. Studies using an OVA-sensitized helminth parasite-infected mice model supported the critical protective role of regulatory B and T cells in allergic airway inflammation.¹³⁷ TGFβ⁺CD5⁺ Bregs did not only increase the number of CD4⁺Foxp3⁺ T cells but also inhibited airway eosinophilia.¹³⁸ CD1d^{hi} Bregs prevent the development of allergic airway inflammation via its interaction with T regulatory cells.¹⁵

6.4 | Allergic rhinitis

Allergic rhinitis (AR) is an IgE-mediated hyper-responsiveness to aeroallergens characterized by symptoms of pruritus, sneezing, rhinorrhea, and nasal obstruction.¹³⁹ AR patients were characterized by an increase in terminally differentiated memory B cells, plasma cells, and CD19⁺CD24^{hi}CD27⁺ Breg cells,^{140,141} and a decrease in CD19⁺CD24^{hi}CD38^{hi} Breg cells in the peripheral blood.¹⁴¹ Kim et al¹⁴² reported that both, CD19⁺CD25⁺CD71⁺CD73⁺ Bregs and CD19⁺CD25^{hi}CD71⁺CD73⁺ Bregs, were reduced in AR patients. In this study, they also found that there were fewer T follicular helper (Tfh)-like cells (CD4⁺PD-1⁺CXCR5⁺) in AR patients. Thus, the reconstitution of nasal mucosal immunity and Bregs number may be facilitated via regulation of the proportion and

TABLE 2 Allergic disease and role of Bregs in human and mouse models

Disease	Human Role of Bregs	Mouse Model	Role of Bregs
Allergic asthma	The frequency of CD24 ^{hi} CD27 ⁺ Bregs was downregulated. This subset of Bregs have a lower IL-10 secreting capacity in response to LPS and could induce IL-10 ⁺ T cells. ¹³⁰	HDM-induced asthma model in wildtype and IL-10 ^{-/-} BALB/c mice	CD9 ⁺ Breg cells prevent the development of asthma and contribute to immunological tolerance. ¹³¹
	CD19 ⁺ CD25 ⁺ CD71 ⁺ CD73 ⁻ Bregs have a profound response both to CpG and α BCR + CD40L. ⁷³	Ova-induced BALB/c and C57BL/6J mice with/ without <i>S mansoni</i> infection	CD19 ⁺ CD9 ⁺ Bregs induce CD3 ⁺ CD4 ⁺ CD25 ⁻ effector T cell apoptosis. ¹³²
	CD9 ⁺ Bregs induce CD3 ⁺ CD4 ⁺ CD25 ⁻ effector T cell apoptosis. ¹³²		Bregs induces IL-10 ⁺ Tregs response and supports Foxp3 ⁺ Tregs to immunosuppress allergic airway inflammation. ¹³⁶
	RSV-infected nBreg cell frequencies correlated with increased viral load and decreased blood memory Th1 cell frequencies. ¹³⁴		TGF β ⁺ CD5 ⁺ Bregs increased the number of CD4 ⁺ Foxp3 ⁺ T cells and inhibited airway eosinophilia. ¹³⁷
Allergic rhinitis		Cockroach allergen sensitized CBA/J and CBA/CaHN ^{btk} ^{-/-} ^{xid} /J ^{xid} mice	CD1d ^{hi} Bregs prevent the development of allergic airway inflammation via T regulatory cells. ¹⁵
	Increased CD19 ⁺ CD24 ^{hi} CD27 ⁺ Breg cells. ^{139,140}	Ova-induced wildtype BALB/c mice	Deficiency of CD5 ⁺ Bregs increases allergic airway inflammation. ¹³⁵
	Decreased CD19 ⁺ CD24 ^{hi} CD38 ^{hi} Breg cells. ¹⁴⁰		TGF- β 1 ⁺ Bregs interact with Treg cells in nasal allergic inflammation and recovery. ¹⁴³
	CD19 ⁺ CD25 ⁺ CD71 ⁺ CD73 ⁻ Bregs and CD19 ⁺ CD25 ^{hi} CD71 ⁺ CD73 ⁻ Bregs were reduced. ¹⁴¹		
	A trend of a lower number of IL-10-producing CD19 ⁺ CD25 ⁺ CD71 ⁺ CD73 ⁻ Bregs, and less induced IL-10 producing Bregs after CpG treatment. ⁷³		
Food allergy and atopic dermatitis	Less CD19 ⁺ CD24 ^{hi} CD38 ^{hi} and CD19 ⁺ CD5 ^{hi} CD1d ⁺ IL-10-secreting Breg cells. ¹⁴²		
	Decreased CD19 ⁺ CD5 ⁺ Breg in the milk allergy group, and increased in the milk-tolerant group. ¹⁴⁸	Hydrolyzed cholera toxin protein sensitized C3H/HeOuJ mice	CD5 ⁺ Bregs contributed to the suppressed clinical symptoms. ¹⁵¹
	Decreased CD19 ⁺ CD5 ⁺ Foxp3 ⁺ B cell fraction in CD5 ⁺ B cells in the milk allergy group, and an increased fraction in the milk-tolerant group. ¹⁴⁷	Ova- and cholera toxin-induced wildtype BALB/c mice	CX3CR1 ⁺ CD5 ⁺ CD19 ⁺ TGF- β ⁺ Bregs induce naive T cells to Tregs, suppress symptoms and Th2 inflammation. ⁵⁶
	Decreased TGF- β 1 ⁺ CD19 ⁺ CD5 ⁺ Bregs in the milk allergy group, and increase in the milk-tolerant group. ¹⁴⁵	Casein-tolerant MLN CD5 ⁺ B cell transplanted naive mice	IL-10 ⁺ CD5 ⁺ Bregs suppress allergic inflammation and induce Foxp3 ⁺ Tregs. ¹⁵²
Atopic dermatitis	CD19 ⁺ CD25 ⁺ CD71 ⁺ CD73 ⁻ Bregs secrete lower IL-10 and increase the proliferation of CD4 ⁺ CD25 ⁻ T cells. ¹⁵⁰		
	CD24 ^{hi} CD38 ^{hi} Bregs correlated inversely with AD severity. ¹⁵⁴	Dinitrofluorobenzene-induced wildtype BALB/c local atopic dermatitis mice	CD19 ⁺ CD5 ^{hi} CD1d ⁺ IL-10 ⁺ Bregs have a decreased IL-10-secreting function and suppressed IgE-regulating function. ¹⁵⁵

function of Tfh cells. Further research is warranted to compare the role of Bregs and Tfh in the development of allergic rhinitis with those of Tregs.

Few reports have focused on the IL-10⁺ Bregs subset, a key player in immunoregulatory functions. Experimental data by Wirz OF et al⁷³ showed a decreasing trend in the numbers of IL-10-producing CD19⁺

CD25⁺ CD71⁺ CD73⁻ Bregs in AR patients. This subset of cells was less induced in AR patients compared with healthy donors after CpG treatment. A recent study reported that seasonal AR patients had lower proportions of CD19⁺CD24^{hi}CD38^{hi} and CD19⁺CD5^{hi}CD1d⁺ IL-10-secreting Breg cells compared with the healthy control group.¹⁴³ TGF- β 1⁺ Bregs were downregulated in an OVA-induced wildtype BALB/c AR mouse model, but increased with prolonged OVA challenge.¹⁴⁴ The TGF- β 1⁺ Bregs interact with Treg cells in the development of allergic inflammation and its recovery in nasal resident tissue. The elevated frequency of TGF- β 1⁺ Bregs in prolonged OVA stimulation is consistent with a study of grass pollen subcutaneous immunotherapy (SCIT) in AR patients.¹⁴³ An increased population of CD19⁺CD24^{hi}CD38^{hi} and CD19⁺CD5^{hi}CD1d⁺ IL-10-secreting Breg cell subsets were observed after SCIT treatment.

6.5 | Food allergy

Food allergy is an adverse immune reaction toward naturally processed food proteins, which can be classified as IgE-mediated, non-IgE-mediated, or mixed-type.⁷⁶ Food allergy symptoms include oropharyngeal pruritus, nausea, urticaria, and angioedema can sometimes involve respiratory symptoms and can be life-threatening due to systemic anaphylaxis.¹⁴⁵ IL-10-secreting CD5⁺Bregs and TGF- β -producing Breg cells have been well characterized in non-IgE-mediated food allergy and atopic dermatitis.¹⁴⁶ It is well known that food allergy is closely related to atopic dermatitis (AD),¹⁴⁷ and AD is thought to be a non-IgE-mediated process in response to Th2 cells.¹⁴⁸ A recent study on food allergy and late eczematous reaction patients found a decreased CD19⁺CD5⁺ Breg cells in the milk allergy group and a increase in the milk-tolerant group.¹⁴⁹ Similarly, a different study found a decreased CD19⁺CD5⁺Foxp3⁺ B cell fraction in CD5⁺ B cells in the milk allergy group, while there was an increased fraction in the milk-tolerant group.¹⁴⁸ Similar responses were observed in TGF- β 1⁺ CD19⁺CD5⁺ Bregs upon allergen stimulation in milk-tolerant subjects.¹⁴⁶

Food allergy is thought to be an important causative factor of inflammatory bowel disease, which mainly consists of ulcerative colitis (UC) and Crohn's disease.¹⁵⁰ A recent study recruited UC and food allergy patients and found that CD19⁺ CD25⁺ CD71⁺ CD73⁻ Bregs had a lower IL-10 secretion capacity and an increased proliferation of CD4⁺CD25⁻ T cells compared with Bregs from healthy donors.¹⁵¹ The impaired function of Bregs in UC patients could be restored by vasoactive intestinal peptide, which blocks the function of tristetraprolin to stabilize the IL-10 secretion.

There are many studies on the role of Bregs in food allergy in mouse models. It has been demonstrated that extensively hydrolyzed proteins can be used to treat cow's milk allergy and partially hydrolyzed proteins prevent the development of allergic symptoms. Kiewiet et al¹⁵² used hydrolyzed whey proteins and cholera toxin as an adjuvant to build a milk allergy mouse model. Although sensitization was established, the partial whey hydrolysate did not induce clinical symptoms. The authors suggest that the increasing CD5⁺

Bregs and Tregs contributed to the suppressed clinical symptoms. Liu et al⁵⁶ found a CX3CR1⁺ CD5⁺ CD19⁺ TGF- β -producing Breg subtype in the intestine of BALB/c mice. These Bregs could induce naive T cells to differentiate into Tregs, suppressing allergic symptoms and Th2 inflammation in the food allergic intestine. Kim et al¹⁵³ also found an increased IL-10-producing CD5⁺ Breg subset in the mesenteric lymph node (MLN). Transferring CD5⁺ B cells from the MLN suppressed the allergic inflammation with casein challenge in an IL-10-dependent manner.

6.6 | Atopic dermatitis

Atopic dermatitis is a chronic relapsing disease characterized by a Th1/Th2 imbalance, and it is closely related to food allergy.¹⁴⁷ Patients' symptoms include erythema, rash, scaling, papules, and fissures.¹⁵⁴ In 1974, B cell-depleted mice were found to inhibit delayed-type hypersensitivity skin reactions.¹ Several studies have focused on the role of Bregs on AD and food allergy (as mentioned above in the food allergy section). However, there are limited reports on exclusively AD. A recent study has demonstrated a decreased frequency of CD24^{hi}CD38^{hi} Bregs in patients with severe AD which was inversely correlated with disease severity, indicating that IL-6 promotes the function of Bregs. Recombinant human IL-6 significantly increased IL-10 production in B10 cells in vitro from healthy donors, but slightly increased in severe AD donors, indicating that IL-6 proceeds through the CD5-STAT3 pathway.¹⁵⁵

Most of the food allergy models in mice have a skin reaction, but in murine models demonstrating all characteristics of AD are rare. Li et al¹⁵⁶ used a dinitrofluorobenzene solution to induce AD-like lesions in BALB/c mice and demonstrated that in the spleen, the percentage of CD19⁺ CD5^{hi} CD1d⁺ IL-10-secreting Breg cells increased, but there was a decreased frequency of IL-10-producing B cells in total CD19⁺B cells. There is also a suppressed regulatory function of CD19⁺ CD5^{hi} CD1d⁺ IL-10-secreting Breg in AD mice, which is consistent with the results from the food allergy mice models.

7 | THE ROLE OF BREG CELLS IN ALLERGEN TOLERANCE

Non-allergic individuals do not develop clinical symptoms in response to allergen exposure. Intuitively one would assume that these individuals simply ignore the allergens that they are exposed to. While this may be true to some extent, it has been demonstrated that allergen tolerance is more complex. Allergen tolerance involves active mechanisms of suppression, particularly in response to high-dose exposure.¹⁵⁷ Understanding the immunological mechanisms involved in tolerance to allergens is paramount to developing intelligent therapeutic strategies to confer tolerance to allergic patients. These mechanisms have been studied in several mouse¹⁵⁸ and human models of high allergen exposure such as non-allergic beekeepers and cat owners.^{11,27,159-161} Another valuable human model to study in vivo

mechanisms of allergen tolerance induction are allergic patients who receive allergen-specific immunotherapy (AIT).¹⁶²⁻¹⁶⁴

7.1 | Models to study allergen tolerance

Models of natural exposure to high doses of allergens, such as in the beekeeper model, have been used to study immune responses to high-dose allergen exposure for many years.^{11,27,165-167} Beekeepers typically receive multiple stings during the beekeeping season (which lasts from spring until late summer), but rarely get stung during the autumn and winter months outside of the beekeeping season. Analysis of bee venom allergen-specific T and B cells in non-allergic beekeepers in and out of the beekeeping season has provided valuable insight into the mechanisms that mediate peripheral tolerance. High-dose exposure in non-allergic beekeepers was associated with reduced T cell-related cutaneous late-phase swelling and a suppression of allergen-specific proliferation and cytokine production. In parallel, there was an increase of allergen-specific IL-10-producing Tr1 cells. IL-10, PD-L1, and CTLA4 were found to play roles in suppression of allergen-specific T cell responses.¹⁵⁹ Apart from the beekeeper model, patients receiving AIT represent another highly valuable model to study allergen-specific immune responses in relation to the development of allergen tolerance. AIT has been used as a treatment option for allergic diseases for more than a century. It is routinely used in clinical practice in the form of subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT), and various other administration routes are under investigation.¹⁶⁸ The immune response to AIT can be roughly divided into four categories: (a) rapid desensitization of mast cells and basophils, (b) induction of allergen-specific Treg and Breg cells, (c) changes in humoral responses characterized by early increases in allergen-specific IgG4 and IgE, in some cases followed by a reduction in allergen-specific IgE, and (d) decreases in mast cells and eosinophils in affected tissues (Figure 3).¹⁶⁸

7.2 | Studies on the role of Bregs in human high-dose allergen exposure models

We, and others, have used the beekeeper model and allergic patients treated with AIT model to study the potential roles of Breg cells in allergen tolerance. Instead of using surrogate markers to isolate B cells that are capable of IL-10 production, we applied a cytokine secretion assay to capture live B cells that secreted IL-10 in response to stimulation with a B type CpG (CpG2006), which is the most potent inducer of IL-10 production in human peripheral B cells. In analogy to inducible regulator T (T_R1) cells, these B cells were referred to as inducible IL-10-secreting B regulatory 1 or B_R1 cells. Transcriptomic analysis of IL-10⁺ and IL-10⁻ B cells led to the identification of a combination of surface markers that were associated with IL-10-producing B cells. Inducible IL-10-producing B_R1 cells were enriched among CD19⁺CD73⁻CD25⁺CD71⁺ B cells. In

addition, B_R1 cells showed increased expression of PD-L1, CD80, and IL-2R beta. CD25 is the IL-2R alpha chain, which is highly expressed on Treg cells, and contributes to Treg-mediated suppression of effector T cells through IL-2 deprivation.¹⁶⁹ CD71 is a receptor for transferrin and mediates cellular iron uptake. Both CD25 and CD71 are known to be upregulated upon activation of B cells, and the increased surface expression of these markers on IL-10⁺ B cells is indicative of an activated state.^{170,171} CD73, also known as ecto-5'-nucleotidase (NT5E), is an enzyme that catalyzes the conversion of adenosine monophosphate (AMP) to free adenosine. CD73 acts in conjunction with CD39, which converts ATP into AMP and is ubiquitously expressed by B cells, to convert ATP to free adenosine. CD73 and CD39 are highly expressed by Foxp3⁺ Tregs and the enhanced capacity to convert ATP to free adenosine contributes to the suppressive capacity of Tregs.¹⁷² IL-10-producing B_R1 cells were mainly CD73⁻, and therefore, this suppressive mechanism is not relevant for these cells. B_R1 cells isolated either through the use of the cytokine secretion assay or through sorting based on CD73, CD25, and CD71 expression were both capable of suppressing antigen-specific CD4⁺ T cell proliferation. This suppressive effect was dependent on IL-10 secretion, as neutralizing anti-IL-10R antibodies reverted this suppressive effect.

We used the beekeeper and venom immunotherapy (VIT) models to study the frequency of IL-10⁺ cells among B cells specific for the major bee venom allergen phospholipase A2 (PLA) in non-allergic beekeepers and bee venom allergic patients before and after 3-4 months of VIT. In beekeeper blood (collected during the beekeeping season), the frequency of IL-10⁺ B cells was higher in PLA-specific than in PLA-non-specific B cells. Interestingly, there was no significant difference between the frequency of IL-10⁺ cells among PLA-specific and non-specific B cells in bee venom allergic patients before VIT. After 110 days of VIT, the frequency of IL-10⁺ B cells significantly increased only in PLA-specific cells.²⁷

In a recent study, we compared the PLA-specific B cell responses to high-dose bee venom exposure in non-allergic beekeepers and in bee venom allergic patients. We found that CD73⁺CD25⁺CD71⁺IL-10⁺ B_R1 cells were more frequent in beekeepers during the season (in the tolerant state) than before the season. This response was remarkably similar to that observed in venom allergic patients before and three months after the start of VIT. These findings strongly suggest that elevated levels of circulating IL-10⁺ B cells were associated with a state of tolerance to allergens.

Humoral responses play an important role in allergy and allergen tolerance. Allergen-specific IgE antibodies are essential contributors to the pathogenesis of allergic diseases, while IgG4 antibodies are associated with tolerance induction and are strongly increased in response to high-dose allergen exposure, for example in AIT.^{173,174} Since IL-10 is known to promote IgG4 production by B cells,^{175,176} we hypothesized that B cells that produce IL-10 may be skewed toward IgG4 production. We indeed found that IL-10⁺ B cells produced more IgG4, but not IgG1 or IgA, than in IL-10⁻ B cells. This effect

was restricted to naive CD27⁺IL-10⁺ B cells and was not observed in CD27⁺IL-10⁺ B cells. This suggests that naive B cells capable of producing IL-10 are more prone to switch to IgG4 than those that do not produce IL-10. PLA-specific B cells isolated from beekeepers also expressed increased levels of IgG4 and IL-10 mRNA. A limitation of this study was that bulk-sorted PLA-specific B cells were analyzed using RT-qPCR, and so it could not be excluded that the IgG4 and IL-10 amplicons were derived from different cells.²⁷ Lighaam et al¹⁷⁷ reported that the majority of IgG4⁺ B cells did not produce IL-10. This is in line with our own recent observation that IgG4⁺ B cell clones did not produce high levels of IL-10. In fact, IL-10 production in most IgG4⁺ clones was lower than the levels measured in IgG1⁺ clones.¹⁷⁸ These data suggest that although IL-10⁺ B cells are more prone to switch to IgG4, class-switched IgG4⁺ B cells do not maintain stable expression of IL-10 throughout their life cycle.

Venom immunotherapy studies that investigated the role of Bregs in relation to allergen tolerance only focused on the changes between baseline and one timepoint relatively early during VIT (3–4 months after start of therapy).^{11,27} Another limitation of these studies stems from the relatively high rate (75%–95%) of responders in VIT, which complicates subgroup analysis of responder and non-responder patients.¹⁷⁹ To investigate the long-term B cell responses in AIT and the differences in these responses between clinical responders and non-responders to AIT, we performed another study in a cohort of house dust mite (HDM) allergic patients treated with AIT. B cell responses were analyzed at different time points (baseline, 10w, 30w, and 2y) during treatment.¹² Responder patients were defined based on a reduction of >50% in symptom medication score at 2 years compared with baseline. B cells specific for the major HDM allergen Der p 1 were significantly increased only in the responder patients at 30w and 2y. In particular, Der p 1-specific IgG4- and IgA-switched B cells showed a significant increase starting from 30w and 10w, respectively, and reached the highest levels after 2 years, while IgG1-switched Der p 1-specific B cells did not change over time. The frequency of IL-10⁺CD73⁺CD25⁺CD71⁺ B cells significantly increased only in responders at 30w and 2y. Interestingly, B cells producing IL-1RA also increased in responder patients. The frequency of CD73⁺CD25⁺CD71⁺ that were double-positive for IL-10 and IL-1RA was almost twofold higher in responder patients than in non-responder patients after 2 years of therapy. IL-1RA can bind to the IL-1 receptor and prevent binding of IL-1 α and IL-1 β , thereby suppressing IL-1-mediated inflammation.³⁹ Thus, IL-1RA may play a role in B-cell-mediated immunosuppression in allergen immunotherapy. IL-10 overexpression in B cells resulted in the development of an anti-inflammatory phenotype in B cells, characterized by increased IL-1RA production and reduced pro-inflammatory cytokine production.¹³

In 2018, Zissler et al¹⁸⁰ published a study in which the frequencies of IL-10-producing B cells were measured in grass pollen AIT over a three-year period. The frequency of IL-10⁺ B cells increased progressively throughout the up-dosing phase but reduced rapidly during the pollen season. After 1 year (at the end of the preseason up-dosing phase), the frequency of IL-10⁺ B cells was significantly

increased while the Foxp3⁺ Treg compartment remained unchanged. Interestingly, a subgroup of patients that developed a strong increase in the frequency of IL-10⁺ B cells showed a significant decrease in circulating CD4⁺Foxp3⁺ T cells that expressed IL-17. A similar negative correlation was observed for Th1 cells. The phenotype of the IL-10⁺ B cells that were induced during the early phase of AIT was characterized by expression of CD1d and CD5, while CD24⁺CD27⁺ and CD24⁺CD38⁺ B cells were not increased among IL-10⁺ B cells. The ratio of IL-10⁺ B cells over IL-17⁺CD4⁺ T cells (Breg/Th17 ratio) at the end of the initial up-dosing phase correlated strongly with allergy symptoms at the end of the study period (3 years). This warrants further studies to determine the potential value of this ratio for the use as a predictive biomarker for grass pollen AIT.

8 | CONCLUSION

It is important to delineate the origin and development of Bregs in order to generate them *in vitro* for future use in cellular immune therapy, particularly against autoimmune diseases. Nevertheless, a separate lineage for Bregs has not been found in either humans or rodents. sRNA sequencing and pathway analysis can be used to visualize the probable pathways which are involved in the maturation stages of B-cell development and to assist in revealing the diverse Breg subsets and their regulatory profiles in various microenvironments. Considering the immunosuppressive function of Bregs, the expansion of Breg cells could be exploited for the treatment of a wide variety of immune disorders and allergic diseases. Induction of Breg cells is achieved by exposing them to inflammatory cytokines, such as BAFF, IFN- α , IFN- β , IL-1 β , IL-6, IL-21, and IL-35. Commensal bacteria were also found to contribute to Breg differentiation in mouse models. However, the over-expansion of Bregs may also result in detrimental side effects. An over-suppressed immunity will cause a wide range of clinical complications, such as malignancies and infections. An improved understanding of the underlying mechanisms that induce Breg expansion and differentiation could lead to the development of novel Breg-based therapeutic strategies.

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CONFLICT OF INTEREST

There is no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

S Ma, P Satitsuksanoa, K Jansen, L Cevhertas, and W van de Veen have contributed to write the sections of manuscript; M Akdis was responsible to put all the sections together with the figures, finalize the manuscript, and perform revision.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this article.

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