

Role of regulatory B cells in immune tolerance to allergens and beyond



Willem van de Veen, PhD,^{a,b} Barbara Stanic, PhD,^{a,c} Oliver F. Wirz, MSc,^a Kirstin Jansen, MSc,^a Anna Globinska, MSc,^{a,d} and Mübeccel Akdis, PhD^a Davos, Switzerland, and Lodz, Poland

Immune tolerance to both self-antigens and innocuous non-self-antigens is essential to protect the host against chronic inflammatory diseases and tissue damage. A wide range of cell types and suppressive molecules are involved in induction and maintenance of tolerance. In addition to their key function in the production of immunoglobulins, B cells can regulate immune responses through their surface molecules and secretion of cytokines. Regulatory B (Breg) cells are characterized by their immunosuppressive capacity, which is often mediated through IL-10 secretion. However, IL-35 and TGF- β have also been associated with B cell-mediated immunosuppression. Several types of murine and human Breg cells have been described, such as mouse CD5⁺CD1d^{hi} B10 cells, CD21^{hi}CD23^{hi}CD24^{hi} transitional stage 2-like B cells, and CD138⁺ plasma cells and plasmablasts. Human Breg cell types include CD27⁺CD24^{high} B10 cells, CD24^{hi}CD38^{hi} immature transitional B cells, and CD73⁻CD25⁺CD71⁺ B_R1 cells and a subset of plasma cells. Support for the *in vivo* existence of

allergen-specific human Breg cells comes from direct detection of their increase during the course of allergen-specific immunotherapy, as well as their increased expression in nonallergic but high-dose allergen-exposed beekeepers. Human B_R1 cells selectively upregulate IgG₄ antibodies on differentiation to plasma cells. This suggests an additional immune regulatory role because of the noninflammatory and blocking antibody function of IgG₄. Taken together, Breg cells appear to be involved in mediating allergen tolerance, but many open questions remain to be answered. (J Allergy Clin Immunol 2016;138:654-65.)

Key words: Regulatory B cells, IL-10, IL-35, TGF- β , IgG₄, tolerance, allergen, autoimmunity, suppression, B_R1, B10

Induction and maintenance of immune tolerance to self-antigens and innocuous environmental antigens is essential to prevent chronic inflammation. Immunosuppressive mechanisms are also critical to dampen inflammatory responses to infections. In healthy subjects a fine balance is maintained between proinflammatory responses required to counter infections and immunosuppressive responses to prevent chronic inflammation and tissue damage.

The prevalence of allergic diseases continues to increase. The reasons why certain persons have allergies and others do not still remain incompletely understood. The mechanisms by which immune responses to innocuous antigens lead to either allergy or tolerance involve complex interactions between genetic background and the exposome.¹ Healthy immune responses to high-dose allergen exposure have been studied in beekeepers and cat owners and are characterized by induction of IL-10-producing type 1 regulatory T (*T_R1*) cells and blocking allergen-specific IgG (particularly IgG₄) antibodies.² Studies involving allergic patients undergoing allergen immunotherapy (AIT) have also provided valuable insight into the mechanisms underlying tolerance induction to allergens.³

The term regulatory B (Breg) cell is used as an umbrella term that comprises the different immunosuppressive B-cell subsets that have been described in mice and human subjects with distinct phenotypic and functional properties. The first reports describing the immunosuppressive potential of B cells date back to the 1970s and essentially resulted from allergen sensitization studies. An ovalbumin (OVA) sensitization model in guinea pigs revealed that adoptively transferred splenocytes lost their suppressive capacity on delayed-type hypersensitivity reactions in the skin when they were depleted of B cells.^{4,5} The precise mechanisms

From ^athe Swiss Institute of Allergy and Asthma Research (SIAF), University of Zürich, Davos; ^bthe Christine Kühne-Center for Allergy Research and Education (CK-CARE), Davos; ^cthe AO Research Institute Davos; and ^dthe Department of Immunology, Rheumatology and Allergy, Medical University of Lodz.

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Corresponding author: Mübeccel Akdis, PhD, Swiss Institute of Allergy and Asthma Research (SIAF), Oberstrasse 22, CH-7270, Davos, Switzerland. E-mail: akdis@siaf.uzh.ch.

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Terms in boldface and italics are defined in the glossary on page 655.

Abbreviations used

AIT: Allergen immunotherapy
BCR: B-cell receptor
Breg: Regulatory B
DC: Dendritic cell
EAE: Experimental autoimmune encephalomyelitis
MZ: Marginal zone
OVA: Ovalbumin
PD-L1: Programmed death ligand 1
PLA: Phospholipase A₂
TIM1: T-cell immunoglobulin and mucin domain 1
Treg: Regulatory T

of suppression were not identified in these early studies, and the study of immunosuppressive B cells did not receive significant attention for more than 20 years.

In addition, reports on exacerbation of colitis and new onset of psoriasis in patients treated with total B-cell depletion therapy using the anti-CD20 mAb rituximab suggest that a subset of B cells has a regulatory function in human subjects.^{6,7} Because the majority of B cells express CD20, most Breg cells are targeted by anti-CD20 depletion therapy. An exception to this could be the

recently described regulatory plasma cells because plasma cells typically downregulate CD20.

The discovery that B cell–deficient mice did not recover from experimental autoimmune encephalomyelitis (EAE) sparked renewed interest in the immunosuppressive capacity of B cells.⁸ Later studies demonstrated a key role for B cell–derived IL-10 in the suppression of different autoimmune models.^{9–11} IL-10 is considered the hallmark cytokine of Breg cells; however, *TGF-β* and *IL-35* have also been associated with B cell–mediated immunosuppression.^{12–14} Studies specifically designed to assess the role of Breg cells in induction and maintenance of tolerance to allergens remain scarce. However, several studies have demonstrated a potential role for Breg cells in induction and maintenance of tolerance to allergens.^{15–22}

The phenotype of Breg cells and the molecular mechanisms underlying B cell–mediated immune suppression have been studied in various human and murine disease models. The fact that Breg cells are found among B lymphocytes at different stages of maturation and differentiation, including early transitional B cells,²³ as well as highly differentiated plasmablasts²⁴ and plasma cells,^{12,25} suggests that there are either multiple distinct lineages of Breg cells or that IL-10 can be induced by environmental stimuli at various stages of B-cell development. Many

GLOSSARY

COMPLEMENT: A factor of the innate immune system whereby plasma proteins can be activated directly by pathogens or indirectly by pathogen-bound antibody, leading to a cascade of reactions that occur on the surfaces of pathogens and generate active components with various effector functions.

CpG SITES: Regions of DNA in which a cytosine nucleotide occurs next to a guanine nucleotide separated by only 1 phosphate. CpG islands are regions with a high frequency of CpG sites. Methylation of the cytosine within a gene can change the gene expression.

CX3CR1: Also known as the fractalkine receptor or G protein–coupled receptor 13, a transmembrane protein and chemokine involved in the adhesion and migration of immune cells.

Fas LIGAND (FasL OR CD95L): A type II transmembrane protein that belongs to the TNF family. Its binding with its receptor induces apoptotic cell death.

FORKHEAD BOX PROTEIN 3 (Foxp3): Also known as scurf, a transcription factor responsible for the development and function of Treg cells.

GM-CSF: Also known as colony-stimulating factor 2 (CSF2), a monomeric glycoprotein secreted by macrophages, T cells, mast cells, natural killer cells, endothelial cells, and fibroblasts that functions as a cytokine. GM-CSF functions as a white blood cell growth factor and stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes.

IFN-α: A type of human type I interferon protein that helps regulate immune system activity. IFN-α is produced by leukocytes and is mainly involved in innate immune responses against viral infection.

IFN-γ: Known as type II interferon, IFN-γ is a cytokine that is critical for innate and adaptive immunity against viral, bacterial, and protozoal infections. It is an important activator of macrophages and inducer of class II MHC molecule expression. IFN-γ is produced predominantly by natural killer and natural killer T cells as part of the innate immune response and by CD4⁺ T_H1 and CD8⁺ cytotoxic T-lymphocyte effector T cells once antigen-specific immunity develops.

IL-1 (FAMILY): A group of 11 cytokines that play a central role in the regulation of immune and inflammatory responses to infections, as well as promotion of fever and sepsis.

IL-1β: A member of the IL-1 cytokine family that is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1. IL-1β is an important mediator of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis.

IL-6: Implicated in a wide variety of inflammation-associated disease states, this cytokine is involved in the maturation of B cells and has been shown to be an endogenous pyrogen capable of inducing fever in patients with autoimmune diseases or infections.

IL-10: A cytokine produced primarily by monocytes and, to a lesser extent, by lymphocytes, which has pleiotropic effects in immunoregulation and inflammation by limiting the immune response to pathogens and thereby preventing damage to the host.

IL-12: A cytokine produced by dendritic cells, macrophages, and human B-lymphoblastoid cells in response to antigen stimulation. IL-12 is involved in the differentiation of naive T cells into T_H1 cells. It is known as a T cell–stimulating factor, which can stimulate the growth and function of T cells. It stimulates the production of IFN-γ and TNF-α from T cells and natural killer cells and reduces IL-4–mediated suppression of IFN-γ.

IL-15: A cytokine with structural similarity to IL-2. Like IL-2, IL-15 binds to and signals through a complex composed of IL-2/IL-15 receptor β chain (CD122) and the common γ chain (gamma-C, CD132). IL-15 is secreted by mononuclear phagocytes (and some other cells) after viral infection. IL-15 induces cell proliferation of natural killer cells, cells of the innate immune system whose principal role is to kill virally infected cells.

IL-21: A member of the common γ chain family of cytokines with immunoregulatory activity. The protein plays a role in both the innate and adaptive immune responses by inducing the differentiation, proliferation, and activity of multiple target cells, including macrophages, natural killer cells, B cells, and cytotoxic T cells.

IL-21 RECEPTOR (IL-21R): This receptor is a ligand for the growth-promoting cytokine IL-21. It belongs to the type I cytokine receptors and has been shown to form a heterodimeric receptor complex with the common γ chain, a receptor subunit also shared by the receptors for IL-2, IL-7, and IL-15, and is important for the proliferation and differentiation of T cells, B cells, and natural killer cells.

IL-35: An IL-12 family cytokine produced by regulatory, but not effector, T cells and B cells that plays a role in immune suppression.

IONOMYCIN: An ionophore produced by the bacterium *Streptomyces conglobatus*. It is used in research to raise the intracellular calcium level and stimulates intracellular production of the cytokines interferon, perforin, IL-2, and IL-4, usually in conjunction with phorbol 12-myristate 13-acetate.

LPS: An endotoxin found in the outer membrane of gram-negative bacteria that elicits a strong immune responses in animals.

MHC-II: A complex found on antigen-presenting cells that presents antigen derived from extracellular proteins to T-cell receptors.

MYELOID DIFFERENTIATION PRIMARY RESPONSE GENE 88 (MyD88): A cytosolic adapter protein that functions as an essential signal transducer in the IL-1 and TLR signaling pathways. These pathways regulate the activation of numerous proinflammatory genes.

OVALBUMIN: The main protein found in egg white and well-characterized allergen used in immunologic studies.

PHORBOL MYRISTATE ESTER: An effective skin irritant and potent tumor promoter in mice through activation of protein kinase C. Together with ionomycin, phorbol myristate ester can also be used to stimulate T-cell activation, proliferation, and cytokine production and is used in protocols for intracellular staining of these cytokines.

T_H3 CELLS: T cells that protect mucosal surfaces in the gut from nonpathogenic non-self-antigens by secreting the anti-inflammatory cytokines TGF- β and IL-10. T_H3 cells inhibit T_H1 and T_H2 cells.

TOLL-LIKE RECEPTOR (TLR) LIGANDS: Ligands associated with a class of receptors expressed on macrophages and dendritic cells that recognize conserved microbial particles that can activate an immune response.

T_R1 CELLS: Inducible Treg cells that have an extrathymic origin, lack Foxp3 expression, and characteristically produce high amounts of IL-10. T_R1 cells play a pivotal role in promoting and maintaining tolerance.

TGF- β : A cytokine secreted by many cell types that controls proliferation, cellular differentiation, and other functions in most cells. It also plays a role in immunity, cancer, bronchial asthma, and various other diseases.

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questions remain to be addressed with regard to the ontogeny, plasticity, and mechanisms of suppression of Breg cells.

In this review we provide an overview of the major Breg cell subsets that have been described in human subjects and mice and their mechanisms of suppression and recapitulate our current understanding of the role of Breg cells in patients with chronic inflammatory conditions, with a specific focus on allergy.

THE (RE)DISCOVERY OF Breg CELLS

After the initial observations that B cell–depleted splenocytes lost their suppressive effect on delayed-type hypersensitivity reactions in a guinea pig OVA sensitization model,^{4,5} it took more than 20 years until B cell–mediated immune suppression once again received serious attention. The use of a B cell–deficient mouse strain in the frequently used EAE model, which mimics the autoimmune disease multiple sclerosis, led to an interesting discovery. In this model mice are immunized with a myelin antigen, which leads to development of T cell–mediated autoimmune disease. Wild-type mice typically have hind limb weakness and paralysis, which is followed by spontaneous remission 1 week after initial onset of disease symptoms. In contrast, B cell–deficient muMT did not recover from EAE, demonstrating a key role for B cells in protection against the inflammatory autoimmune response.⁸ Later, it was shown that IL-10 produced by autoantigen-specific B cells was responsible for suppression of T cell–mediated autoimmune responses.⁹ Around the same time, the protective effects of IL-10–producing B cells were reported in models of intestinal inflammation and arthritis.^{10,11}

Since then, much effort has been made to characterize Breg cells both functionally and phenotypically. This has resulted in identification of different types of Breg cells both in mice and human subjects with distinct (and partially overlapping) characteristics. Tables I^{12,16,17,19,23,24,26–42} and II^{15,24,43–45} provide a summary of different types of Breg cells in mouse and humans respectively. Types of Breg cells are listed along with their associated phenotypic markers, suppressive molecules, associated disease models, and stimuli that induce their activation. Table III shows basic information regarding the function of Breg cell–associated surface markers that have been reported.

SUPPRESSOR MOLECULES OF Breg CELLS

Three cytokines have been identified as suppressive cytokines produced by Breg cells: IL-10, TGF- β , and IL-35 (Fig 1).⁴⁶ IL-10 is a potent immunosuppressive cytokine involved in the maintenance of homeostasis by dampening inflammatory responses to infections and inducing immune tolerance in patients with chronic inflammatory diseases, including allergy, autoimmunity, organ transplantation, and tumor tolerance. It has direct and indirect suppressive effects on proliferation and cytokine production of effector T cells.^{47,48} Interestingly, B-cell survival and class-switch recombination, as well as plasma cell differentiation, are positively regulated by IL-10.^{47,48} Many cell types, including monocytes, T cells, dendritic cells (DCs), natural killer cells, macrophages, mast cells, and B cells, can produce IL-10.^{47,48} TGF- β is a pleiotropic cytokine that plays a role in a wide range of processes, including wound healing and tissue remodeling, as well as immune regulation. Importantly, it is involved in the conversion of naive CD4⁺ T cells into functional regulatory T (Treg) cells. Treg and T_H3 cells are the major sources of TGF- β .⁴⁸ IL-35 plays a role in immune suppression through induction of Treg cell proliferation while suppressing T_H17 responses. It is primarily produced by Treg cells.⁴⁷ In addition to secreted cytokines, increased expression of surface CD19,²⁶ CD62 ligand and MHC-II,⁴⁹ Fas ligand,^{27,28} T-cell immunoglobulin and mucin domain (TIM1),^{50,51} and programmed death ligand 1 (PD-L1),^{15,29,43,52} as well as the signaling molecules signal transducer and activator of transcription 3⁴⁴ and myeloid differentiation response gene 88 (MyD88),^{25,53} have been implicated in B cell–mediated immunosuppression (Fig 1).

TYPES OF Breg CELLS

Several types of Breg cells with distinct phenotypic characteristics and mechanisms of suppression have been described both in mice and human subjects (Tables I and II). Some Breg cell types have overlapping phenotypes with previously described B-cell subsets. Murine B1 cells, which can be subdivided into CD5⁺ B1a and CD5[−] B1b cells, are primarily found in the peritoneal and pleural cavities. There is still no consensus regarding the existence of a separate human B1 lineage. B1a-like cells have been identified as a relevant source of IL-10.^{30,54} Murine CD5⁺CD1d^{hi}

TABLE I. Mouse Breg cell subsets

Name	Phenotype	Suppressor molecules	Mouse model	Induction
B1a-like B phenotype (CD5 ⁺)				
B10 cells	CD19 ⁺ CD1d ^{hi} CD5 ⁺	IL-10	Contact hypersensitivity, ²⁶ EAE, ³¹ lupus, ³⁴ myasthenia gravis, ³⁶ collagen-induced arthritis, ³⁵ colitis, ³⁷ allergic inflammation ^{16,19}	LPS, anti-CD40, CpG, TLR ligation, <i>S. mansoni</i> infection
B1a cells	CD19 ⁺ CD5 ⁺	IL-10	Neonatal infection, ³⁰ colitis ⁴⁰	TLR ligation anti-CD40 LPS
Killer B1a cells	IgM ^{hi} CD5 ⁺ CD1d ^{hi} FasL ⁺	FasL	<i>S. mansoni</i> infection ^{27,28}	IL-4, IL-10, IL-5
TolBC	CD19 ⁺ CD5 ⁺ CX3CR1 ⁺	TGF-β	Allergy-induced intestinal inflammation ¹⁷	αvβ6 plus anti-IgM
Immature/transitional B-cell phenotype				
T2 B cells	CD19 ⁺ CD21 ^{hi} CD23 ^{hi} CD24 ^{hi} IgM ^{hi} IgD ^{hi} CD1d ^{hi}	IL-10 IL-10-independent	Collagen-induced arthritis ²³ Lupus ³³ Allograft survival ⁴¹	Anti-CD40
MZ-like phenotype				
MZP B cells	CD19 ⁺ CD21 ^{hi} CD23 ⁻	IL-10	Allograft survival ³⁹	
MZ B cells	CD24 ^{hi} IgM ^{hi} IgD ^{lo} CD1d ^{hi}	IL-10	<i>Leishmania donovani</i> infection ³²	
GIFT-15	B220 ⁺ CD21 ⁺ CD22 ⁺ CD23 ⁺ CD24 ⁺ CD1d ⁺ CD138 ⁺ IgD ⁺ IgM ⁺	IL-10	EAE ⁴²	GM-CSF-IL-15 fusokine
Plasma cell/plasmablast phenotype				
Plasma cells	IgM ⁺ CD138 ^{hi} TACI ⁺ CXCR4 ⁺ CD1d ^{int} TIM1 ^{int} CD138 ^{hi} PD-L1 ⁺ B220 ⁺ IgA ⁺	IL-10 and IL-35 IL-10 and PD-L1	EAE and <i>S. enterica</i> infection ¹² Tumors ²⁹	Oxaliplatin
Plasmablasts	CD138 ⁺ CD44 ^{hi}	IL-10	EAE ²⁴	
Nondefined phenotype				
I35		IL-35	EAU ³⁸	IL-35

EAU, Experimental autoimmune uveitis; MZP, Marinal zone precursor; TolBC, tolerogenic B cell.

TABLE II. Human Breg cell subsets

Name	Phenotype	Suppressor molecules	Associated disease	Induction
B10/pro-B10	CD27 ⁺ CD24 ^{hi} CD148 ^{hi} CD48 ^{hi}	IL-10	Healthy subjects and upregulated in patients with lupus, rheumatoid arthritis, Sjögren syndrome, autoimmune skin disease, and multiple sclerosis ⁴³	LPS, CpG-ODN (+ CD40 ligation)
Immature cells	CD19 ⁺ CD24 ^{hi} CD38 ^{hi}	IL-10	SLE, ⁴⁴ RA, ⁴⁴ CHB ⁴⁵	CD40 ligation
B _R 1 cells	CD73 ⁻ CD25 ⁺ CD71 ⁺	IL-10 IgG ₄	Allergic subjects, healthy beekeeper ¹⁵	CpG-ODN
Plasmablast	CD27 ^{int} CD38 ⁺	IL-10 Immunoglobulins	Healthy subjects ²⁴	CpG-ODN + IL-2, IL-6, and IFN-α/γ

CHB, Chronic hepatitis B; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

B10 cells²⁶ share some characteristics (in particular CD5 expression) with B1a B cells but also with other B-cell subsets.

Most other types of Breg cells described to date have a phenotype that more closely resembles B2 B cells, which represent the majority of B cells. B2 cells can be divided into follicular B cells, which are found in the circulation and lymphoid follicles of secondary lymphoid tissues and marginal zone (MZ) B cells. Follicular and MZ B cells both originate from immature precursors that emerge from the bone marrow. These immature B cells are called transitional B cells and are typically characterized as transitional stage 1 and transitional stage 2 B cells, depending on their maturation stage.⁵⁵ Follicular B cells mount humoral responses to thymus-dependent antigens and require T-cell help to enter germinal center reactions and produce high-affinity antibodies. On activation, these different B cells can undergo terminal differentiation to antibody-secreting plasma cells. MZ B cells are mainly located in the MZ of the spleen. The human counterparts of MZ B cells are considered IgM memory cells, which express IgM and CD27. Together with MZ B cells, B1 cells

facilitate humoral responses to thymus-independent antigens, resulting in production of broadly reactive low-affinity antibodies.

In mice both transitional stage 2 MZ precursor B cells (defined as CD19⁺CD21^{hi}CD23^{hi}CD24^{hi}IgM^{hi}IgD^{hi}CD1d^{hi})²³ and MZ B cells (identified as CD19⁺CD21^{hi}CD23⁻CD24^{hi}IgM^{hi}IgD^{lo}CD1d^{hi})⁵⁶ have been associated with IL-10-mediated immunoregulatory functions. Furthermore, human Breg cells have been found among immature transitional B cells (identified as CD19⁺CD24^{hi}CD38^{hi}).⁴⁴ Immunoregulatory B cells have been also found among both mouse and human plasma cells.^{12,24,29} Human B_R1 cells are characterized by a CD73⁻CD25⁺CD71⁺ phenotype and have been studied in the context of allergen tolerance induction.¹⁵

ORIGIN AND INDUCTION OF Breg CELLS

The diversity of phenotypes of Breg cells that have been described in both human subjects and mice make it increasingly clear that B cell-mediated immunosuppression is not restricted to one clearly defined B-cell subset. There are multiple types of

TABLE III. Functions of cell-surface markers associated with Breg cells

Marker	Alternative name	Function
CD1d	R3G1	Presentation of lipid antigens to T cells
CD5		Attenuation of BCR signaling in B1 cells
CD21	Complement receptor 2	B-cell coreceptor enhancing BCR signaling to T cell–dependent to T cell complement–tagged antigens
CD22	SIGLEC2	Inhibition of BCR signaling
CD23	FcεRII	Receptor for IgE
CD24		Promotion of antigen-dependent proliferation of B cells and prevention of terminal differentiation into plasma cells
CD25	IL-2RA	α Chain of the IL-2 receptor, which mediates IL-2 signaling
CD27	TNFRSF7	Memory B-cell marker; functions as regulator of B-cell activation and antibody production
CD38	ADPRC1	Regulation of intracellular Ca ²⁺ through catalyzing synthesis and hydrolysis of cyclic ADP-ribose
CD44	HCAM/Pgp-1/HUTCH-1	Lymphocyte activation, recirculation, and homing
CD48	SLAMF 2/BLAST-1	Promotion of immunologic synapse formation through interaction with CD2 on T cells
CD71	TFRC	Regulation of cellular iron uptake
CD73	NTSE	Enzymatic conversion of adenosine monophosphate to adenosine
CD138	Syndecan-1	Expressed on plasma cells; mediates adhesion of plasma cells to extracellular matrix proteins
CD148	PTPRJ	Tyrosine phosphatase regulating cell growth and proliferation
CD274	PD-L1	Inhibition of TCR-mediated signaling through binding to PD1 expressed on T cells
IgM/IgG/IgA/IgD		BCR functions through binding of cognate antigens, leading to activation of B cells
TIM1	HAVCR1/CD365	Costimulatory molecule modulating CD4 ⁺ T-cell effector differentiation
FasL	CD95L	Induction of apoptosis on target cells expressing Fas receptor

B cells that are capable of regulating immune responses through a variety of mechanisms. Thus far, no Breg cell–defining transcription factor has been identified, and most Breg cell subsets that have been described share only partially overlapping phenotypes. Mouse splenic B10 cells share similar phenotypic features with CD5⁺ B1 cells,^{26,54} CD1d^{hi}CD21⁺CD23[−] MZ B cells,⁵⁶ and CD1d^{hi}CD21⁺CD23⁺ transitional stage 2 MZ precursor B cells.²³ The discovery of IL-10–producing Blimp-1^{lo}CD138⁺ plasmablasts/plasma cells that predominantly produce IL-10 on B-cell receptor (BCR) stimulation further complicates the idea of a unique Breg cell lineage.²⁴

In particular, BCR-derived signals are a prerequisite for selected mouse B cells, both for their development and acquisition of regulatory B10-like competence.^{26,57} In addition to BCR signals, so-called pro-B10 cells need other molecular triggers (eg, *LPS*, *phorbol myristate ester*, and *ionomycin in vitro*) to facilitate a transcriptionally active conformation of the *IL10* gene.⁴⁹ For generation of fully functional mouse B10 cells, crosstalk with T cells is required because MHC-II– or *IL-21R*–deficient mice do not control autoimmunity-related inflammation in a B cell–dependent manner.⁴⁹

Thus far, several molecules have been identified as important factors for the development of B10 cells. It was shown that *IL-21R*, *MHC-II*, and *CD40* are required for B10 cell effector function in patients with EAE. Furthermore, the ligation of Toll-like receptor (*TLR*) 4 and *TLR9* induces *IL-10* production in B10 cells.³¹ *TIM1* was found to be essential for the optimal production of *IL-10* in Breg cells, and *TIM1* defects increase the production of the proinflammatory cytokines *IL-1* and *IL-6*.⁵⁸ Another interesting study was recently published.⁵⁹ Microarray analysis of purified *IL-10*⁺ and *IL-10*[−] B cells revealed that the phosphoinositide 3-kinase–Akt pathway in B cells is critical for the development of *IL-10*–producing B cells.

Overexpression of *IL-10* in primary human peripheral B cells demonstrated that solely *IL-10* expression in B cells conferred a suppressive profile comprising upregulation of suppressor of cytokine signaling 3, glycoprotein A repetitions predominant, *CD25*, and *PD-L1*. These cells had suppressive effects on

TLR–mediated cytokine production from PBMCs and T-cell proliferation and drove DCs toward an immunoregulatory phenotype.⁶⁰ This indicates that *IL-10* itself supports the induction of an immunoregulatory phenotype in B cells that exerts substantial anti-inflammatory and immunosuppressive functions.

The gut microbiota can also play a role in Breg cell induction. Induction of arthritis led to *IL-1β* and *IL-6* production, which induced Breg cell differentiation and *IL-10* production in conventionally housed mice, whereas antibiotic-treated or sterile housed mice did not experience this response. This indicates that inflammatory signals induced by both the gut flora and arthritis lead to induction of Breg cells and restrain excessive inflammation.⁶¹ More recently, an “autoinflammatory loop” between plasmacytoid DCs and CD24⁺CD38^{hi} Breg cells was reported. DC-derived *IFN-α* promoted maturation of CD24^{hi}CD38^{hi} immature B cells to CD24⁺CD38^{hi} Breg cells producing *IL-10*, which in turn suppressed *IFN-α* production by DCs. Breg cell development in patients with systemic lupus erythematosus was impaired, possibly because of hyperactive plasmacytoid DCs producing *IL-6* and *IL-12* in addition to *IFN-α*.⁶²

Monitoring of intracellular pathways can be used to “trace back” the fates of late premature stages in B-cell development. Nevertheless, the question of whether there is a common pre-B-cell lineage ancestor that can give rise to a compartment-specific or maturation/activation-defined B cell with a set of regulatory features still remains open. The currently available data favor the idea of the intrinsic plasticity of diverse B-cell subsets, allowing them to acquire distinct regulatory profiles in particular inflammatory microenvironments.

Breg CELLS IN IMMUNE TOLERANCE TO ALLERGENS

Allergic responses are the result of an imbalanced response to innocuous environmental antigens that leads to the production of T_H2 cells and IgE antibodies. Understanding the mechanisms driving healthy versus allergic responses to allergens is critical for the development of novel strategies to induce allergen

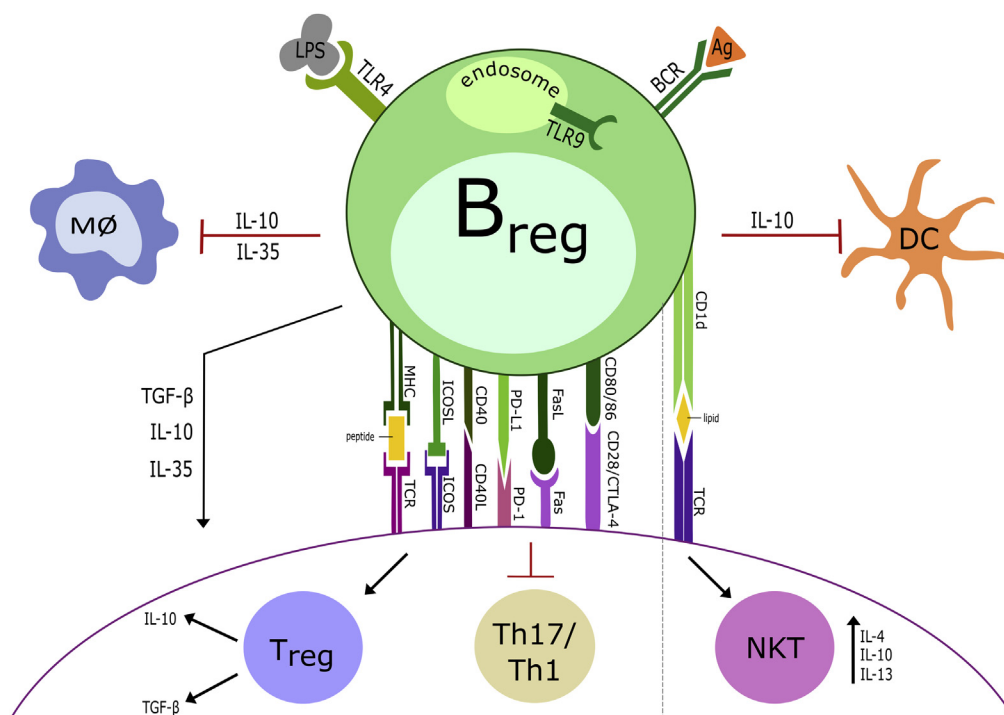


FIG 1. Mechanisms of suppression by Breg cells. Expression and secretion of suppressive mediators in Breg cells can be induced by TLR ligands, such as LPS and CpG, as well as BCR and CD40 signaling. Breg cells can regulate T-cell responses through inhibition of effector T-cell (in particular T_H1 and T_H17) responses while promoting Treg cell expansion. These effects are mediated by secreted factors, such as IL-10, TGF- β , and IL-35. Moreover, 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. CD1d, which is expressed by certain Breg cells, can activate natural killer T (*NKT*) cells with suppressive function. Furthermore, Breg cells can inhibit DC and macrophage activation, primarily through secreted IL-10. Ag, Antigen; *MΦ*, macrophages.

tolerance. Mechanisms of development of allergen tolerance can be studied in mouse models.⁶³ Furthermore, several interesting *in vivo* models have been used to study this process in human subjects.

Models for studying human allergen tolerance induction

Healthy immune responses to high-dose allergen exposure in human subjects have been studied in 2 different models: immune responses to bee venom allergens in beekeepers and immune responses to cat allergens in cat owners.⁶⁴⁻⁶⁶ Allergic patients can benefit from therapies that have a disease-modifying effect on allergic immune responses toward a healthy response. AIT is the only curative treatment for allergic diseases that is currently applied in the clinic and represents another highly relevant human *in vivo* model for allergen tolerance induction.^{67,68} Many regulatory mechanisms are involved in the induction of allergen tolerance in allergic patients through AIT, including induction of allergen-specific T_R1 cells, which produce IL-10 and TGF- β .²

Breg cells might also play a key role in the induction of immune tolerance to allergens. As discussed below, several studies have demonstrated that B cells can suppress allergen-mediated inflammation through secretion of IL-10 and TGF- β , thereby suppressing effector T-cell responses and inducing Treg cells.

Furthermore, Breg cells might promote allergen tolerance through preferential production of IgG₄ antibodies on differentiation to plasma cells.

Role of Breg cells in high-dose allergen exposure models

In 1998, it was first reported that the frequency of IL-10-expressing human B cells was increased in patients with bee venom allergy who received AIT.⁶⁹ More evidence for a potential role for Breg cells in allergen tolerance induction was obtained when B cells that secreted IL-10 on ligation of TLR9 were purified by using an IL-10 secretion assay, which allowed isolation of live IL-10⁺ and IL-10⁻ B cells. These B-cell fractions were subsequently compared by using whole-transcriptome analysis, revealing that these inducible IL-10⁺ B cells were enriched among CD19⁺CD73⁻CD25⁺CD71⁺ cells.¹⁵ Furthermore, they expressed increased levels of PD-L1. Some of these surface molecules have a known role in immune regulation.

CD25 is expressed by natural Treg cells and plays a role in Treg cell-mediated suppression through IL-2 deprivation, which induces apoptosis in effector T helper cells.⁵¹ CD25 expression is also required for IL-10 expression by CD4⁺ T cells and thereby is critical for the generation of T_R1 cells.⁵² Increased CD25 expression on human B cells has been associated with enhanced

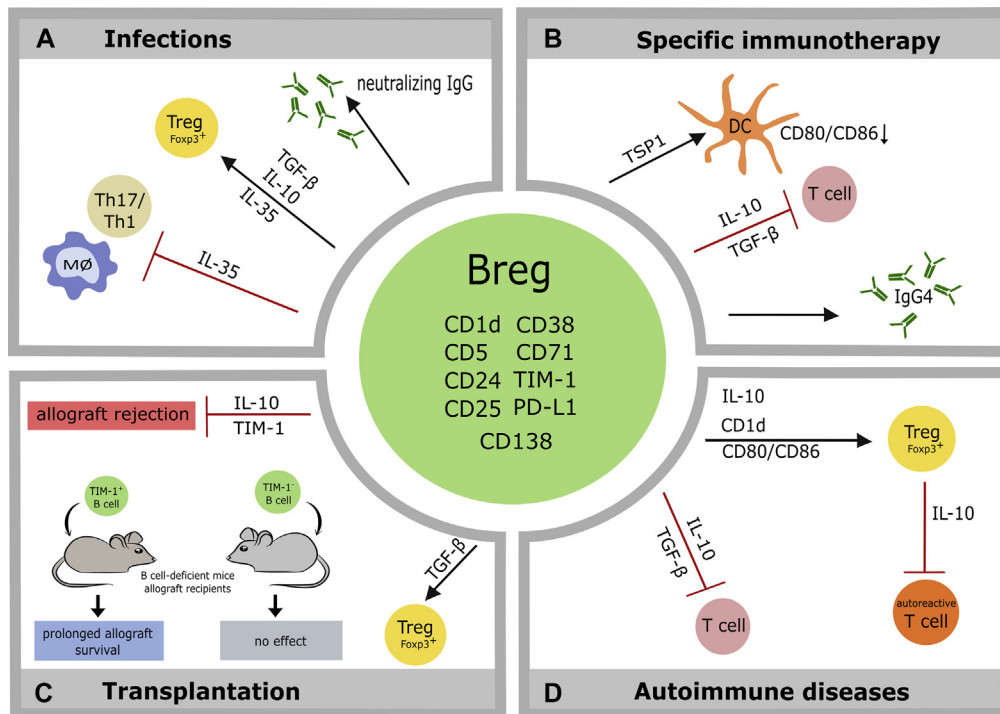


FIG 2. Role of Breg cells in regulation of inflammatory responses. Breg cells have been implicated in restoring homeostasis after infections and induction of tolerance to allergens, autoantigens, and transplanted tissue grafts. **A**, Numerous pathogens have been demonstrated to induce Breg cells. B cell-derived IL-35 can suppress T-cell and monocytic responses. IL-10, IL-35, and TGF- β induce the Treg cell population. **B**, Specific immunotherapy induces development of thrombospondin-producing B cells that can downregulate costimulatory molecules (CD80/CD86) in DCs. IL-10-producing B cells have a potent suppressive capacity on T-cell response and produce anti-inflammatory IgG₄. IL-10- and TGF- β -producing Breg cells can suppress T-cell responses. **C**, Transfer of TIM⁺ B cells into B cell-deficient allograft recipients results in prolonged allograft survival, whereas transfer of TIM⁻ B cells has no effect. Breg cell-mediated tolerance can be dependent on TGF- β -driven induction of Treg cells. **D**, Breg cells activate Treg cells through IL-10 production and B7 costimulatory molecules. Activated Treg cells release IL-10, suppresses the autoreactive T_H1 response in patients with EAE. IL-10- and TGF- β -producing Breg cells can suppress T-cell responses. MØ, Macrophage; TSP1, thrombospondin 1.

in vitro antigen-presenting capacity,⁷⁰ as well as increased B-cell IL-10 production in a murine model for intestinal inflammation.⁷¹

CD71 is the major transferrin receptor and mediates cellular uptake of iron. CD25 and CD71 are activation markers of T and B cells, and their increased expression might reflect an increased activation state.²⁹

CD274, also named PD-L1, is an inhibitory costimulatory molecule that counteracts T-cell activation through binding to PD-1 on T cells.⁴⁴

CD73 is a cell-surface enzyme that converts adenosine monophosphate to adenosine, which has potent immunosuppressive effects. CD73 is expressed on Treg cells and might be responsible for part of their immunosuppressive effects.⁵⁴ On the other hand, B_R1 cells express low levels of surface CD73, and therefore adenosine-mediated immune suppression is unlikely to play an important role in suppression of T-cell responses. These inducible IL-10⁺ B cells were named B_R1 cells in analogy to inducible T_R1 cells. B_R1 cells potently suppressed *in vitro* antigen-specific T-cell proliferative responses in an IL-10-dependent manner (Fig 2).¹⁵

The frequency of inducible IL-10⁺ cells among B cells specific for the major bee venom allergen phospholipase A2 (PLA) showed a 2- to 5-fold increase in patients with bee venom allergy

3 to 4 months after the start of venom immunotherapy, reaching levels comparable with those observed in healthy beekeepers.¹⁵ The total frequencies of B_R1 cells (CD73⁻CD25⁺CD71⁺IL-10⁺) were also significantly upregulated in venom immunotherapy-treated patients.⁷² These observations indicate that Breg cells expand in response to high-dose allergen exposure and support a functional role for IL-10-producing B cells in inducing and maintaining allergen tolerance. In parallel, CCR5 expression was also upregulated on PLA-specific B cells in patients after AIT and in healthy beekeepers.⁷² CCR5 expression by Treg cells is essential for their migration to inflammatory sites, where they exert their suppressive function *in vivo*.⁷³ Whether CCR5 expressed on allergen-specific B cells in allergen tolerance induction has a similar function remains to be addressed.

Breg cells in humoral responses to allergens

The humoral response that marks allergen tolerance is characterized by a transient increase in allergen-specific IgE levels, followed by a decrease in IgE and a gradual increase in allergen-specific IgG antibody levels. During AIT, IgG₄ levels increase particularly, resulting in a 10- to 100-fold decreased allergen-specific IgE/IgG₄ ratio.² IgG₄ antibodies have several

noninflammatory properties, including low affinity for activating Fc γ receptors, inability to activate **complement**, and their unique capacity to form bispecific and functionally monovalent antibodies through Fab arm exchange.⁷⁴ Because of these factors, allergen-specific IgG₄ is considered anti-inflammatory and might protect against allergic responses by competing with IgE for allergen binding.

IL-10 has been shown to promote IgG₄ production by B cells,⁶⁹ which led to the question of whether B cells that can produce IL-10 are more prone to become IgG₄-producing plasma cells. This was indeed the case because purified alive naive IL-10⁺ B_R1 cells produced more than 15-fold more IgG₄ than IL-10⁻ B cells, whereas other immunoglobulin isotypes were produced at equal rates.¹⁵ B cells specific for the major bee venom allergen PLA, which were isolated from healthy beekeepers, expressed increased levels of IL-10 and mature IgG₄ mRNA. The frequency of IgG₄-switched memory B cells was significantly increased among PLA-specific B cells in venom immunotherapy-treated patients.⁷² Taken together, these data demonstrate that B_R1 cells merge 2 key elements of a healthy immune response to allergens: suppression of effector T cells and induction of an IgG₄-skewed humoral response.

Breg cells in models of allergic airway inflammation

Mouse models focusing on the role of B cells in allergic airway disease have yielded several interesting findings regarding the immunoregulatory role of Breg cells mediated through IL-10 secretion. CD9⁺ B cells were identified as a source of critical suppressive mediators in a house dust mite model of allergic airway hyperresponsiveness. CD9 is an adhesion molecule expressed primarily on MZ B cells, B1 cells, and plasma cells. Adoptive transfer of CD9⁺ B cells suppressed T_H2- and T_H17-mediated airway inflammation through provision of IL-10. The extent of overlap of these CD9⁺ B cells with previously described Breg cell subsets remains to be elucidated.⁷⁵

In addition to IL-10, TGF- β produced by B cells can also mediate tolerance in the setting of allergic airway inflammation. Mice subjected to short-term exposure to aerosolized OVA (daily challenge for 7 days) induced sensitization, whereas chronic exposure (daily challenge for 42 days) resulted in tolerance, as characterized by reduced eosinophilia and lymphocytosis and lack of airway hyperresponsiveness. The chronic antigen exposure used in this model can be considered a high-dose allergen tolerance induction regimen. Adoptive transfer of B cells isolated from hilar lymph nodes of chronically exposed mice to sensitized mice could suppress allergic airway inflammation. Interestingly, this protective effect was not dependent on IL-10, as previously demonstrated by the intact suppression seen after transfer of B cells from tolerant IL-10^{-/-} mice.²⁰ Later, it was found that TGF- β -producing CD5⁺CD1d^{hi} B cells, which were selectively expanded in tolerant mice, mediated this suppressive effect. These cells could induce expression of *forkhead box protein 3* (*Foxp3*) in CD4⁺ T cells *in vitro*. Unlike CD5⁺CD1d^{hi} B10 cells, which produce IL-10 rather than TGF- β and are mainly found in the spleen, these cells were primarily localized in the regional lymph nodes.⁷⁶

Breg cells in models of allergic intestinal inflammation

Studies with murine models of food allergy and food AIT also demonstrated a possible involvement of antigen-specific Breg

cells that produced TGF- β in tolerance induction to food allergens. A subpopulation of intestinal CD5⁺CX3CR1⁺ B cells that expressed TGF- β was identified in naive BALB/c mice. TGF- β expression could be induced by stimulation with $\alpha\beta$ 6 and anti-IgM. Intestinal epithelial cells can produce both $\alpha\beta$ 6 and fractalkine (the ligand for CX3CR1). Therefore CX3CR1⁺ B cells might be recruited to the intestinal epithelium, where they can be activated to produce TGF- β . Activated CD5⁺CX3CR1⁺ B cells promoted *in vitro* induction of CD4⁺CD25⁺Foxp3⁺ cells and suppressed T-cell proliferation. Their adoptive transfer led to suppression of food allergy-related T_H2-mediated intestinal inflammation.¹⁷ A potential factor that might promote TGF- β production by B cells is thrombospondin 1, an immunoregulatory protein that binds latent TGF- β to generate active TGF- β . This was demonstrated when mice were sensitized to OVA in the presence of cholera toxin to induce allergic intestinal inflammation. Sensitized mice were subsequently treated with an AIT regimen, which consisted of increasing doses of gavage-fed OVA. AIT-receiving mice showed a significant increase in CD35⁺ lamina propria B cells, which produced thrombospondin 1. CD35 is a regulator of complement activation and plays a role in memory B-cell development. No additional phenotypic markers associated with these Breg cells were reported in this study.⁷⁷

A similar murine model for intestinal allergic inflammation was used to study the effect of the probiotic strain *Clostridium butyricum* in AIT. OVA-sensitized mice were treated with an AIT regimen consisting of increasing doses of gavage-fed OVA with or without *C butyricum*. Intestinal allergic inflammation was moderately reduced in AIT-receiving mice but strongly suppressed after combined treatment. This was accompanied by an increased frequency of OVA-specific IL-10⁺ B cells in the lamina propria, suggesting that *C butyricum* promotes the effect of AIT on allergic inflammation through induction of Breg cells.⁷⁸

Thus B cells producing the immunoregulatory cytokines IL-10 and TGF- β are expanded in response to high-dose allergen exposure both in human subjects and mice. The mechanisms that drive this effect remain to be further elucidated. This response appears to be, at least in part, antigen specific because frequencies of allergen-specific IL-10-producing B cells specifically increased after AIT. Breg cells can, in turn, induce Treg cells and suppress effector T-cell activation. The phenotypes of the Breg cells involved in these responses remain poorly defined. CD5⁺ B cells can suppress OVA-induced allergic airway inflammation through TGF- β secretion,⁷⁶ whereas CD9⁺ B cells conferred protection against house dust mite-induced allergic airway inflammation through secretion of IL-10.⁷⁴ TGF- β -producing B1a-like Breg cells with gut-homing potential (CD5⁺CX3CR1⁺), as well as TGF- β -producing Breg cells expressing CD35, have been reported as potentially relevant regulatory cells in murine food allergy models.^{17,77}

Breg CELL RESPONSES DURING INFECTIONS

Although Breg cells provide benefits for the host in the context of chronic inflammatory conditions and autoimmunity, their presence during infection might impair pathogen clearance (Fig 2). A wide range of pathogens, including bacteria, viruses, helminths, and protozoa, induce distinct Breg cell subsets. Helminth infections induce strong T_H2 responses and IgE production. Interestingly, the incidence of allergic disorders is lower among

persons infected by helminths than among noninfected subjects.⁷⁹ Helminths use specific mechanisms to downregulate protective immune responses to infect and coexist with their hosts. Several studies have reported the induction of Breg cell responses on parasite infection that were associated with reduced symptoms in diverse models of inflammation, including allergic airway inflammation and anaphylaxis. Helminth infection has been shown to induce both IL-10- and TGF- β -producing B cells, which in turn dampen inflammatory responses, leading to suppression of allergic inflammation and impaired antiparasite immune responses.

Infection with *Schistosoma mansoni* has been associated with high levels of IL-10- and LAP/TGF- β 1-producing B cells that can inhibit production of IL-4, IFN- γ , and IL-17 by T cells and mediate conversion of effector T cells into CD25^{hi}Foxp3⁺ IL-10-producing Treg cells. Thereby helminth infections can contribute to an immunoregulatory environment and promote dampening of T_H1 and T_H17 but also T_H2-skewed pathologies.^{21,22,80,81} Mice infected with *S. mansoni* were protected in an experimental model of systemic anaphylaxis. This effect was dependent on induction of IL-10-producing B cells, which were 2-fold increased in *S. mansoni*-infected mice. B2 cells were responsible for this protective effect because *Xid* mice, which lack B1 cells, still showed B cell-mediated protection against anaphylaxis.²²

Protective effects of B cells on allergic airway inflammation have also been reported in *S. mansoni*-infected mice. A model for acute, intermediate, or chronic helminth infection was developed. Helminth-infected C57BL/6 mice were sensitized and challenged with OVA. Acutely infected mice had OVA-induced airway hyper-responsiveness, as well as eosinophilia and bronchial inflammation, whereas chronic helminth exposure led to suppression of these symptoms. Adoptive transfer of spleen-derived B cells (as well as CD4⁺ T cells) isolated from chronically infected mice to OVA-sensitized mice led to reduced symptoms in recipient mice, which was IL-10 dependent.²¹ A later study showed that *S. mansoni* infection in particular induced IL-10-producing CD5⁺CD1d^{hi} B10 cells and that these cells were able to prevent OVA-induced allergic airway inflammation after passive transfer to sensitized recipients in an IL-10-dependent manner.¹⁹ Microarray analysis revealed that CD1d^{hi} B cells had increased expression of *Tlr7*. It was further demonstrated that ligation of CD19⁺CD1d^{hi} B cells with TLR7 increased IL-10 production in these cells. Adoptive transfer of these TLR7-elicited B10 cells was able to reduce airway inflammation through induction of Treg cells.¹⁶

Breg cells that are induced on helminth infection typically have a B10 CD5⁺CD1d^{hi} phenotype and mostly mediate their effect through IL-10 secretion. In addition, other types of Breg cells can be induced on helminth infection. CD19⁺CD23^{hi} B cells (a phenotype that is indicative of follicular B cells), which were isolated from *Heligmosomoides polygyrus*-infected mice, could suppress inflammatory responses as well. Adoptive transfer of these cells potentially suppressed both allergic airway inflammation in an OVA model and autoimmune inflammation in an EAE model. Interestingly, CD19⁺CD23^{hi} B cells isolated from infected IL-10^{-/-} mice retained their suppressive capacity.⁸¹ The responsible factor for this suppressive effect remains to be identified.

There is an obvious potential downside to the aforementioned immunosuppressive effects caused by helminth infections, which promote Breg responses and allergen tolerance. Parasite-induced Breg cell induction might impair protective T-cell responses to parasite infections and thereby prevent clearance of the infection.

On *Leishmania donovani* infection, MZ B cells are able to suppress T-cell function. After infection, MZ B cells upregulate surface IgM and produce MyD88-dependent IL-10. Depletion of MZ B cells enhanced T-cell responses.³² B1a cells expressing Fas ligand were found during *S. mansoni* infection. This type of cell was induced on IL-4, IL-5, and IL-10 stimulation and was able to induce apoptosis in CD4⁺ T cells.^{27,28} Because of this killer-like function, this subset has also been described as killer B cells. These studies suggest that parasite infections induce both suppressive and cytotoxic Breg cells, which in turn impair antiparasite T-cell responses.

Breg cell responses have been studied in various other infection models. A number of recent studies have shown that plasma cells can produce cytokines and regulate infectious diseases *in vivo*. *Salmonella typhimurium* infection promoted the generation of IL-10⁺CD138⁺ plasma cells, which impaired an efficient host antibacterial immune response mediated by combined actions of neutrophils, natural killer cells, and inflammatory T cells.²⁵ CD138^{hi} plasma cells were the main source of B cell-derived IL-35 and IL-10 in a *Salmonella enterica* infection model, as well as in an EAE model.¹² Breg cells might also have a detrimental role in mounting antiviral responses. In patients with chronic hepatitis B infection, a positive correlation between viral load and serum IL-10 levels was observed. A similar correlation was found between the frequencies of circulating IL-10-producing B cells (which were primarily immature CD19⁺CD24^{hi}CD38^{hi} B cells). Purified CD19⁺CD24^{hi}CD38^{hi} cells suppressed CD8⁺ T-cell responses, suggesting that Breg cells can impair antiviral T-cell responses.⁴⁵

IMMUNE REGULATION BY Breg CELLS IN PATIENTS WITH OTHER INFLAMMATORY CONDITIONS

Breg cells in patients with autoimmune disease

Much of the pioneering work on the identification and functional characterization of Breg cells was done in the context of autoimmune disease models (Fig 2). CD19⁺CD21^{hi}CD23^{hi}CD24^{hi} transitional stage 2 MZ precursor B cells had a preventive and therapeutic effect in a collagen-induced arthritis model through IL-10. Interestingly, these cells could suppress T_H1 responses *in vitro* without the requirement of cell-to-cell contact. These B cells could only suppress T-cell responses to an antigen to which they were exposed during the induction phase of the disease, indicating that this regulatory response is antigen specific.²³ These cells could be selectively expanded by short-term culture with an anti-CD40 mAb, as well as its *in vivo* administration. These expanded Breg cell suppressed autoimmune disease in a murine lupus model.⁵³

A functionally similar subset of immature human CD19⁺CD24^{hi}CD38^{hi} B cells has been identified in the context of autoimmune disease.^{44,82} IL-10 production in these cells was induced by CD40 stimulation. Coculture experiments with anti-CD3-stimulated CD4⁺ T cells in the presence of neutralizing antibodies against CD80, CD86, IL-10, and TGF- β demonstrated that CD19⁺CD24^{hi}CD38^{hi}-mediated T-cell suppression is dependent on CD80, CD86, and IL-10 but not TGF- β . Interestingly, the regulatory capacity of this subset was impaired in patients with systemic lupus erythematosus. CD19⁺CD24^{hi}CD38^{hi} B cells were also responsible for regulating CD8⁺ T cell-mediated immune responses against chronic hepatitis B virus infection.⁴⁵ It was shown that these Breg cells promote conversion of

CD4⁺CD25⁻ T cells into CD4⁺CD25⁺ Treg cells, whereas conversion into T_H1 and T_H17 cells was selectively inhibited.⁸³ Patients with rheumatoid arthritis had reduced numbers of circulating CD19⁺CD24^{hi}CD38^{hi} B cells. Furthermore, these Breg cells were functionally impaired in patients because they were not able to promote conversion of T_H cells into Treg cells.

A functional Breg cell subset termed B10 cells was identified as CD19⁺CD1d^{hi}CD5⁺ B cells in mice. These cells produced IL-10 in response to LPS stimulation and, to a lesser extent, to anti-IgM antibody plus CD40 mAb stimulation. B10 cells, which represent 1% to 2% of splenic B220⁺ cells and 7% to 8% of peritoneal B cells efficiently suppressed T cell–mediated inflammatory responses in an oxazolone-induced contact hypersensitivity model.²⁶ An immunosuppressive effect of B10 cells has been observed in several autoimmune models in mice. In the setting of murine lupus, B10 cell deficiency exacerbated systemic autoimmunity.³⁴ In the setting of collagen-induced arthritis, IL-10–producing B10 cells were able to suppress T_H17 cells and thereby dampen disease symptoms,³⁵ whereas adoptively transferred B10 cells were able to suppress autoimmune myasthenia gravis.³⁶ In addition to their protective function in patients with autoimmune diseases, suppressive effects of B10 cells have been demonstrated in dextran sulfate sodium–induced intestinal inflammation in mice, which is used as a model for human ulcerative colitis.³⁷

A human Breg cell subset analogous to mouse B10 cells has been identified.⁵⁴ The phenotypic markers that identify this human B10 population differ from those described in mice. Human B10 cells are CD27⁺CD24^{hi}, with about 60% expressing CD38 as well. Furthermore these cells expressed CD27, CD148, and CD48, suggesting a mature or activated state. The expression of CD27 is particularly interesting because it is considered a memory B-cell marker. This indicates that human B10 cells might be part of the memory B-cell compartment or they upregulate this memory marker in an unconventional manner. In addition, the transitional B-cell marker CD10 was expressed at very low levels in B10 cells, suggesting that these cells did not recently emigrate from the bone marrow. Thus these human B10 cells are significantly different from immature CD19⁺CD24^{hi}CD38^{hi} B cells. IL-10 production in these cells was induced in response to LPS, CpG, phorbol myristate ester, and ionomycin stimulation. B cells that produced IL-10 after short-term stimulation (several hours) were called B10 cells. A larger percentage of IL-10⁺ B cells were induced after prolonged stimulation with especially LPS or CpG (48 hours), followed by short-term phorbol myristate ester and ionomycin stimulation. The B cells that expressed IL-10 in the latter condition were called pro-B10 cells because they required prolonged stimulation to mature into IL-10–competent cells. In human blood B10 cells were found at similar frequencies as in mice.⁴³ B10 cells suppressed TNF- α production both in CD3-activated CD4⁺ T cells and LPS-stimulated monocytes, indicating that they can directly suppress both T-cell responses and innate responses. The frequencies of pro-B10 cells were significantly increased in several groups of patients with autoimmune diseases, such as rheumatoid arthritis, lupus, Sjögren syndrome, autoimmune skin disease, and multiple sclerosis.⁴³

Plasma cells can produce IL-10 and have been shown to suppress autoimmune responses. In a murine EAE model a population of IL-10–expressing plasmablasts emerged in the draining lymph nodes that exerted a suppressive effect on the autoimmune response. B cell–specific Blimp1- or interferon regulatory factor 4–deficient mice, which lack plasmablasts,

had exacerbated EAE, indicating that IL-10–producing plasmablasts can control autoimmune inflammation.²⁴

In most studies investigating the role of Breg cells in autoimmune models, IL-10 is the key immunomodulatory cytokine. A recent study identified B cell–derived IL-35 as an important factor in the regulation of autoimmune responses. Stimulation with IL-35 induced Breg cells that produce both IL-35 and IL-10, called i35 B cells. Mice treated with IL-35 were protected from experimental autoimmune uveitis. This protective effect was dependent on IL-35 because mice with a B cell–specific IL-35 deficiency, as well as mice lacking the IL-35 receptor, still had the disease. Adoptive transfer of these i35 Breg cells suppressed disease symptoms in mice with established experimental autoimmune uveitis through suppression of T_H1 and T_H17 responses and expansion of Treg cells.³⁸

Breg cells in patients undergoing organ transplantation and those with cancer

Breg cells have been shown to exert regulatory functions in relation to allograft survival (Fig 2). Both skin and cardiac allograft survival were prolonged by transferring CD19⁺CD21^{hi}CD23^{hi}CD24^{hi} transitional stage 2 MZP B cells.^{39,41} However, these studies do not provide a consensus regarding the role of IL-10 in B cell–mediated allograft rescue. Several types of Breg cells also play a role in tumor development by impairing antitumor T-cell responses (this topic is reviewed extensively elsewhere⁸⁴). An interesting recent finding in this area was that CD138⁺IgA⁺ plasma cells were able to suppress cytotoxic T-cell responses through exhaustion of CD8⁺ cells in prostate tumors. IL-10 and PD-L1 were identified as responsible factors for this suppression.²⁹

TARGETED THERAPIES BASED ON Breg CELLS

There are currently no Breg cell–based therapies used in clinical practice. Given the strong immunosuppressive capacity of Breg cells, *ex vivo* or *in vivo* Breg cell expansion holds promise for the treatment of chronic inflammatory conditions. Successful *ex vivo* expansion of functional Breg cells was demonstrated in several different models. Treatment of mouse splenocytes with a *GM-CSF* and *IL-15* “fusokine” leads to generation of IL-10–producing Breg cells, which suppressed *in vitro* antigen-specific T-cell responses and reversed EAE.⁴² Another study showed that functionally suppressive transitional stage 2 like IL-10–producing Breg cells were selectively expanded on *in vitro* culture, as well as *in vivo* administration of an agonistic anti-CD40 antibody.³³ Moreover, IL-10–producing B cells could be very efficiently expanded by culturing spleen-derived B cells in the presence of CD40L with IL-4, followed by *IL-21* stimulation. The IL-10–producing B cells expanded in patients with these conditions were primarily CD5⁺ and could inhibit the initiation and progression of EAE.⁴⁹ Such approaches could possibly be extended to human subjects to develop therapeutic options for the treatment of chronic inflammatory conditions. To our knowledge, there are currently no interventional clinical trials underway that target Breg cells.

CONCLUSION

During the last 20 years, significant progress has been made in understanding the role of Breg cells in the regulation of chronic inflammatory conditions. It is becoming increasingly clear that

Breg cells do not form a single uniform cell subset. Several different sets of surface markers have been described that identify B-cell populations that suppress inflammatory responses. Thus far, no Breg cell-specific transcription factor has been identified, which makes it difficult to conclude whether Breg cells form a distinct lineage. It is reasonable to hypothesize that Breg cells represent an immune regulatory state because of B-cell plasticity in response to several environmental, inflammatory, and tissue-damaging signals that induce immune tolerance, such as allergens, infections, organ transplantation, and tumors.

Taken together, there are now substantial data demonstrating that B cells play their part in the maintenance and induction of tolerance to many different self- and non-self-antigens. Therapeutic strategies specifically targeting Breg cells can be developed in the future and hold promise as treatment options for chronic inflammatory diseases, including allergies.

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