Regulatory B Cells

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Abstract

B cells are typically characterized by their ability to produce antibodies, function as secondary antigen-present cells, and produce various immunoregulatory cytokines. The regulatory B (Breg)-cell population is now widely accepted as an important modulatory component of the immune system that suppresses inflammation. Recent studies indicate that Breg-cell populations are small under physiological conditions but expand substantially in both human patients and murine models of chronic inflammatory diseases, autoimmune diseases, infection, transplantation, and cancer. Almost all B-cell subsets can be induced to form Breg cells. In addition, there are unique Breg-cell subsets such as B10 and Tim-1^+ B cells. Immunoregulatory function may be mediated by production of cytokines such as IL-10 and TGF-β and ensuing suppression of T cells, by direct cell–cell interactions, and (or) by altering the immune microenvironment. In this chapter, we describe in detail the discovery of Breg cells, their phenotypes, differentiation, function, contributions to disease, and therapeutic potential.

Keywords

Regulatory B cells · IL-10 · Diseases

8.1 Introduction

Morris et al. were the first to identify antibodies (Abs) with both pro- and anti-inflammatory activities in mice (Morris and Moller 1968). Subsequently, several groups described the ability of splenic B cells to suppress delayed-type hypersensitivity (DTH) responses in guinea pigs (Katz et al. 1974; Neta and Salvin 1974). Kennedy et al. also demonstrated that memory B cells can act as suppressors–inducers of feedback control (Kennedy and Thomas 1983). These and subsequent studies demonstrated that B cells could exert immunosuppressive effects, at least in part by regulating T-cell function. However, it has taken several decades to definitively identify regulatory B (Breg) cells and their immunosuppressive mechanisms. In the 1990s, Janeway and colleagues first reported the existence of an immunoregulatory B-cell subset that when transplanted induced complete recovery of acute experimental autoimmune encephalomyelitis

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J.-Y. Wang (ed.), *B Cells in Immunity and Tolerance*, Advances in Experimental Medicine and Biology 1254, https://doi.org/10.1007/978-981-15-3532-1_8

(EAE) in mice (Wolf et al. 1996). Mizoguchi and Bhan then demonstrated that B-cell-deficient mice with experimental inflammatory bowel disease (IBD) had more severe colitis symptoms, again suggesting that some B-cell populations have suppressive activity in immunological diseases. These authors also first coined the term "regulatory B cells" to designate B cells with immunoregulatory properties (Mizoguchi et al. 1997).

Since these initial discoveries, it has been established that B cells can contribute to the maintenance of immune tolerance and the suppression of inflammatory responses (Mizoguchi et al. 2002). However, there is still no consensus phenotypic definition of Breg cells. Immunomodulatory B cells have been ascribed various phenotypes, ranging from immature B2-lineage cells such as transitional-2 marginal zone precursor (T2-MZP) cells to mature B1-lineage B cells (Evans et al. 2007; Lenert et al. 2005; Lundy and Boros 2002). In 2008, the Tedder group identified a unique $CD19^{+}CD5^{+}CD1d^{hi}$ B-cell subset that produced IL-10 and functioned as a suppresser of diseases (Yanaba et al. 2008). In the following decade, several distinct Breg-cell subtypes and functions were identified in various disease models, including models of IBD, EAE, and cancer. Breg cells regulate disease development through multiple mechanisms, such as by producing IL-10, IL-35, and IL-21 (Shen et al. 2014; Tedder and Leonard 2014; Wang et al. 2014). Several research groups have examined Breg-cell function by treating isolated cells with exogenous factors followed by adoptive transfer into disease model mice to test for therapeutic effects. For instance, granulocyte—macrophage colony-stimulating factor and interleukin-15 fusokine (GIFT-15) converted naïve B cells into Breg cells that were subsequently shown to suppress EAE (Rafei et al. 2009). Human Breg cells with distinct phenotypes have also been identified (Blair et al. 2010; Bouaziz et al. 2010; Iwata et al. 2011). Tedder et al. defined human $CD19^{+}CD24^{+}CD27^{hi}$ B cells as Breg cells (Iwata et al. 2011), and Mauri et al. found that $CD19^{+}CD24^{hi}CD38^{hi}$ cells are immunosuppressive (Blair et al. 2010). Subsequently, a large number of studies confirmed the presence of Breg cells in the peripheral blood of patients with different diseases, suggesting broad clinical importance and potential therapeutic utility. Milestones and highlights in Breg-cell research are summarized in Table 8.1.

8.2 Breg-Cell Phenotype

Unlike Treg cells, there are no unique surface markers or released factors (i.e., specific cytokine profiles) that define Breg cells. Thus, the term Breg refers to B cells with regulatory functions. Nonetheless, multiple Breg-cell subsets with distinct phenotypes and effector functions have been described (summarized in Table 8.2).

8.2.1 Breg Cells in Mouse

8.2.1.1 Common Breg-Cell Subsets

Unlike natural Treg cells, there are a few if any "natural Breg cells." However, any B-cell subset can differentiate into Breg cells under the appropriate stimulus environment, such as the presence of toll-like receptor (TLR) ligands and anti-CD40 stimuli (Rosser and Mauri 2015). Bulk $CD19^{+}$ B cells can produce IL-10 upon lipopolysaccharide (LPS) stimulation (Matsushita and Tedder 2011). Innate B-cell subsets such as B-1, marginal zone (MZ), and transitional (T) B cells reportedly can be induced into Breg cells. For instance, stimulation of MZ B cells with TLR agonists facilitated production of IL-10, TGF-β, and IL-6, cytokines released by Breg cells (Tian et al. 2001). Further, these B-cell-derived populations have ameliorative effects in disease models. For example, CD40-stimulated T2 B cells suppressed lupus in MRL/lpr mice (Blair et al. 2009), while oral immunization with ovalbumin (OVA) enabled B-1 cells to negatively regulate intestinal immunity (De-Gennaro et al. 2009). Even follicular B (FOB) cells can express high levels of CD11b in response to TLR ligands plus IL-10, thus "acquiring" strong regulatory functions and suppressing autoimmune disease (Liu et al. 2015; Wang et al. 2018).

Table 8.1 Milestones and highlights in Breg-cell research

Year	Mouse	Human
1968	Inhibitory antibodies are exist (Morris and Moller 1968)	
1974	B cells suppress DTH (Katz et al. 1974; Neta and Salvin 1974)	
1983	Memory B cells are immunosuppressive (Kennedy and Thomas 1983)	
1996	B-cell-deficient mice develop more severe EAE (Wolf et al. 1996)	
1997	B-cell-deficient mouse develop more severe colitis (Mizoguchi et al. 1997)	
2002	The term "Breg" coined (Mizoguchi et al. 2002)	
2007	Transitional 2 B cells suppress arthritis (Evans et al. 2007)	
2008	Breg cells isolated with a unique $CD19^{+}CD1d^{hi}CD5^{+}$ phenotype (Yanaba et al. 2008)	
2010	Breg cells differentiate via TLR or CD40 stimulation (Neves et al. 2010; Zheng et al. 2010)	$CD19^{+}CD24^{hi}CD38^{hi}$ B cells exhibit regulatory capacity in human blood (Blair et al. 2010)
2011	Regulatory B cells identified in mice (Matsushita and Tedder 2011)	Identification of regulatory B cells in humans that resemble mouse-regulatory B cells (Iwata et al. 2011)
2012	IL-21 involved in Breg-cell development (Yoshizaki et al. 2012)	
2015	IL-35 involved in Breg-cell development (Wang et al. 2014) $CD11b^{+}$ Breg-cell-subset suppresses EAH (Liu et al. 2015)	
2018	LAG-3 expression identifies regulatory plasma cells (Lino et al. 2018)	

8.2.1.2 The Unique $CD5^{+}CD1d^{hi}$ Breg Cells

Currently, there are no known phenotypic or lineage markers that are unique to Breg cells; rather, Breg cells are more likely defined by their function and behavior. Tedder and colleagues found a specific Breg-cell subset, $CD5^{+}CD1d^{hi}$, present only in the mouse spleen and showing dramatic changes under disease conditions (Yanaba et al. 2008). Under physiological conditions, $CD5^{+}CD1d^{hi}$ Breg cells accounted for only 1–3% of the total B-cell population, but this proportion rose to 10–20% under various disease conditions (Yanaba et al. 2008). These $CD5^{+}CD1d^{hi}$ Breg cells highly expressed IL-10, and transferring these cells into mice with autoimmune diseases effectively alleviated disease symptoms (Kalampokis et al. 2013; Watanabe et al. 2010). Since the first report in 2008, many research groups have found this Breg subset and documented a regulatory role in various diseases. For example, Sheng et al. reported that $CD5^{+}CD1d^{hi}$ Breg cells regulate experimental autoimmune myasthenia gravis (MG) via IL-10 release (Sheng et al. 2015). In addition, B-cell activating factor (BAFF) induced IL-35 production by $CD5^{+}CD1d^{hi}$ Breg cell in lupus (Zhang et al. 2017). This $CD5^{+}CD1d^{hi}$ Breg-cell subset also appears indispensable for maintaining immune homeostasis (Xing et al. 2014). Further, $CD5^{+}CD1d^{hi}$

Table 8.2 Breg-cell phenotypes

Location	Subset	Phenotypes
Peritoneal cavity	B-1 cells	$CD19^+CD5^+IgM^{hi}CD23^-$ $CD21^-CD11b^+$ (Shimomura et al. 2008; Yanaba et al. 2008)
Spleen	$CD1d^{hi}CD5^+$ B cells	$CD19^{hi}CD1d^{hi}CD5^+IgM^{hi}CD24^{hi}B220^{hi}$ (Yanaba et al. 2008)
	MZ B cells	$CD19^+CD21^+CD1d^{+/-}CD5^+CD23^{low}$ (Bankoti et al. 2012)
	T2-MZP B cells	$CD19^+CD21^{hi}CD23^{hi}CD24^{hi}IgM^{hi}IgD^{hi}CD1d^{hi}$ (Evans et al. 2007; Moreau et al. 2015)
Intestine	IgA^+ plasmacytes	$CD19^+B220^+IgA^+PD\text{-}1^+$ (Liu et al. 2017)
"New"	$PD\text{-}1^+$	$CD19^+PD\text{-}1^+$ (Xiao et al. 2016)
	$CD73^+$ B cells	$CD19^+CD39^+CD73^+$ (Kaku et al. 2014)
	$TIM\text{-}1^+$ B cells	$CD19^+TIM^+$ (Ding et al. 2011)
	GrB^+ B cells	$CD19^+CD38^+CD1d^+IgM^+CD147^+$ (Lindner et al. 2013)
	$Foxp3^+$	$CD19^+$ $Foxp3^+$ (Guo et al. 2015; Vadasz and Toubi 2016)
	Killer B cells	$CD19^+Fas^+$ (Zhang et al. 2018)
	$CD11b^+$ B cells	$CD19^+CD11b^+$ (Liu et al. 2015)
Human blood	$CD27^+$ B cell	$CD19^+CD24^{hi}CD27^+$ (Iwata et al. 2011)
	$CD38^{hi}$ B cell	$CD19^+CD24^{hi}CD38^{hi}$ (Blair et al. 2010)

Breg cells have been found in humans where they likely exert inhibitory effects on various diseases (Chen et al. 2017).

8.2.1.3 Other New Breg-Cell Subsets

In addition to the $CD5^+CD1d^{hi}$ Breg-cell subset, several additional Breg-cell subsets have been reported in recent years, such as $Tim\text{-}1^+$, $CD11b^+$, $LAG\text{-}3^+$, $foxp3^+$, and $PD\text{-}1^+$ Breg cells. Ding et al. first reported Breg cells expressing TIM-1 and that TIM-1 ligation could promote Breg-cell expansion in mice (Ding et al. 2011). Kuchroo and colleagues confirmed that Tim-1 is essential for induction and maintenance of IL-10 expression by Breg cells and for their function in regulation of tissue inflammation (Xiao et al. 2012, 2015). Our group has been investigating a Breg-cell subset since 2015 (Liu et al. 2015; Qian et al. 2019; Wang et al. 2018) with high surface expression of CD11b under disease conditions. These $CD11b^+$ Breg cells have a crucial regulatory role in experimental autoimmune hepatitis (EAH) and are able to inhibit inflammation by disrupting T-cell receptor (TCR) signaling. In a colitis model, $CD11b^+$ B cells cooperate with Tregs in the maintenance of gut homeostasis. Using epigenomic detection, Lino et al. (2018) reported that LAG3 (CD223) identified another subset of regulatory plasma cell in vivo and in vitro. Other Breg cells express Treg-specific features such as $Foxp3^+$ expression (Noh et al. 2010; Vadasz and Toubi 2016). Finally, $PD\text{-}1^+$ B cells have been identified and functions in tumor immunity reported (Ren et al. 2016; Wang et al. 2019; Xiao et al. 2016).

8.2.2 Breg Cells in Human

Studies showing that Breg-cell populations expand under disease conditions in mice have led to numerous investigations on the presence and functions of Breg-cell subsets in humans, including various disease patients. However, the phenotypes of human Breg cells, their resemblances to mouse subsets, and relationships to specific diseases are controversial (Blair et al. 2010; Bouaziz et al. 2010; Iwata et al. 2011). Both $CD19^+CD24^{hi}CD27^+$ and $CD19^+CD24^{hi}CD38^{hi}$ Breg cells have been identified and shown to

inhibit the proliferation and function of pro-inflammatory cells, but do not show consistent expression in disease. In the healthy population, $CD19^{+}CD24^{hi}CD27^{+}$ B cells account for about 25% of total B cells, but the proportion can either increase or decrease in specific diseases (de Masson et al. 2015; Yang et al. 2015, 2017; Zha et al. 2012). Further, immunosuppressive functions are only partially dependent on IL-10. In addition, $CD19^{+}CD24^{hi}CD38^{hi}$ B-cell function is actually impaired under disease conditions in contrast to most murine Breg-cell subsets (Blair et al. 2010; Hasan et al. 2019; Li et al. 2019b; Yu et al. 2017; Zhu et al. 2014).

8.2.3 Regulatory and Effector B Cells

With the discovery of Breg cells, B cells are now divided into effector (Beff) and regulatory subsets. Given that nearly every B-cell subset can "acquire" regulatory or effector functions, Breg and Beff cells are difficult to distinguish. Normally, cytokine expression is used to distinguish between these two groups since Breg-cell subsets predominantly produce IL-10, TGF-β, and IL-35, while Beff subsets produce IL-6, TNF-α, and IFN-γ (Matsushita 2019). The Tedder group demonstrated complex relationships between Breg and Beff cells in an EAE model (Matsushita et al. 2008). However, due to the lack of Breg-cell lineage commitment factors, it is difficult to distinguish these two B cell groups for further study of cell–cell interactions, a condition essential for future therapeutic applications.

8.3 Development and Differentiation of Breg Cells

Compared to Tregs, relatively little is known about Breg-cell biology as there are not known reliable surface markers or master transcription factors. Further, the Breg cell may be a transitional state rather than an independent stable B-cell lineage (Berthelot et al. 2013; Rosser and Mauri 2015), creating additional difficulties for studies on basic biology, particularly in vivo. Thus, it is believed that all Breg-cell subsets arise from a common progenitor by external stimulation and that all B cells can differentiate into Breg cells (Fig. 8.1). Immature B cells like T2-MZP B cells can develop into Breg cells after TLR ligand stimulation, and mature B cells and even

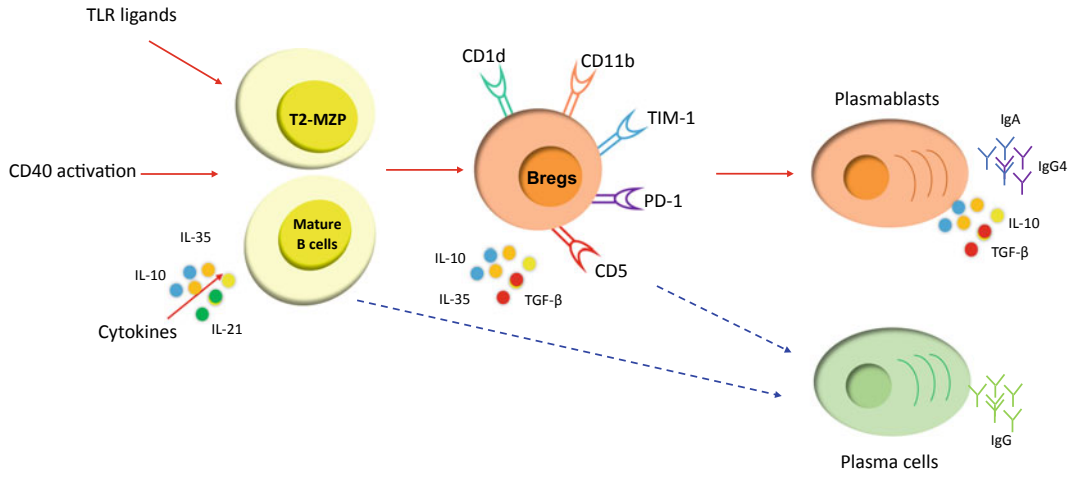

Fig. 8.1 Proposed development and differentiation pathways of Breg cells. Both T2-MZP B cells and mature B cells can differentiate into Breg cells under TLR ligand stimulation and CD40 activation by cytokines. Also, IL-10- and/or IL-35-producing plasmablasts can develop from mature B cells. All Breg-cell types can also differentiate into conventional plasma cells

plasma B cells can be transformed into Breg cells by appropriate stimuli. B cells transitioning into Breg cells are called B10 progenitor or B10pro cells. Functional Breg cells can be induced by CD40 ligand, LPS, or CpG oligonucleotides (Kalampokis et al. 2013). Tedder's group reported that mouse and human B10pro cells matured after in vitro stimulation by TLRs, disease-specific stimuli such as LPS in infection promote Breg-cell maturation. Myeloid differentiation primary response gene 88 (MyD88) signaling is required for IL-10 production in Breg cells but not for Breg-cell development. Thus, similar to Tregs, MyD88 is thought to be involved but not critical for Breg-cell development (Lino et al. 2018). Stromal interaction via stromal interaction molecules 1(STIM1) and 2(STIM2) is also necessary for IL-10 production by Breg cells (Matsumoto et al. 2011), while the cytokines IL-21 (Yoshizaki et al. 2012) and IL-35 (Shen et al. 2014) are essential for Breg-cell development. Lack of IL-21 or IL-35 decreases Breg-cell numbers both in vivo and in vitro. Furthermore, an earlier report suggested that splenic Breg cells eventually differentiate into antibody-producing plasmablasts after stimulation in vivo and in vitro (Maseda et al. 2012). Plasmablasts also have regulatory functions in autoimmune diseases and cancer (Liu et al. 2017; Matsumoto et al. 2014). Recent studies have suggested similarities in the in vivo development of Breg cells in mice and in humans.

8.4 The Mechanisms of Breg-Cell Regulation

Production of IL-10 is crucial for the immunosuppressive actions of Breg cells, although questions remain as to the intracellular pathways that induce IL-10 secretion. The target cells of Breg-cell immunomodulation and fundamental mechanisms are shown schematically in Fig. 8.2.

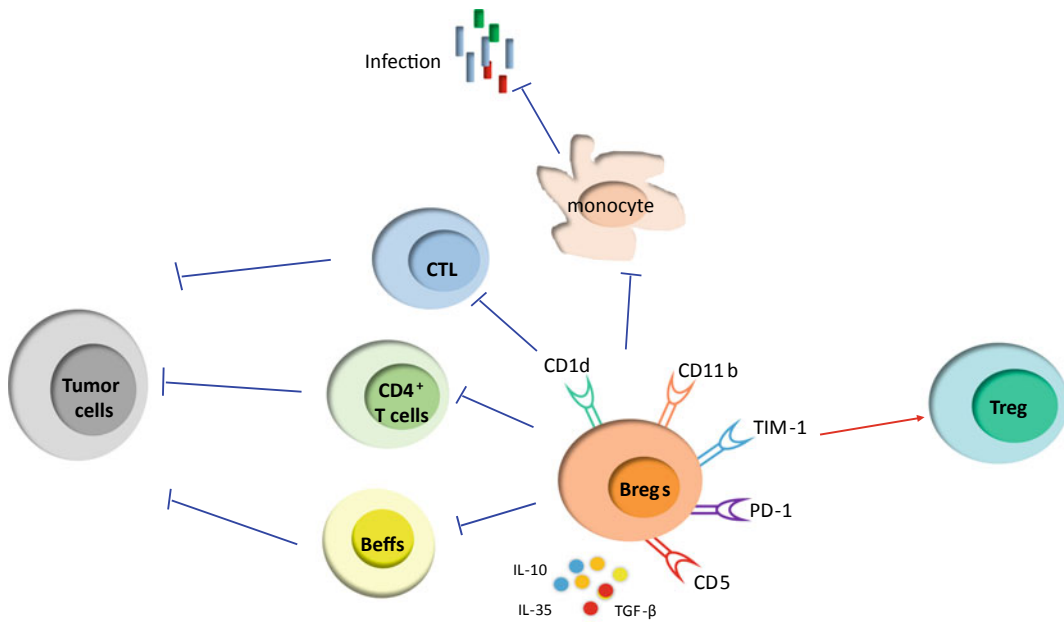

Fig. 8.2 Effects of Breg cells on other immune cells and tumor cells. Breg cells suppress the differentiation of monocytes, $CD4^+$ T cells, and cytotoxic T lymphocytes (CTLs), but promote Treg differentiation by secreting IL-10, IL-35, and TGF-β as well as through cell–cell contact, thereby inhibiting antitumor activity and inflammation

8.4.1 Cytokine-Producing Breg Cell

B cells release a variety of cytokines after stimulation (such as IL-4, IL-6, IL-10, IFN-γ, TNF-α, and TGF-β) and are classified into Breg or Beff subsets depending on cytokine release profile. Although the cellular origins of cytokine-producing B-cell subsets are still unclear and there are no definitive cell surface or transcription factor biomarkers for these cells, accumulating evidence indicates that B cells are critical for both innate and adaptive immune regulations (Bao and Cao 2014). Among them, B cells secreting IL-10, TGF-β, and IL-35 are defined as Breg cells.

It is now widely accepted that most primary Breg-cell functions are dependent on IL-10 expression and release. Indeed, several studies have used IL-10 expression as a marker for Breg cells, and the *il-10* reporter mouse has been used to study the phenotype and molecular mechanisms of immunomodulation by Breg cells. Fillatreau et al. first demonstrated that B cells regulate autoimmunity by IL-10 release. Subsequently, dozens of studies reported the role of IL-10 in B-cell regulation (Fillatreau et al. 2002). It has been reported that LAG-3, STIM1, and MYD88 are involved in mediating B-cell production of IL-10 (Kirkland et al. 2012; Lino et al. 2018; Matsumoto et al. 2011). However, other studies suggest IL-10-independent Breg-cell functions. Teichmann et al. performed lineage-specific deletion of *Il10* from B cells, and found that IL-10^+ B cells were rare in vivo and that Breg-cell phenotypic subsets, such as $CD1d^{hi}CD5^+$ and $CD21^{hi}CD23^{lo}$, were not enriched in *Il10* transcription (Teichmann et al. 2012), indicating that Breg-cell development does not rely on IL-10. These IL-10-independent mechanisms include promotion and maintenance of Tregs by production of TGF-β, IL-35, IgA, and adenosine as well as by surface expression of CD11b, Tim-1, PD-L1, and FasL (Ray et al. 2012, 2015; Wang et al. 2015; Zhao et al. 2019).

8.4.2 Regulatory Plasma Cells and Anti-Inflammatory Antibodies

Plasmablasts can also suppress inflammatory responses. Maseda et al. reported that splenic B10 cells differentiated into antibody-producing plasmablasts after stimulation in vivo and in vitro (Maseda et al. 2012). Mice deficient in B-cell *Irf4* and *Prdm1* expression exhibited defective plasma cell differentiation, and consequently developed exacerbated EAE (Matsumoto et al. 2014), again suggesting that some antibodies have anti-inflammatory functions. Moreover, IL-10- and IL-35-producing $CD138^+$ plasma cells suppressed pro-inflammatory responses during EAE and Salmonella infection (Shen et al. 2014). Matsumoto et al. suggested that human $CD19^+CD24^{hi}CD38^{hi}$ B cells are actually IL-10-producing plasmablasts (Matsumoto et al. 2014), and that $CD19^+CD24^{hi}CD27^+$ Breg cells belong to memory B cells. Antibodies can also induce immunoregulation by B cells, one of the earliest Breg studies found that antibodies can have regulatory functions in vivo (Morris and Moller 1968). IgG4 has been repeatedly implicated in B-cell regulatory function, and IgG4-expressing B cells are confined to the IL-10^+ B-cell subset in human subjects (Lin et al. 2014, 2017; van de Veen et al. 2013). Our group proposed a role for Breg-cell-derived IgA in maintaining mucosal immunity. In colitis, IgA-expressing B cells maintain intestinal homeostasis and suppress IBD (Wang et al. 2015). Alternatively, these cells may have deleterious effects in cancer by suppressing tumor inflammation, as IgA^+ B cells promoted colorectal tumors by inhibiting CTLs (Liu et al. 2017).

8.5 Cellular Targets of Breg Cell-Mediated Suppression

8.5.1 Suppression of Effector T Cells

Several disease model studies have demonstrated that controlling the overactivation of $CD4^+$ T cells is one of the most important regulatory functions of B cells (Lund and Randall 2010; Rosser et al. 2014). Adoptive transfer of Breg cells suppresses inflammatory responses and $CD4^+$ T-cell activation. For instance, adoptive transfer of $CD11b^+$ B cells to EAH model mice suppressed $CD4^+$ T-cell proliferation and IFN-γ/TNF-α production, ameliorating the disease (Liu et al. 2015). Moreover, Tim-1^+ Breg cells isolated from mice receiving MHC-mismatched islet allografts prolonged islet graft survival in secondary graft recipients (Ding et al. 2011). The same study reported that $CD4^+$ T cells from graft recipients that received Tim-1^+ Breg cells produced lower levels of IFN-γ and $CD4^+$ T polarization was tend to a type 2 response compared to T cells from control mice (Ding et al. 2011). Similarly, transfer of generated Breg cells which induced by GIFT-15 in vitro, suppressed the function of Teffs and consequently alleviate EAE symptoms (Rafei et al. 2009). In addition, Breg cells can also suppress inflammatory responses by inducing T-cell death. Studies revealed that after LPS stimulation, B cells express Fas ligand (FasL) and TGF-β, induces apoptosis of both B and T cells in diabetic environment (Tian et al. 2001). In addition, studies have found that B1 cells express FasL and target $CD4^+$ T cells for apoptosis in schistosomiasis, preventing the development of schistosome granulomatous disease (Lundy and Boros 2002).

Breg-cell inhibition of $CD8^+$ T cells and suppression of inflammation have been studied extensively in infectious diseases and cancers. In μMT mice, B cells can limit $CD8^+$ T-cell-mediated immune surveillance, resulting in more frequent tumorigenesis (Schioppa et al. 2011). For instance, B-cell-deficient mice were resistant to the development of breast tumors in mouse. The B-cell-deficient mouse has an increased CTL response to breast-tumor antigen, leading to effective clearance of tumor cells (Qin et al. 1998). Similarly, Liu et al. reported that IgA^+ B cells effectively inhibited CTLs, leaded to poor prognosis of colorectal tumors (Liu et al. 2017).

8.5.2 Enhancing the Production of Tregs

Breg cells can also promote the production and activity of Tregs (Chien and Chiang 2018), Breg and Treg cells cooperate to control inflammation in infection (Jeong et al. 2012) and diseases such as EAE (Matsushita et al. 2010). Reichardt et al. found that B-cell-induced Tregs, termed "bTregs," secreted IL-10 and suppressed inflammation. Further, Shao et al. found that $Foxp3^-IL\text{-}10^-$ bTregs suppress inflammation by expressing high levels of CTLA, GITR, ICOS, LAG3, and OX40, but not IL-10 (Shao et al. 2016). In addition to inducing naive T cells to differentiate into Tregs, B cells can also promote the proliferation of Tregs. In μMT mouse models of oral tolerance and arthritis, reconstitution of wild-type (WT) B cells restored $Foxp3^+$ Treg expansion (Carter et al. 2011; Sun et al. 2008). Moreover, B cells mitigate autoimmune diseases including IBD by promoting Treg proliferation through GITR ligand signaling (Ray et al. 2012; Shao et al. 2016; Wang et al. 2015). In mouse-tumor models, the absence of B lymphocytes reduced the number and function of Tregs, thus enhancing the antitumor response (Tadmor et al. 2011). The therapeutic effects of bTregs have been described in a variety of other mouse disease models, such as allergic asthma (Chu and Chiang 2012), rheumatoid arthritis (Chen et al. 2016), and breast cancer (Olkhanud et al. 2011). These functions in animal models of disease suggest that bTregs are a promising target for broad-based clinical treatments.

8.5.3 B Cells

In many Breg-cell-mediated immunosuppressive animal models, the production of pathogenic

antibodies is reduced, which may be the indirect effect of inhibiting helper T cells but may also be the result of Breg-cell acting directly on the antibody-secreting B cells (Rosser et al. 2014). In the rat model of cardiac transplantation, Breg cells inhibited the production of B-cell antibodies. Antibody unable to class switch from IgM to IgG, thus the graft can survive for a long time (Le Texier et al. 2011). Furthermore, adoptive transfer of Breg cells into graft-recipient mice prevented cardiac allograft rejection, and the suppression was related to the decrease in total IgG levels and increase in Th2-related antibody isotype IgG1 (Le Texier et al. 2011). In other animal models of Breg-cell-mediated immunosuppression, the transfer of Breg cells also elicits a similar type 2 antibody response (Evans et al. 2007; Gray et al. 2007; Mauri et al. 2003; Miles et al. 2012). These studies suggest that Breg cells can alter B-cell effector functions directly or indirectly via helper T cells.

8.5.4 Other Cell Targets of Breg Cell-Mediated Suppression

Breg cells have been shown to suppress IL-12 production by dendritic cells (DCs). Lenert et al. reported that MZ B cells from lupus mice produced larger amounts of IL-10 than WT mice, and IL-10 suppressed IL-12 production by total splenocytes in vitro (Lenert et al. 2005). Further studies have shown that CpG-stimulated $CD5^+$ B cells inhibit the response of neonatal DCS to IL-12 in an IL-10-dependent manner, thereby activating Th1 cells. This interaction helps prevent neonatal death by suppressing excessive inflammation after infection (Sun et al. 2005; Zhang et al. 2007). In addition, leishmania infection induces a large number of IL-10-producing B cells in vitro, which reduce IL-12 secretion through DCs (Ronet et al. 2010). In contrast to T cells and DCs, Breg-cell effects on monocytes and macrophages are largely uninvestigated. However, $CD19^{-/-}$ mice lacking $CD1d^{h}iCD5^+$ B cells demonstrated promoted phagocytosis of *Listeria monocytogenes* by macrophages, possibly due to the lack of a Breg-cell response. In vitro, LPS-activated $CD1d^{hi}CD5^+$ B cells suppressed macrophage produced nitric oxide, TNF-α, and IFN-γ production (Horikawa et al. 2013), which may explain the enhanced phagocytosis and cytotoxicity in the absence of Breg cells. This aspect of Breg-cell function warrants more intensive investigation. In tumor and infection models, Breg cells are able to suppress immune responses by natural killer (NK) cells (Bankoti et al. 2012; Inoue et al. 2006). The interaction between B cells and CD40L on tumor cells induces IL-10 production, consequently suppresses NK cell produced IFN-γ (Inoue et al. 2006). Reconstitution of WT B cells into μMT mice restored breast-tumor growth, while inhibited NK cell activity (Inoue et al. 2006). B cells can also inhibit the infiltration of $CD49^+$ NK cells by interacting with Treg cells, thereby preventing the effective elimination of tumor cells in and out of the body (Zhang et al. 2013). Breg-cell-mediated IL-10-dependent suppression of neutrophil responses has also been demonstrated in murine infection models (Neves et al. 2010). Both B-$Myd88^{-/-}$and B-$TLR2^{-/-}$chimeric mice showed defects in the IL-10 response of B cells and increased activity of neutrophils, NK cells and T cells, resulting in more effective clearance of *salmonella typhimurium.* In this model, there were more neutrophils and a high production of TNF-α (Neves et al. 2010).

8.6 Breg Cells in Diseases

Among the most interesting aspects of Breg-cell behavior is expansion and functional activation in disease-associated microenvironments, suggesting contributions to disease pathogenesis or amelioration and underscoring Breg cells as promising targets from immunotherapy.

8.6.1 Breg Cell in Autoimmune Diseases

As described before, the existence of Breg cells was first posited in an autoimmune disease model and their functions have been most extensively

studied in various autoimmune disease models and human patients (Rosser and Mauri 2015). B cells and plasma cells are considered the primary drivers of autoimmune diseases through the production of autoantibodies, so B-cell depletion and reduced autoantibody titers should theoretically be an effective treatment. However, such treatments have not demonstrated experimental or clinical success. In fact, Goetz et al. found that ulcerative colitis was exacerbated by depletion of peripheral B cells using rituximab (RTX) (Goetz et al. 2007), and Lehmann-Horn and colleagues found that B-cell depletion accentuated pro-inflammatory reactions in neuroimmunological disorders (Lehmann-Horn et al. 2011). These studies indicate that Breg cells are of vital importance for controlling autoimmune inflammation.

In terms of specific autoimmune diseases, Tedder and colleagues have examined the interactions of regulatory and pathogenic B cells in the EAE disease environment and how Treg and Breg cells contribute to disease progression (Matsushita et al. 2008, 2010). In IBD, the Breg subsets B-1 (Shimomura et al. 2008), $CD19^{+}CD5^{+}CD1d^{hi}$ (Yanaba et al. 2011), and CD11b (Wang et al. 2018) have strong inhibitory effects on autoimmune processes when transferred in vivo. Mauri and colleagues suggested that murine tissue-resident T2-MZP cells (Evans et al. 2007) and peripheral blood $CD19^{+}CD24^{+}CD38^{+}$ cells (Flores-Borja et al. 2013) are essential for arthritis remission (Mauri and Menon 2017). In addition, contributions of Breg cells to the amelioration of other autoimmune diseases such as autoimmune hepatitis (Liu et al. 2015) and thyroiditis (Zha et al. 2012) have been suggested.

8.6.2 Breg Cells in Cancer

In B-cell- or Breg-cell-deficient mice, enhanced antitumor immunity is associated with increased activity of CTLs (Liu et al. 2017) and NK cells (Terabe et al. 2005). The antitumor effects of immune cells are suppressed by IL-10 and TGF-β produced by B cells (Cai et al. 2019; Inoue et al. 2006; Liu et al. 2017). For instance, in ovarian cancer, Breg cells significantly suppressed IFN-γ production by CTLs via IL-10 release (Wei et al. 2016). Breg cells also promote the transformation of naïve T cells into Tregs through TGF-β release, which in turn inhibits the proliferation of CTLs and increases tumor metastasis (Olkhanud et al. 2011). In the mouse model of breast cancer, tumor-environment-induced Breg cells produced TGF-β and conversion of Teffs to Tregs, suppressing the proliferation of T cells and NK cells (Zhang et al. 2016). Similarly, PD-1 transforms B cells into Breg cells, resulting in the suppression of tumor-specific T cells and the promotion of human hepatoma growth (Ren et al. 2016). Also, tumor cells can convert normal B cells into Breg cells, inhibiting the antitumor immune process (Olkhanud et al. 2011). Nonmetastatic cancer cells express and utilize metabolites of the 5-lipoxygenase (5-LO) pathway to induce generation of Breg (Wejksza et al. 2013). Similarly, glioma cell-derived placenta growth factors (PlGFs) can induce Breg cells to suppress $CD8^{+}$ T-cell antitumor activities (Ye et al. 2014). Moreover, GrB^{+} Breg cells are found in many tumor microenvironments, where they contribute to escape from the antitumor immune response (Lindner et al. 2013). Collectively, these results suggest that Breg cells suppress immune responses to murine and human tumors and thus may contribute to carcinogenesis, progression, and metastasis.

8.6.3 Breg Cell in Transplantation

Breg-cell transplantation is a potential strategy for enhancing graft tolerance (Chu et al. 2018; Li et al. 2019a; Mohib et al. 2018; Wortel and Heidt 2017). In two recent clinical studies, a few patients with stable graft function for years after immunosuppressive drug withdrawal demonstrated greater absolute numbers and proportions of peripheral B cells compared to recipients with graft rejection (Chesneau et al. 2013; Viklicky et al. 2013). In addition, B-cell depletion with RTX can promote transplant rejection. For

example, B-cell depletion plus RTX treatment accelerated allograft rejection in a skin graft model (DiLillo et al. 2011; Marino et al. 2016). Alternatively, B cells reconstituted after depletion, differentiated into an immunosuppressive phenotype and promoted long-term survival of allogeneic islets in a nonhuman primate model (Liu et al. 2007). Moreover, the stimulated transitional B cells from graft-tolerant patients expressed higher levels of IL-10, suggesting that Breg cells may help prevent rejection following organ transplantation (Chesneau et al. 2013).

A number of experimental tolerogenic agents, including anti-TIM-1, anti-TIM-4, and anti-CD45, all require B cells for tolerance induction in mice (Ding et al. 2011, 2017; Lee et al. 2012). For instance, anti-TIM1 Ab induced transplant tolerance to allogeneic islets only when B cells were present in recipients (Lee et al. 2012). Furthermore, the synergistic effect of combining anti-CD45RB with anti-TIM1 mAbs for tolerance of allogeneic islets also depended on the presence of recipient B cells (Lee et al. 2012). Mice T2 B cells that tolerant to MHC-mismatched skin grafts expressed lower levels of CD86 and higher levels of TIM-1, and transfer of T2 B cells prolonged skin allograft survival by suppression T-cell activation (Moreau et al. 2015).

8.6.4 Breg Cell in Infection

Breg cells also modulate inflammatory responses to parasitic, viral, and bacterial infections. In *Babesia microti* infection, B cells produce more IL-10 and adoptive transfer of IL-10-producing B cells increases the susceptibility to infection (Jeong et al. 2012). The functions of Breg cells in bacterial infection have been investigated mainly using B-cell-deficient mouse models (lMT, JHD, or JHT). Breg cells can be either beneficial or deleterious for infection outcome due to functional diversity. For instance, Breg cells can either suppress anti-infective immunity and/or overactive cellular immunity (Fillatreau 2011). Virus such as HIV and HBV can induce the development of IL-10-producing B cells, which are able to inhibit effective anti-HIV-1 T-cell responses (Liu et al. 2014). Multiple subsets of Breg cell are induced by infection; for instance, peritoneal B-1 cells produce high levels of IL-10 upon stimulation by TLR ligands (Sindhava et al. 2010). Marginal zone B cells (MZBs) participate in the early immune response to several pathogens. Bankoti et al. (2012) reported that depletion of MZBs enhanced T-cell responses and led to insufficient resistance to parasitic infections.

8.6.5 Other Conditions

Pregnancy also influences the development and function of Breg cells. The pregnancy-associated hormones estradiol, progesterone, and human chorionic gonadotropin are known to suppress the capacity of both innate and adaptive immune cells (Guzman-Genuino and Diener 2017; Lima et al. 2016; Muzzio et al. 2014). In a murine model of pregnancy loss, Jensen et al. reported that $CD19^{+}CD5^{+}CD1d^{+}$ B cells were diminished in abortion-prone animals, while transfer of IL-10-producing Breg cells prevented fetal rejection (Jensen et al. 2013). This same group found that $CD19^{+}CD24^{hi}CD27^{+}$ Breg-cell number increased during the first trimester of human pregnancy, while cell numbers in spontaneous abortion patients remained as low as in non-pregnant women (Rolle et al. 2013). It is not yet clear why Breg-cell numbers change during pregnancy. B cells express receptors for hormones and are critical regulators of immune status during pregnancy. Further studies on the effects of pregnancy-associated hormones on regulatory B cells may facilitate novel preventative treatments for spontaneous abortion (Muzzio et al. 2014).

8.7 Breg Cell-Targeted Therapies

Studies on Breg cells in health and disease have provided compelling evidence for possible therapeutic applications. However, since the phenotype and transcription factors are not yet clear, there have been no clinical trials on Breg cell-based treatments. Several studies have shown that

RTX therapy induces repopulation of B10 cells. Sun et al. demonstrated that RTX therapy for MG delayed IL-10-producing cell repopulation (Sun et al. 2013). Targeting Breg cells may benefit multiple sclerosis and colitis (Goetz et al. 2007; Lehmann-Horn et al. 2013). Many studies in humans have shown significant changes in the number of Breg cells over the course of disease, suggesting that Breg cells are crucial to disease progression (Mauri and Blair 2014). Moreover, numerous studies in murine models clearly demonstrate that transferring B cells with induced regulatory function can effectively mitigate disease processes (Matsushita 2019; Zhao et al. 2019). Given this clinical potential, it is critical to identify biomarkers for more precise phenotypic analysis and purification of Breg cells.

8.8 Conclusions and Outstanding Questions

While Breg cells may have a myriad of clinical applications, clinical trials are hampered by the lack of reliable biomarkers and distinct phenotypes. In fact, Breg-cell subsets are most likely B-cell populations induced by specific inflammatory environments during disease. Therefore, there are many questions to be solved before such therapeutic applications can be considered, such as what biomarkers can be used for identification and purification, what transcription factors control differentiation, and the precise environmental conditions required for Breg-cell differentiation. Moreover, it must be determined if these processes are organ- and disease-specific. Also, the metabolic and epigenetic pathways involved in Breg-cell differentiation must be elucidated. Answers to these questions could facilitate the development of Breg-cell-based immunotherapies for a variety of diseases, including autoimmune disorders, other inflammatory disorders, and cancer.

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