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REVIEW

Suppressive mechanisms of regulatory B cells in mice and humans

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Abstract

B cells include immune-suppressive fractions, called regulatory B cells (Bregs), which regulate inflammation primarily through an interleukin 10 (IL-10)-mediated inhibitory mechanism. Several B-cell fractions have been reported as IL-10-producing Bregs in murine disease models and human inflammatory responses including autoimmune diseases, infectious diseases, cancer and organ-transplant rejection. Although the suppressive functions of Bregs have been explored through the hallmark molecule IL-10, inhibitory cytokines and membrane-binding molecules other than IL-10 have also been demonstrated to contribute to Breg activities. Transcription factors and surface antigens that are characteristically expressed in Bregs are also being elucidated. Nevertheless, defining Bregs is still challenging because their active periods and differentiation stages vary among disease models. The identity of the diverse Breg fractions is also under debate. In the first place, since regulatory functions of Bregs are mostly evaluated by *ex vivo* stimulation, the actual *in vivo* phenotypes and functions may not be reflected by the *ex vivo* observations. In this article, we provide a historical overview of studies that established the characteristics of Bregs and review the various suppressive mechanisms that have been reported to be used by Bregs in murine and human disease conditions. We are only part-way through but the common phenotypes and functions of Bregs are still emerging.

Keywords: B cells, interleukin 10, interleukin 35, TGFβ

Introduction

B cells are among the main players in the adaptive immune system. Their main functions in protecting against pathogens include antigen presentation, cytokine secretion and antibody production after differentiation into plasma cells (1). These mechanisms induce immune reactions resulting in inflammation and elimination of foreign antigens. X-linked agammaglobulinemia (XLA) is a genetic disorder caused by defects in Bruton's tyrosine kinase, which is essential for B-cell differentiation and results in the absence of antibody-producing plasma cells. Patients with XLA reveal recurrent bacterial and viral infections and need appropriate treatments such as antibiotics and immunoglobulin replenishment (2).

The clinical manifestations of XLA suggest the crucial precipitative function of B cells in the control of infection. On the other hand, disorders of B-cell functions also cause autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), diabetes mellitus (DM), autoimmune skin blistering diseases, multiple sclerosis (MS) and vasculitis (3, 4). These autoimmune conditions are regarded to correlate with overactivation of pathogenic B cells, and based on the historical concept of effector versus suppressor cells, the existence of B cells with suppressive functions towards the convergence of inflammation have been implied in maintaining healthy conditions.

In this review, we outline the history of studies that established the existence and characteristics of regulatory B cells (Bregs), and review the molecules related to regulatory function of Bregs [interleukin 10 (IL-10) and others] in various murine and human disease conditions.

Establishing the existence and characteristics of Bregs

The regulatory function of B cells was first reported in the 1970s in the delayed hypersensitivity reaction (DHS) model in guinea pigs (5–7). In these studies, cyclophosphamide and adoptive transfer of splenocytes depleted of B cells were used to diminish the dominance of B cells. The animals with a reduced ratio of B cells revealed a more intense and elongated DHS reaction compared with controls, suggesting the B cell-mediated suppression of T-cell activity. However, the detailed suppressive mechanism was not clarified.

Table 1. Breg subsets in murine models and humans, and the involved immune-suppressive molecules

	Organ	Fraction	Phenotype	Molecule	Model	Ref.
Mouse	Sp	B10	CD1d^{hi}CD5^{+}	IL-10	EAE	(30)
	Sp	B10	CD1d^{hi}CD5^{+}CD9^{+}	IL-10	CHS	(111)
	Sp	B10	CD1d^{hi}CD5^{+}	IL-10	CHS	(38)
	Sp	N/A	TIM-1^{+}	IL-10	EAE	(100)
	Sp	B10	CD1d^{hi}CD5^{+}TIM-1^{+}	IL-10	Transplantation	(98)
	Sp	T2	CD21^{hi}CD24^{hi}	IL-10	SLE	(129)
	Sp	T2MZP	CD23^{+}CD21^{hi} IgM^{+}	IL-10	CIA	(32)
	Sp	N/A	N.D.	IL-10	EAE	(16)
	LN	PB	CD138^{+}CD44^{hi}	IL-10	EAE	(28)
	Sp	PC/PB	CD138^{+}	IL-10	Infection	(45)
	Sp/LN	PB	CD19^{lo}CD138^{hi}CD44^{+}	IL-10	SLE	(130)
	Tumor	PC	CD138^{+}IgA^{+}PD-L1^{+}	IL-10	Tumor	(89)
	Sp	PC	CD138^{hi}CD1d^{int} TIM-1^{int}	IL-35 IL-10	Infection	(21)
	Sp	PC	LAG-3^{+}PD-L1^{+} PD-L2^{+}IgM^{+}	IL-10	Infection	(20)
	Sp	T2MZP	CD1d^{+}	CD1d	Arthritis	(94)
	Sp	N/A	PD-L1^{hi}	PD-L1	EAE	(87)
	N/A	N/A	N.D.	CD80/CD86	EAE	(92)
	Perit. cavity	B-1	N.D.	CD39/CD73	Colitis	(19)
	Sp	B-1/MZ/PC	N.D.	CD9	Normal	(110)
	Sp	B-1a	CD5^{+}	FasL	Infection	(82)
	LN	PC/other	N.D.	GABA	DTH, Tumor	(23)
	Sp	MZ	CD21^{hi}CD23^{lo}	N.D.	CHS	(35)
	Sp	N/A	N.D.	TGFβ	Transplantation	(18)
	Sp	N/A	FasL	TGFβ	DM	(65)
	N.D.	N/A	N.D.	TGFβ	EAE	(70)
Human	Bld	PB	CD27^{+}CD38^{hi}	IL-10	Normal	(28)
	Bld	Memory	CD24^{hi}CD27^{hi}	IL-10	Normal	(59)
	Bld	Immature	CD24^{hi}CD38^{hi}	IL-10	SLE	(46)
	Tumor	PC	CD138^{+}IgA^{+}PD-L1^{+}	IL-10	Tumor	(89)
	Bld	Memory	CD24^{hi}CD27^{+}CD39^{hi}	TIGIT	Normal	(24)
	Bld	N/A	CD19^{+}	CD39/CD73	Normal	(106)
	Bld	PB	CD24^{-}CD38^{+}	GzmB	Normal	(73)
	Bld	PB-like	CD38^{+}CD1d^{+} IgM^{+}CD147^{+}	GzmB	Tumor	(77)
	Bld	N/A	CD19^{+}	TGFβ	Normal	(71)
	Bld	Immature	CD24^{hi}CD38^{hi}	TIM-1	SSc	(101)

Sp, spleen; N/A, not applicable; N.D., not determined; LN, lymph node; PB, plasmablast; PC, plasma cell; Bld, blood.

In the 1990s, a B cell-deficient mouse strain μMT was established by disruption of the immunoglobulin μ chain gene (8). Mature B cells are absent in μMT mice because of the impaired differentiation of the B-cell lineage. In the same decade, the experimental autoimmune encephalomyelitis (EAE) model was established, which is mediated by CD4^{+} T cells and represents MS (9, 10). Although the severity of EAE was comparable between wild-type and μMT mice, it turned out that μMT mice showed longer disease duration than wild-type counterparts (11). It was thus suggested that B cells take part in the convergence of disease activity.

In the 2000s, progressive studies focusing on the suppressive mechanisms mediated by B cells were published. Chronic colitis and arthritis models, as well as EAE, were adapted in these studies, and the involvement of the same molecule—IL-10, an immune-regulatory cytokine (12, 13)—in the suppressive function of B cells was demonstrated (14–16). When the humanized anti-CD20 antibody rituximab (which depletes B cells) was used for the purpose of avoiding antibody-mediated rejection of kidney transplants, most of the recipients surprisingly developed acute T cell-mediated rejection (17). This observation also gave rise to ideas about the potential regulatory function of B cells.

Since then, several phenotypically variable B-cell fractions have been identified as IL-10-producing Bregs (14). Furthermore, other immune-suppressive molecules and IL-10-independent regulatory mechanisms of B cells have also been reported in both human and murine models (18–24). These Breg fractions are found in various differentiation stages and are distributed across a wide range of organs, either transiently or continuously, depending on different disease and experimental conditions. Their identity and fates, and the differences between human and murine models are yet to be fully elucidated. The reported Breg fractions, mediators and functions are summarized in Table 1 and Fig. 1.

IL-10-producing Bregs

Mouse IL-10-producing Bregs

IL-10 was first reported as a suppressive cytokine secreted from Th2 cells that inhibits cytokine production by Th1 cells (13, 25). μMT mice reconstructed with IL-10-deficient B cells

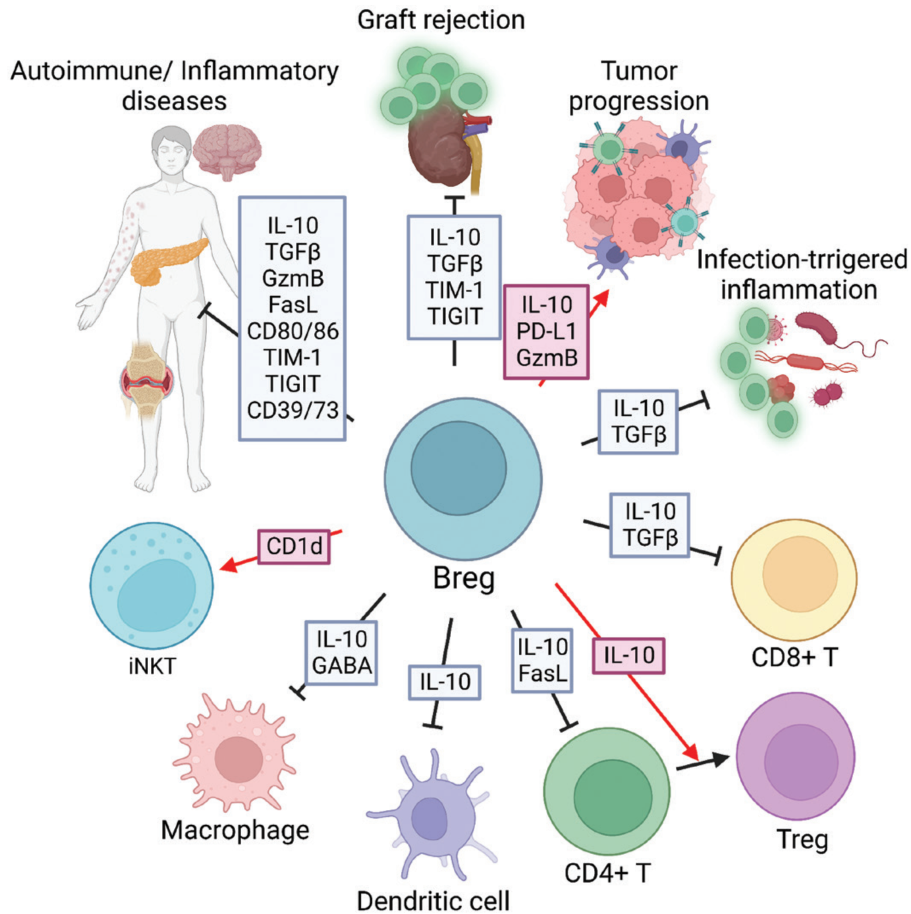

Fig. 1. Immune-suppressive molecules involved in Breg functions and their target cells. Breg definition depends on the expression of various immune-suppressive molecules. Their effects on disease conditions and on distinct immune cells are summarized.

revealed exacerbated arthritis compared with those reconstructed with wild-type B cells (26), demonstrating the importance of IL-10 production from B cells in controlling the disease activity.

The IL-10 signal is mediated by IL-10 receptor (IL-10R), a member of the interferon (IFN) receptor family. The activation of Jak1/Tyk2 that follows IL-10R signaling leads to the phosphorylation of the downstream STAT3. Although the IL-10 signaling cascade shares the STAT3-mediated signal transduction with the cascades activated by pro-inflammatory cytokines IL-6, IL-21 and IL-23, STAT3 phosphorylation by IL-10 signaling lasts longer than the activation by IL-6 and this difference in the duration of STAT3 activity generates diversity in gene transcription (27).

B cell-derived IL-10 suppresses T-cell activation by dendritic cells (DCs) (28) as well as directly affecting T-cell functions via IL-10R (29–31). Several B-cell fractions have been reported as being Bregs on the basis of IL-10 expression. Transitional 2-marginal zone precursor (T2MZP) B cells characterized by the phenotype $CD21^{hi}CD23^{hi}IgM^{+}$ in the spleen and lymph nodes are potent producers of IL-10 among B cells in a murine model of collagen-induced arthritis (CIA) (32). Marginal zone (MZ) B cells in spleen also produce IL-10 when activated by apoptotic cells via the Toll-like receptor 9 (TLR9)-mediated signaling cascade and these B cells are involved in the amelioration of CIA (33, 34). The exacerbated contact hypersensitivity reaction (CHS) in CD19-deficient mice can be ameliorated by wild-type MZ B cells, possibly by representing the Breg fraction (35).

B-1 cells predominate over B-2 (‘conventional’) cells in the peritoneal cavity. MZ B cells and B-1 cells possess innate immune characteristics, with a capacity to produce IL-10 potently upon innate stimulation (12, 36). IL-10-producing B-1 cells can also exert suppressive function in other organs such as skin by immigrating from the peritoneum (37). It is of note that both MZ B cells and B-1 cells include $CD5^{+}$ populations and they overlap with a population of $CD5^{+}CD1d^{hi}$ IL-10-producing B cells that were designated as ‘B10’ cells. B10 cells suppress the reactivity of $CD4^{+}$ T cells to IFNγ and tumor necrosis factor α (TNFα) and, through IL-10 production, the B10 cells diminish the differentiation of the T cells into Th17 cells (30, 36, 38, 39). CD5 itself also serves as a negative regulator of the B-cell receptor (BCR) signal (40).

Studying IL-10-producing Bregs in vitro

The major issue in determining the roles of these fractions as Bregs is that IL-10 production is evaluated after artificial *ex vivo* stimulation with lipopolysaccharides (LPS), with CpG motif-containing oligodeoxynucleotides (CpG) or with

phorbol myristate acetate (PMA) plus ionomycin, which may also affect the phenotypic characteristics of B cells. In addition, in many cases their regulatory functions are evaluated only by adoptive transfer into other mice during inflammatory disease conditions. It is thus unclear if these Bregs actually exert their regulatory function by IL-10 production *in vivo* (41–43). Given this situation, IL-10 reporter mice, in which IL-10-expressing cells are recognizable in the absence of stimulation, make it easier to evaluate IL-10 production from Bregs during the natural disease course (44). So far, plasma cells/plasmablasts are the only established lineages of B cells that reliably produce IL-10 without *ex vivo* stimulation (28, 42, 43).

IL-10-producing plasmablasts appear in the draining lymph nodes after the induction of EAE (28). IRF4, which is an essential transcription factor for plasmablast differentiation, is required for IL-10 production from these plasmablasts. Plasma cells in the spleen are also reported to produce IL-10 *in vivo* from day 1 of *Salmonella typhimurium* infection. In this infection model, the IL-10-producing phenotype of plasma cells is regulated by the MyD88-dependent pathway, and the produced IL-10 suppresses IFNγ secretion from natural killer cells (NK cells) and the accumulation of neutrophils in the site of infection (45). On the basis of these results, IL-10-producing plasmablasts/plasma cells are recognized to be involved in both adaptive and innate immune reactions.

Human IL-10-producing Bregs

In humans, the findings on IL-10-producing B cells are mostly restricted to those in peripheral blood. Immature transitional B cells ($CD19^{+}CD24^{hi}CD38^{hi}$) were the first reported human Breg fraction that produces IL-10 following CD40 stimulation (46). Their dysfunction is reported in several human immune-mediated diseases including RA (47), MS (48–50), SLE (46, 48, 51), type 1 DM (52), inflammatory bowel disease (53) and pemphigus vulgaris (54, 55). In SSc subjects, the decreased IL-10-producing Bregs and increased IL-6-producing effector B cells correlate with the severity of skin fibrosis and interstitial lung disease (56). Human Bregs are also recognized to play roles in infectious diseases. For example, the numbers of IL-10-producing B cells are elevated in the peripheral blood of the HIV-1-infected subjects and suppress the activity of HIV-1-specific T cells (57, 58).

Using another phenotypic definition, the $CD19^{+}CD24^{hi}CD27^{+}$ fraction is reported as representing human B10 cells because of its IL-10-producing potency although the differentiation status of human B10 and murine B10 cells does not necessarily overlap. B10 cell maturation into functional IL-10-secreting effector cells that inhibit *in vivo* autoimmune disease requires IL-21-dependent and CD40-dependent cognate interactions with T cells (30). The upregulation of IL-10 from this fraction, which downregulates Th1 and Th17 activity by affecting the production of inflammatory mediators from other cells such as TNFα from monocytes, has been demonstrated in autoimmune disorders including RA, SLE and skin blistering disorders (59). Although both $CD24^{hi}CD38^{hi}$ and $CD24^{hi}CD27^{+}$ B cells are able to produce IL-10 after stimulation with CD40 ligand (CD40L), CpG and anti-BCR antibody, and although both subsets suppress proliferation and proinflammatory cytokine production from $CD4^{+}$ T cells, the $CD24^{hi}CD27^{+}$ B cells exert more efficient regulatory function than $CD24^{hi}CD38^{hi}$ B cells, possibly due to higher expression levels of transforming growth factor β (TGFβ) and granzyme B (GzmB), as well as various cell surface integrins and CD39 in $CD24^{hi}CD27^{+}$ B cells (60).

Plasmablasts in the blood of healthy humans, which are possibly differentiated from mature naive or immature naive B cells, also secrete IL-10 and soluble IgM after stimulation with IL-2, IL-6, IFNα and CpG (28). Differentiation into $CD24^{hi}CD38^{hi}$ Bregs and $CD24^{-}CD38^{hi}$ plasmablasts is promoted by IFNα secreted from plasmacytoid DCs (61).

Whether the regulatory role of Bregs is antigen-specific or not is still controversial. CD40 stimulation without antigen and BCR interaction, which mimics the stimulation of bystander B cells, reportedly induces IL-10 from B cells, whereas CD40 plus BCR-mediated stimulation leads to the decreased production of IL-10 and the augmented production of IL-6, TNFα and lymphotoxin (62). In another study, stimulation mediated by CpG and CD40L alongside BCR cross-linking induces more IL-10 than CpG- and CD40L-mediated stimulation alone (60). Further detailed investigation is awaited.

Other regulatory molecules in Bregs

In addition to IL-10, other secreted mediators, cell surface molecules and signaling molecules have been reported as defining regulatory functions in B cells. However, as described below, in many cases their expression was evaluated in relation to the $IL-10^{+}$ Breg phenotypes and knowledge about IL-10-independent regulatory mechanisms is quite limited.

Interleukin 35

IL-35 is a heterodimeric cytokine composed of IL-12α and IL-27β (63). IL-35 serves as a suppressive factor of T-cell proliferation and contributes to the augmented regulatory function of regulatory T cells (Tregs). IL-35 promotes the conversion of B cells to Bregs with CD5 and/or T-cell immunoglobulin and mucin domain 1 (TIM-1) expression that also produces IL-35 besides IL-10. Adoptive transfer of Bregs induced by recombinant IL-35 ameliorates experimental autoimmune uveitis by inhibiting the pathogenic Th17 and Th1 cells while promoting Treg expansion (64) although the sites where they function have not been determined.

IL-35-producing B cells have also been identified as negative regulators in the EAE model. Mice in which IL-35 is specifically deleted in B cells fail to recover from the induced EAE. On the other hand, these mice obtain increased resistance to *S. typhimurium* infection. IL-35 is induced by B cells via TLR4 and CD40 signaling, and plasma cells are regarded as the IL-35-producing population in this model (21). The homology of IL-35- and IL-10-producing Bregs is yet to be elucidated.

In humans, the plasma levels of IL-35 level and the frequency of circulating $IL-35^{+}$ B cells are decreased in SLE and inversely correlate with disease activity (22). The detailed phenotype of the IL-35-producing B cells has not been identified.

Transforming growth factor β

The effect of TGFβ produced by B cells was first described in a type 1 DM model—non-obese diabetic (NOD) mice (65). TGFβ receptor signaling leads to the transcription of target genes via the complex of phosphorylated Smad2/3 and Smad4 (66,67), and TGFβ from B cells induces anergy in CD8^{+} T cells (68). TGFβ^{+}CD5^{+} Bregs in murine lymph nodes induce naive CD4^{+} T cells to differentiate into Tregs *in vitro* in a model of allergic airway disease (69). Immune tolerance in a murine transplantation model is also mediated by TGFβ from Bregs (18). Mice in which TGFβ is specifically depleted in B cells show an earlier onset of EAE (70) although, unlike IL-10 deficiency, TGFβ deficiency does not affect the elongation of inflammation, suggesting distinct regulatory functions of IL-10 and TGFβ.

TGFβ-producing Bregs have been demonstrated in humans, too. Human blood B cells stimulated with CpG suppress the proliferation of T cells and increase the expression of Foxp3 and CTLA-4 in Tregs via the TGFβ signaling pathway, independent of the IL-10 pathway (71). The phenotypical characteristics of TGFβ^{+} B cells have not been clarified.

Granzyme B

GzmB is a member of the serine protease family that induces apoptosis in target cells (72). The expression of GzmB in B cells has been explored in humans. IL-21 is regarded as the main stimulant of GzmB^{+} B cells with diminished CD40 signaling. The major GzmB^{+} B cells are plasmablasts in healthy human blood. Although *in vitro* plasmablast differentiation does not reproduce GzmB production, GzmB^{+} B cells induced by stimulation with IL-21, CD40L, anti-BCR antibodies, CpG and IL-2 suppress the proliferation of T cells in a GzmB-dependent and contact-dependent manner, independent of the IL-10 pathway (73).

In SLE subjects, GzmB is produced from CD5^{+} B cells and the viability and proliferation of this population is rather suppressed by IL-21 *in vitro* (74). As for their function, the degree of GzmB^{+} Breg impairment correlates with the disease activity in RA (75). These results suggest that impaired development and regulatory functions occur in GzmB^{+} B cells in autoimmune conditions.

Interestingly, untreated HIV subjects, whose CD4^{+} T cells highly express IL-21, have increased blood GzmB^{+} B-cell numbers. A subject with a mutation in NF-κB essential modulator, whose cells lack CD40 signaling, also shows GzmB production from the majority of blood B cells (76), leading to the suppression of T-cell proliferation. GzmB^{+} B cells have also been reported within tumor-infiltrating B cells. They show a CD38^{+}IgM^{+}CD1d^{+}CD5$^{+/-}$ plasmablast-like phenotype and are found adjacent to IL-21-producing Tregs. They in turn limit T-cell proliferation by degrading the T-cell receptor (TCR) ζ chain (77). GzmB^{+} Bregs also suppress Th1 and Th17 cells at least partially by degrading the TCR ζ chain and inducing T-cell apoptosis.

γ-*Aminobutyric acid*

Not only cytokines but also neurotransmitters serve as signaling molecules from B cells. In the DHS model, γ-aminobutyric acid (GABA) is produced by activated B cells in various sites—not only in draining lymph nodes but also in the spleen, bone marrow and Peyer's patches. IgA^{+} plasma cells also produce GABA. B cell-derived GABA induced monocytes to differentiate into anti-inflammatory macrophages, which suppress the activity of CD8^{+} T cells. In a tumor implant model, B cell-derived GABA promoted tumor growth by diminishing the activity of tumor-infiltrating macrophages and then T cells. The infiltration of B cells or plasma cells was limited in this tumor model, suggesting that GABA from B cells regulates these effector cells during the priming or migrating phase, possibly in the draining lymph nodes (23).

GABA production from B cells and IgA^{+} plasma cells is observed in human tonsils and renal cell tumors. The differentiation of human monocytes into macrophages was promoted by GABA and thus a similar mechanism is presumed in humans, too.

Fas ligand

Fas ligand (FasL, CD178) is a mediator of activation-induced cell death and is expressed mainly in cytotoxic cells including T cells and NK cells (78–80). Murine splenic B cells also express FasL after antigenic stimulation and IL-4/IL-10 from CD4^{+} T cells, and in turn exert a suppressive function by inducing the apoptosis of CD4^{+} T cells in a FasL-dependent manner. However, the activity of apoptosis induction is blocked by IL-4, suggesting an elaborate function of IL-4 not only taking part in upregulating FasL in B cells but also suppressing the cell-killing activity of FasL^{+} B cells (81). The potency of inducing apoptosis in CD4^{+} T cells is augmented in splenic B-1a cells during schistosome infection (82). On the other hand, CD8^{+} T cells are not directly suppressed by FasL on B cells (68).

Most, but not all, FasL^{+} B cells are found in the B10 population but do not always co-express IL-10. In autoimmune disease models, numbers of splenic FasL^{+}CD5^{+} B cells are reduced in the CIA model, and FasL in B cells correlates with a reduction of IL-17 production from antigen-specific T cells. On the other hand, overexpression of FasL in splenic B cells was reported in the MRL/*lpr* murine SLE model and these FasL^{+} B cells contribute to the cytotoxic destruction of Fas^{+} tissues (83). Similar to the other FasL^{+} immune cells, FasL can be a mediator of both effector and regulatory mechanisms in B cells, too. Actually, in humans, splenic FasL^{+}CD5^{+} B-cell numbers are increased in type 1 DM subjects accompanied with the reduction of IL-10^{+}CD5^{+} B cells, implying a positive role for FasL^{+} B cells in disease activity (84).

Programmed death-ligand 1

Programmed death-ligand 1 (PD-L1) is one of the ligands of programmed death 1 (PD-1). PD-1 is expressed on T cells and PD-1 signaling inhibits the activation and proliferation of T cells (85). In the murine EAE model, PD-L1^{hi} splenic Bregs regulate CD4^{+}CXCR5^{+}PD-1^{+} follicular helper T cells (Tfh cells), which are involved in B-cell activation and the generation of antibody-secreting plasma cells, through PD-L1–PD-1 signaling (86, 87). PD-L1^{hi} Bregs found in the spleen after immunization include both Blimp1^{low}CD138^{low} B cells

and Blimp1^{hi}CD138^{hi}B220^{low} plasma cells (87). B cells found in murine models of malignant tumors also express PD-L1 in addition to IL-10, IL-35 and TGFβ, and associate with the progression of tumors (88). In another study, IgA^{+}PD-L1^{+}IL-10^{+} plasma cells in the tumor microenvironment induce CD8^{+} T-cell exhaustion via PD-L1 and IL-10 in treatment-resistant prostate cancer both in humans and in murine models (89).

Programmed death 1

PD-1 expression has also been reported in association with human tumor-infiltrating Bregs. CD5^{hi}CD24$^{+/-}$CD27^{+}CD38^{dim}PD-1^{+} B cells, which upregulate PD-1 upon encountering PD-L1 via a BCL6-mediated pathway, are involved in tumor progression in advanced-stage hepatocellular carcinoma subjects by suppressing the activity of tumor-specific T cells via IL-10 signals (88). As another fraction, PD-1^{+} Bregs that were found to be increased in untreated thyroid tumor subjects suppressed the proliferation of T cells via the PD-1–PD-L1 pathway, independent of IL-10. This population is rare in the blood circulation and is further reduced after tumor treatment (90), suggesting its association with tumor activity.

CD80/CD86

CD80 and CD86 (B7.1 and B7.2) are expressed on B cells and are co-stimulatory molecules that bind to CD28 on T cells (91). In the murine EAE model, Bregs induce Tregs to migrate into the central nervous system through CD80/CD86–CD28 interaction and resolve the inflammation via IL-10 from Tregs (92). It is of note that the induction of Tregs was delayed in the central nervous system but not in cervical lymph nodes by B-cell deficiency in this model. However, since these findings were based on cell-transfer experiments, the priming sites of Tregs could not be determined.

In humans, blood CD19^{+}CD24^{hi}CD38^{hi} Bregs were demonstrated to inhibit the differentiation of T cells into Th1 cells *in vitro* and this suppressive mechanism is mediated by IL-10 and CD80/CD86 signaling (46). Further regulatory mechanisms have not been disclosed yet.

CD1d

CD1d, which is a major histocompatibility complex (MHC) class I (MHC I)-like molecule, presents lipid antigens to invariant NK T cells (iNKT cells) via an invariant TCR (93). Bregs include a CD1d^{hi} fraction, and lipid-antigen presentation by CD1d^{hi} splenic Bregs activates iNKT cells and downregulates Th1 and Th17 cell activation in terms of adaptive immune responses in a murine arthritis model, partially via IFNγ production from iNKT cells (94). Although CD1d is a definitive marker for B10 cells, the expression of CD1d seems to be dispensable for IL-10 production from CD5^{+} B cells (95). This observation also supports an independent regulatory role for CD1d in Bregs.

T-cell immunoglobulin and mucin domain 1

TIM-1 is preferably expressed on Th2 cells and is proposed to have dual positive and negative immunoregulatory roles (96, 97). In murine spleen, the TIM-1^{+} B-cell population largely overlaps with IL-10-secreting B cells. In a murine islet-transplantation model, TIM-1^{+} B cells participate in suppressing immune rejection accompanied by high production levels of IL-4 and IL-10. A low-affinity anti-TIM-1 antibody that promotes immune tolerance augments the frequency and regulatory function of TIM-1^{+} B cells (98) although the functioning sites of TIM-1^{+} B cells is not determined.

Another report shows the induction of IL-10^{+} antigen-specific Bregs by anti-TIM-1 and anti-CD45RB antibodies in the same islet-transplantation model (99). TIM-1 deficient mice reveal a loss of IL-10 production in B cells and upregulated Th1 and Th17 reactions. IL-10 production from splenic B cells by administration of apoptotic cells is mediated by binding of TIM-1 and phosphatidyl serine on apoptotic cells, and is involved in ameliorating EAE severity (100). On the basis of these observations, TIM-1 can be a mediator inducing IL-10-producing Bregs.

In humans, CD24^{hi}CD38^{hi} B cells from peripheral blood preferably express TIM-1 (42,101), and a skew in frequency and function of TIM-1^{+} B cells was reported in relation to SSc and to allograft rejection in kidney transplantation. The regulatory function of TIM-1 occurs via STAT3 signaling, which also overlaps with IL-10 signaling, and TIM-1^{+} B cells are also enriched within the peripheral blood from cutaneous squamous cell carcinoma subjects (102).

T-cell immunoreceptor with Ig and ITIM domains

The role of T-cell immunoreceptor with Ig and ITIM domains (TIGIT) has been explored on T cells and NK cells. TIGIT works as a checkpoint receptor by competing with a co-stimulatory immunoreceptor CD226 for the ligands CD155 and CD112 (103). Mice with TIGIT specifically deleted in B cells develop inflammation in the central nervous system with infiltration of Th1 and Th17 cells. The expression of TIGIT is enriched on TIM-1^{+} B cells, and aryl hydrocarbon receptor regulates the expression of TIGIT and IL-10 in TIM-1^{+} B cells (104). Thus, TIGIT expression can also be downstream of TIM-1 signaling. TIGIT expression does not totally overlap with IL-10 production in B cells, suggesting that the inhibitory function of TIGIT is independent of IL-10.

In humans, CD19^{+}CD24^{hi}CD27^{+}CD39^{hi}IgD^{-}IgM^{+}CD1c^{+} blood memory B cells express TIGIT. In renal and liver allograft subjects, a lack or decrease of TIGIT^{+} memory B cells is associated with increased donor-specific antibodies and Tfh cell responses, and decreased Treg responses (24), suggesting some regulatory function of TIGIT^{+} memory B cells in humans, too.

CD39/CD73

Whereas extracellular ATP works as a co-stimulatory factor on T cells, extracellular adenosine has an immunosuppressive function via adenosine receptor signaling (105). CD39 hydrolyzes extracellular ATP and ADP to AMP. CD73 is one of the ecto-5′-nucleotidases and catalyzes the conversion of extracellular AMP to adenosine. Both CD39 and CD73 are expressed on Tregs and coordinately work to generate immunosuppressive adenosine pericellularly (105). CD39 expression is also generally found on murine splenic B cells. On the other hand, high expression of CD73 is restricted to

Table 2. Breg subsets in human disease conditions

	Organ	Fraction	Phenotype	Molecule	Ref.
SLE	Bld	Immature	$CD24^{hi}CD38^{hi}$	IL-10	(46)
	Bld	N/A	N.D.	IL-10, IL-35	(22)
MS	Bld	Immature	$CD24^{hi}CD38^{hi}$	IL-10	(116)
SSc	Bld	Immature	$CD24^{hi}CD38^{hi}$,	IL-10	(115)
	Bld	B10	$CD27^{+}CD24^{hi}$	IL-10	(131)
	Bld	Immature	$CD24^{hi}CD38^{hi}$	TIM-1	(101)
RA	Bld	N/A	N.D.	GzmB	(75)
T1DM	Bld	Immature	$CD24^{hi}CD38^{hi}$,	IL-10	(52)
	Sp	N/A	$FasL^{hi}CD5^{+}$	IL-10	(117)
PV, BP	Bld	Immature	$CD24^{hi}CD38^{hi}$	IL-10	(54, 55)
IBD	Bld	N/A	N.D.	IL-10	(53)
Atopic dermatitis	Bld	N/A	N.D.	IL-10	(119)
Psoriasis	Bld	Immature	$CD24^{hi}CD38^{hi}$	IL-10	(118)
Infectious disease	Bld	N/A	N.D.	IL-10	(57)
Cancer	Tumor	PC	$CD138^{+}IgA^{+}PD\text{-}L1^{+}$	IL-10	(89)
	Bld	N/A	N.D.	IL-35	(124)
	Bld	PB-like	$CD38^{+}CD1d^{+}$ $IgM^{+}CD147^{+}$	GzmB	(77)
Transplant reject	Bld	N/A	N.D.	TIM-1	(42)

Bld, blood; N/A, not applicable; N.D., not determined; T1DM, type 1 diabetes mellitus; Sp, spleen; PV, pemphigus vulgaris; BP, bullous pemphigoid; IBD, inflammatory bowel disease; PC, plasma cell; PB, plasmablast.

splenic B10 cells and peritoneal B-1 cells under stable conditions. Whereas IL-10 production from B cells is not affected by CD73 deficiency, CD73 expression is diminished in IL-10 deficient B cells (19), suggesting that CD73 expression on B cells occurs in an IL-10-dependent manner. However, the actual regulatory mechanisms mediated by CD39/CD73 on B cells have not been clarified.

Most healthy human peripheral blood B cells express both CD39 and CD73. B cells activated *in vitro* by CD40L and IL-4 increase CD39 expression and suppress proliferation and cytokine production in T cells. In this stimulation model, CD73 is downregulated in the activated B cells, suggesting that not only adenosine but also AMP generated by the activated B cells has a suppressive function on T cells (106). Adenosine produced by CD39/CD73 can also cause autocrine regulation in B cells themselves through the adenosine receptor. Increased CD39 expression on blood B cells has been reported in RA subjects after successful treatments (107) although the causative roles of CD39 downregulation in disease activity have not been addressed. Since CD39/CD73 expression is also related to IL-10 production and TIGIT/TIM-1 expression in B cells from tonsils and peripheral blood (24), the independent roles of CD39/CD73 from other regulatory molecules are still obscure.

Lymphocyte-activation gene 3

Lymphocyte-activation gene 3 (LAG-3, CD223) has been regarded as a CD4-related molecule that binds to the stable complex of MHC II and the peptide presented by antigen-presenting cells. The binding affinity of LAG-3 to MHC II is higher than that of CD4, and LAG-3 suppresses the activation of T cells by inhibiting MHC-II–CD4 interaction (108, 109). $CD138^{+}LAG\text{-}3^{+}$ IL-10-producing plasma cells exist among naive murine spleen cells and are named as natural regulatory plasma cells. However, the direct involvement of LAG-3 in immune regulation has not been identified (20).

CD9

A tetraspanin family member, CD9 is expressed in MZ B cells, B-1 cells and plasma cells (110). $CD9^{+}$ B cells are abundant among IL-10-expressing B cells (111), and the co-expression of CD80 and CD9 is regarded as characteristic of murine splenic B10 and MZ cells (112). $CD9^{+}$ B cells suppress T-cell proliferation *in vitro* and also suppress *in vivo* ear swelling during CHS, as assessed by a cell-transfer experiment. However, CD9-deficient mice reveal no abnormality in B-cell development or humoral immunity (113, 114), and the detailed function of CD9 in B-cell immunoregulation is not clear yet.

Bregs and human diseases

As above, the function of Bregs has been explored from the aspect of their dysfunction in immune-mediated conditions including autoimmune/inflammatory disorders, infectious diseases, cancer, and organ-transplant rejection. The reported characteristics of Bregs in human disease conditions are summarized in this section and in Table 2.

Autoimmune/inflammatory disorders

In the context of autoimmune disorders, impaired IL-10 production from $CD24^{hi}CD38^{hi}$ Bregs in peripheral blood has mainly been explored in SLE, MS, SSc and autoimmune skin-blistering disorders (46, 48, 54, 55, 115). Impairment of TIM-1 expression from this fraction is also suggested in SSc (101). As for the other fractions, the upregulation of IL-10 from $CD27^{+}CD24^{hi}$ Bregs in autoimmune disorders SLE, RA, MS, Sjögren syndrome and skin-blistering disorders is regarded as the reactive consequence of systemic inflammation (59). On the other hand, diminished IL-10 production is reported from $CD27^{+}CD24^{hi}$ Bregs in SSc (116), and the increased splenic $FasL^{hi}CD5^{+}$ fraction inversely correlates with the downregulation of IL-10 from $CD5^{+}$ B cells in type 1 DM

(117). However, the other observations on Bregs have not strictly defined the subpopulation of B cells with regulatory fractions. Although the decline of IL-35 in SLE and GzmB in RA is suggested to inversely correlate with disease severity, these reports are based on the skewed development of B-cell fractions and serum concentration of the targeted molecules (22, 75). Considering the possibility that the profile of Bregs is affected by the severity and the phase of diseases, further investigation on the time course changes of Breg fractions and their acting regulatory molecules is awaited.

In addition to these autoimmune disorders, the impairment of Bregs is reported in inflammatory disorders such as inflammatory bowel diseases, psoriasis and atopic dermatitis (53, 118, 119). Again, in these disease conditions, the results are restricted to the impaired IL-10 expression in either $CD24^{hi}CD38^{hi}$ Bregs or bulk B cells.

Infectious diseases

The role of IL-10-producing Bregs is suggested in persistent viral infection. In chronic hepatitis B virus (HBV) infection, the frequency of IL-10-producing B cells, which are predominantly $CD24^{hi}CD38^{hi}$ Bregs, parallels hepatic flares (120). They suppress the activity of HBV-specific CD8 T cells via IL-10. The enhancement of Tregs is also reported as a function of Bregs in HBV infection (121). The suppressive role of $CD24^{hi}CD38^{hi}$ Bregs on virus-specific T cells was shown in HIV-1-infected subjects (57, 58), too. IL-10 from the $TIM\text{-}1^{+}$ fraction (58) and the involvement of PD-L1 was suggested to be responsible for the suppression of T-cell activity (57). As a parasite, *Schistosoma Haematobium* infection induces the production of IL-10 and TGFβ in $CD1d^{hi}$ B cells, which leads to reduced T-cell activity (122). Although these infectious conditions are chronic, the antigen specificity of Bregs themselves has not been clarified.

As for an acute infectious condition, $CD10^{-}CD5^{+}$ neonatal Bregs with polyreactive BCRs upregulate IL-10 production after infection with respiratory syncytial virus (RSV) and correlate with diminished memory Th1 activity (123). It is possible that this neonatal Breg fraction can serve as first-line immune modulation independently of pathogen memory.

Cancer

The involvement of Bregs in cancer progression has mostly been evaluated in the cancer microenvironment as tumor-infiltrating Bregs. The reported Breg fractions are thus in a later differentiation stage, such as memory B cells or plasmablasts/plasma cells, which potentially have tissue-tropism (89). In addition to IL-10, IL-35 and TGFβ secretion, $PD^{-}L1^{+}$ plasma cells and $PD\text{-}1^{+}$ memory B cells correlate with tumor progression in prostate cancer and hepatocellular carcinoma, respectively (88, 90). GzmB from tumor-infiltrating plasmablast-like Bregs suppress T-cell functions and induce T-cell apoptosis, leading to tumor progression (77).

Although the evaluation of peripheral blood Bregs is limited, the increase in $IL\text{-}35^{+}$ B cells and $TIM\text{-}1^{+}$ B cells has been suggested in gastric cancer and cutaneous squamous cell carcinoma subjects, respectively (102, 124). However, their acting points in the tumor progression have not been clarified yet.

Organ-transplant rejection

Maintenance of tolerance in transplant recipients is one of the major factors for successful organ transplantation. A regulatory phenotype of B cells was shown to be associated with tolerance in kidney transplant subjects (125–128). Generally, $CD24^{hi}CD38^{hi}$ B cells and naive B cells are increased, and plasma cells are decreased in tolerant recipients, and IL-10 production from B cells associates with immune tolerance (127). The expression of ICOS-L and CD1b in $CD24^{hi}CD38^{hi}$ B cells coincides with IL-10 production, implying the involvement of IL-10-independent regulatory pathways (128). The evaluation of the graft-infiltrating memory B cells or plasmablasts/plasma cells with a potential of exerting regulatory functions is awaited.

Conclusion

Although IL-10 is the most well-studied inhibitory cytokine in the Breg fraction, other inhibitory mechanisms independent of IL-10 have also been found in B cells. However, because these molecules have been evaluated mainly in relation to the traditional Breg phenotypes or IL-10-producing signaling pathways, their independent regulatory mechanisms are still to be revealed. It is also of note that the reported inhibitory mechanisms in Bregs could be shared with those in different immune cells and in interactions between them, and these mechanisms have been demonstrated to not only function alone but also work in concert with other inhibitory cascades.

Bregs are well-analyzed in the spleen, lymph nodes, peritoneal cavity and peripheral tissues including inflamed gut, skin, tumor sites and transplants from murine models, and in peripheral blood and peripheral tissues from humans. Thus, the evaluation of murine Breg equivalents in humans is impossible in many cases. In addition, the timings of Breg emergence, their phenotypes and the involved organs are diverse depending on the pathological conditions. However, the accumulated studies both in murine models and humans imply that the characteristics of B cells in the analyzed lymphoid tissues can reflect those in the involved peripheral organs. Further multifaceted studies would help clarify the specific Breg phenotypes and functions in a wide range of disease conditions including autoimmune disorders, infectious diseases and malignancies.

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References

1 LeBien, T. W. and Tedder, T. F. 2008. B lymphocytes: how they develop and function. *Blood* 112:1570.
2 Suri, D., Rawat, A. and Singh, S. 2016. X-linked agammaglobulinemia. *Indian J. Pediatr.* 83:331.
3 Mauri, C. and Menon, M. 2017. Human regulatory B cells in health and disease: therapeutic potential. *J. Clin. Invest.* 127:772. doi:10.1172/JCI85113.
4 Matsushita, T. 2018. Regulatory and effector B cells: friends or foes? *J. Dermatol. Sci.* 93:2.

5 Turk, J. L., Parker, D. and Poulter, L. W. 1972. Functional aspects of the selective depletion of lymphoid tissue by cyclophosphamide. *Immunology* 23:493.
6 Katz, S. I., Parker, D. and Turk, J. L. 1974. B-cell suppression of delayed hypersensitivity reactions. *Nature.* 251:550.
7 Neta, R. and Salvin, S. B. 1974. Specific suppression of delayed hypersensitivity: the possible presence of a suppressor B cell in the regulation of delayed hypersensitivity. *J. Immunol.* 113:1716.
8 Kitamura, D., Roes, J., Kühn, R. *et al.* 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature* 350:423. doi:10.1038/350423a0.
9 Martin, R. and McFarland, H. F. 1995. Immunological aspects of experimental allergic encephalomyelitis and multiple sclerosis. *Crit. Rev. Clin. Lab. Sci.* 32:121. doi:10.3109/10408369509084683.
10 Viskochil, D., Cawthon, R., O'Connell, P. *et al.* 1991. The gene encoding the oligodendrocyte-myelin glycoprotein is embedded within the neurofibromatosis type 1 gene. *Mol. Cell. Biol.* 11:906. doi:10.1128/mcb.11.2.906-912.1991.
11 Wolf, S. D., Dittel, B. N., Hardardottir, F. *et al.* 1996. Experimental autoimmune encephalomyelitis induction in genetically B cell–deficient mice. *J. Exp. Med.* 184:2271. doi:10.1084/jem.184.6.2271.
12 O'Garra, A., Chang, R., Go, N. *et al.* 1992. Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. *Eur. J. Immunol.* 22:711. doi:10.1002/eji.1830220314.
13 Saraiva, M., Vieira M., O'Garra P. 2020. Biology and therapeutic potential of interleukin-10. *J. Exp. Med.* 217:e20190418.
14 Mizoguchi, A., Mizoguchi, E., Takedatsu, H. *et al.* 2002. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity* 16:219.
15 Mauri, C., Gray, D., Mushtaq, N. *et al.* 2003. Prevention of arthritis by interleukin 10–producing B cells. *J Exp. Med.* 197:489.
16 Fillatreau, S., Sweenie, C. H., McGeachy, M. J. *et al.* 2002. B cells regulate autoimmunity by provision of IL-10. *Nat. Immunol.* 3:944.
17 Clatworthy, M. R., Watson, C. J., Plotnek, G. *et al.* 2009. B-cell-depleting induction therapy and acute cellular rejection. *N. Engl. J. Med.* 360:2683.
18 Lee, K. M., Stott, R. T., Zhao, G. *et al.* 2014. TGF-beta-producing regulatory B cells induce regulatory T cells and promote transplantation tolerance. *Eur. J. Immunol.* 44:1728.
19 Kaku, H., Cheng, K. F., Al-Abed, Y. *et al.* 2014. A novel mechanism of B cell-mediated immune suppression through CD73 expression and adenosine production. *J. Immunol.* 193:5904.
20 Lino, A. C., Dang, V. D., Lampropoulou, V. *et al.* 2018. LAG-3 inhibitory receptor expression identifies immunosuppressive natural regulatory plasma cells. *Immunity* 49:120.
21 Shen, P., Roch, T., Lampropoulou, V. *et al.* 2014. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 507:366.
22 Ye, Z., Jiang, Y., Sun, D. *et al.* 2019. The plasma interleukin (IL)-35 level and frequency of circulating IL-35(+) regulatory B cells are decreased in a cohort of Chinese patients with new-onset systemic lupus erythematosus. *Sci. Rep.* 9:13210. doi:10.1038/s41598-019-49748-z.
23 Zhang, B., Vogelzang, A., Miyajima, M. *et al.* 2021. B cell-derived GABA elicits IL-10(+) macrophages to limit anti-tumour immunity. *Nature* 599:471.
24 Hasan, M. M., Nair, S. S., O'Leary, J. G. *et al.* 2021. Implication of TIGIT(+) human memory B cells in immune regulation. *Nat. Commun.* 12:1534.
25 Fiorentino, D. F., Bond, M. W. and Mosmann, T. R. 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 170:2081.
26 Carter, N. A., Vasconcellos, R., Rosser, E. C. *et al.* 2011. Mice lacking endogenous IL-10-producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells. *J. Immunol.* 186:5569.
27 Braun, D. A., Fribourg, M. and Sealfon, S. C. 2013. Cytokine response is determined by duration of receptor and signal transducers and activators of transcription 3 (STAT3) activation. *J. Biol. Chem.* 288:2986.
28 Matsumoto, M., Baba, A., Yokota, T. *et al.* 2014. Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation. *Immunity* 41:1040.
29 Matsushita, T., Horikawa, M., Iwata, Y. *et al.* 2010. Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune encephalomyelitis initiation and late-phase immunopathogenesis. *J. Immunol.* 185:2240.
30 Yoshizaki, A., Miyagaki, T., DiLillo, D. J. *et al.* 2012. Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. *Nature* 491:264.
31 Yang, M., Deng, J., Liu, Y. *et al.* 2012. IL-10-producing regulatory B10 cells ameliorate collagen-induced arthritis via suppressing Th17 cell generation. *Am. J. Pathol.* 180:2375.
32 Evans, J. G., Chavez-Rueda, K. A., Eddaoudi, A. *et al.* 2007. Novel suppressive function of transitional 2 B cells in experimental arthritis. *J. Immunol.* 178:7868.
33 Gray, M., Miles, K., Salter, D. *et al.* 2007. Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. *Proc. Natl. Acad. Sci. U.S.A.* 104:14080. doi:10.1073/pnas.0700326104.
34 Miles, K., Heaney, J., Sibinska, Z. *et al.* 2012. A tolerogenic role for Toll-like receptor 9 is revealed by B-cell interaction with DNA complexes expressed on apoptotic cells. *Proc. Natl. Acad. Sci.* 109:887.
35 Watanabe, R., Fujimoto, M., Ishiura, N. *et al.* 2007. CD19 expression in B cells is important for suppression of contact hypersensitivity. *Am. J. Pathol.* 171:560.
36 Zhang, X. 2013. Regulatory functions of innate-like B cells. *Cell. Mol. Immunol.* 10:113. doi:10.1038/cmi.2012.63.
37 Geherin, S. A., Gomez, D., Glabman, R. A. *et al.* 2016. IL-10+ innate-like B cells are part of the skin immune system and require alpha4beta1 integrin to migrate between the peritoneum and inflamed skin. *J. Immunol.* 196:2514.
38 Yanaba, K., Bouaziz, J. D., Haas, K. M. *et al.* 2008. A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity* 28:639.
39 Tedder, T. F. 2015. B10 cells: a functionally defined regulatory B cell subset. *J. Immunol.* 194:1395.
40 Burgueno-Bucio, E., Mier-Aguilar, C. A. and Soldevila, G. 2019. The multiple faces of CD5. *J. Leukoc. Biol.* 105:891.
41 Fillatreau, S. 2018. Natural regulatory plasma cells. *Curr. Opin. Immunol.* 55:62. doi:10.1016/j.coi.2018.09.012.
42 Cherukuri, A., Mohib, K and Rothstein, D. M. 2021. Regulatory B cells: TIM-1, transplant tolerance, and rejection. *Immunol. Rev.* 299:31.
43 Mohib, K., Cherukuri, A., Zhou, Y. *et al.* 2020. Antigen-dependent interactions between regulatory B cells and T cells at the T:B border inhibit subsequent T cell interactions with DCs. *Am. J. Transplant.* 20:52.
44 Atarashi, K., Tanoue, T., Shima, T. *et al.* 2011. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* 331:337.
45 Neves, P., Lampropoulou, V., Calderon-Gomez, E. *et al.* 2010. Signaling via the MyD88 adaptor protein in B cells suppresses protective immunity during Salmonella typhimurium Infection. *Immuniy* 33:777.
46 Blair, P. A., Norena, L. Y., Flores-Borja, F. *et al.* 2010. CD19(+) CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity* 32:129.
47 Flores-Borja, F., Bosma, A., Ng, D. *et al.* 2013. CD19+CD24hiCD38hi B cells maintain regulatory T cells while limiting TH1 and TH17 differentiation. *Sci. Transl. Med.* 5:173.
48 Duddy, M., Niino, M., Adatia, F. *et al.* 2007. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J. Immunol.* 178:6092.
49 Ray, A., Mann, M. K., Basu, S. *et al.* 2011. A case for regulatory B cells in controlling the severity of autoimmune-mediated inflammation in experimental autoimmune encephalomyelitis and multiple sclerosis. *J. Neuroimmunol.* 230:1.
50 Knippenberg, S., Peelen, E., Smolders, J. *et al.* 2011. Reduction in IL-10 producing B cells (Breg) in multiple sclerosis is

accompanied by a reduced naive/memory Breg ratio during a relapse but not in remission. *J. Neuroimmunol.* 239:80.
51 Fillatreau, S., Gray, D. and Anderton, S. M. 2008. Not always the bad guys: B cells as regulators of autoimmune pathology. *Nat. Rev. Immunol.* 8:391.
52 Wang, Y., Qin, Y., Wang, X. *et al.* 2020. Decrease in the proportion of CD24(hi) CD38(hi) B cells and impairment of their regulatory capacity in type 1 diabetes patients. *Clin. Exp. Immunol.* 200:22.
53 Oka, A., Ishihara, S., Mishima, Y. *et al.* 2014. Role of regulatory B cells in chronic intestinal inflammation: association with pathogenesis of Crohn's disease. *Inflamm. Bowel Dis.* 20:315. doi:10.1097/01.MIB.0000437983.14544.d5.
54 Zhu, H. Q., Xu, R. C., Chen, Y. Y. *et al.* 2015. Impaired function of CD19+CD24hiCD38hi regulatory B cells in patients with pemphigus. *Br. J. Dermatol.* 172:101.
55 Kabuto, M., Fujimoto, N., Takahashi, T. *et al.* 2017. Decreased level of interleukin-10-producing B cells in patients with pemphigus but not in patients with pemphigoid. *Br. J. Dermatol.* 176:1204. doi:10.1111/bjd.15113.
56 Horii, M., Fushida, N., Ikeda, T. *et al.* 2022. Cytokine-producing B-cell balance associates with skin fibrosis in patients with systemic sclerosis. *J. Dermatol.* 49:1012.
57 Liu, J., Zhan, W., Kim, C. J. *et al.* 2014. IL-10-producing B cells are induced early in HIV-1 infection and suppress HIV-1-specific T cell responses. *PLoS One* 9:e89236.
58 Siewe, B., Wallace, J., Rygielski, S. *et al.* 2014. Regulatory B cells inhibit cytotoxic T lymphocyte (CTL) activity and elimination of infected CD4 T cells after in vitro reactivation of HIV latent reservoirs. *PLoS One* 9:e92934.
59 Iwata, Y., Matsushita, T., Horikawa, M. *et al.* 2011. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 117:530.
60 Hasan, M. M., Thompson-Snipes, L., Klintmalm, G. *et al.* 2019. CD24(hi)CD38(hi) and CD24(hi)CD27(+) human regulatory B cells display common and distinct functional characteristics. *J. Immunol.* 203:2110.
61 Menon, M., Blair, P. A., Isenberg, D. A. *et al.* 2016. A regulatory feedback between plasmacytoid dendritic cells and regulatory B cells is aberrant in systemic lupus erythematosus. *Immunity* 44:683.
62 Duddy, M. E., Alter, A. and Bar-Or, A. 2004. Distinct profiles of human B cell effector cytokines: a role in immune regulation? *J. Immunol.* 172:3422.
63 Collison, L. W., Workman, C. J., Kuo, T. T. *et al.* 2007. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450:566.
64 Wang, R. X., Yu, C. R., Dambuza, I. M. *et al.* 2014. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat. Med.* 20:633.
65 Tian, J., Zekzer, D., Hanssen, L. *et al.* 2001. Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. *J. Immunol.* 167:1081. doi:10.4049/jimmunol.167.2.1081.
66 Chen, W., Jin, W., Hardegen, N. *et al.* 2003. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* 198:1875.
67 Kanamori, M., Nakatsukasa, H., Okada, M. *et al.* 2016. Induced regulatory T cells: their development, stability, and applications. *Trends Immunol.* 37:803. doi:10.1016/j.it.2016.08.012.
68 Parekh, V. V., Prasad, D. V., Banerjee, P. P. *et al.* 2003. B cells activated by lipopolysaccharide, but not by anti-Ig and anti-CD40 antibody, induce anergy in CD8+ T cells: role of TGF-beta 1. *J. Immunol.* 170:5897.
69 Natarajan, P., Singh, A., McNamara, J. T. *et al.* 2012. Regulatory B cells from hilar lymph nodes of tolerant mice in a murine model of allergic airway disease are CD5+, express TGF-beta, and co-localize with CD4+Foxp3+ T cells. *Mucosal Immunol.* 5:691.
70 Bjarnadottir, K., Benkhoucha, M., Merkler, D. *et al.* 2016. B cell-derived transforming growth factor-beta1 expression limits the induction phase of autoimmune neuroinflammation. *Sci. Rep.* 6:34594.
71 Nouel, A., Pochard, P., Simon, Q. *et al.* 2015. B-cells induce regulatory T cells through TGF-beta/IDO production in a CTLA-4 dependent manner. *J. Autoimmun.* 59:53. doi:10.1016/j.jaut.2015.02.004.
72 Velotti, F., Barchetta, I., Cimini, F. A. *et al.* 2020. Granzyme B in inflammatory diseases: apoptosis, inflammation, extracellular matrix remodeling, epithelial-to-mesenchymal transition and fibrosis. *Front. Immunol.* 11:587581.
73 Chesneau, M., Mai, H. L., Danger, R. *et al.* 2020. Efficient expansion of human granzyme B-expressing B cells with potent regulatory properties. *J. Immunol.* 205:2391.
74 Hagn, M., Ebel, V., Sontheimer, K. *et al.* 2010. CD5+ B cells from individuals with systemic lupus erythematosus express granzyme B. *Eur. J. Immunol.* 40:2060.
75 Xu, L., Liu, X., Liu, H. *et al.* 2017. Impairment of Granzyme B-producing regulatory b cells correlates with exacerbated rheumatoid arthritis. *Front. Immunol.* 8:768. doi:10.3389/fimmu.2017.00768.
76 Kaltenmeier, C., Gawanbacht, A., Beyer, T. *et al.* 2015. CD4+ T cell-derived IL-21 and deprivation of CD40 signaling favor the in vivo development of granzyme B-expressing regulatory B cells in HIV patients. *J. Immunol.* 194:3768.
77 Lindner, S., Dahlke, K., Sontheimer, K. *et al.* 2013. Interleukin 21-induced granzyme B-expressing B cells infiltrate tumors and regulate T cells. *Cancer Res.* 73:2468.
78 Volpe, E., Sambucci, M., Battistini, L. *et al.*. 2016. Fas-Fas ligand: checkpoint of T cell functions in multiple sclerosis. *Front. Immunol.* 7:382. doi:10.3389/fimmu.2016.00382.
79 Rouvier, E., Luciani, M. F. and Golstein, P. 1993. Fas involvement in Ca(2+)-independent T cell-mediated cytotoxicity. *J. Exp. Med.* 177:195. doi:10.1084/jem.177.1.195.
80 Ramsdell, F., Seaman, M. S., Miller, R. E. *et al.* 1994. gld/gld mice are unable to express a functional ligand for Fas. *Eur. J. Immunol.* 24:928. doi:10.1002/eji.1830240422.
81 Klinker, M. W., Reed, T. J., Fox, D. A. *et al.* 2013. Interleukin-5 supports the expansion of fas ligand-expressing killer B cells that induce antigen-specific apoptosis of CD4(+) T cells and secrete interleukin-10. *PLoS One* 8:e70131.
82 Lundy, S. K. and Boros, D. L. 2002. Fas ligand-expressing B-1a lymphocytes mediate CD4(+)-T-cell apoptosis during schistosomal infection: induction by interleukin 4 (IL-4) and IL-10. *Infect. Immun.* 70:812.
83 Bonardelle, D., Benihoud, K., Kiger, N. *et al.* 2005. B lymphocytes mediate Fas-dependent cytotoxicity in MRL/lpr mice. *J. Leukoc. Biol.* 78:1052. doi:10.1189/jlb.0904536.
84 Saxena, A., Yagita, H., Donner, T. W. *et al.* 2017. Expansion of FasL-expressing CD5(+) B cells in type 1 diabetes patients. *Front. Immunol.* 8:402.
85 Freeman, G. J., Long, A. J., Iwai, Y. *et al.* 2000. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* 192:1027. doi:10.1084/jem.192.7.1027.
86 Tangye, S. G., Ma, C. S., Brink, R. *et al.* 2013. The good, the bad and the ugly - TFH cells in human health and disease. *Nat. Rev. Immunol.* 13:412.
87 Khan, A. R., Hams, E., Floudas, A. *et al.* 2015. PD-L1hi B cells are critical regulators of humoral immunity. *Nat. Commun.* 6:5997.
88 Xiao, X., Lao, X. M., Chen, M. M. *et al.* 2016. PD-1hi identifies a novel regulatory B-cell population in human hepatoma that promotes disease progression. *Cancer Discov.* 6:546.
89 Shalapour, S., Font-Burgada, J., Di Caro, G. *et al.* 2015. Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. *Nature* 521:94.
90 Wang, X., Wang, G., Wang, Z. *et al.* 2019. PD-1-expressing B cells suppress CD4(+) and CD8(+) T cells via PD-1/PD-L1-dependent pathway. *Mol. Immunol.* 109:20. doi:10.1016/j.molimm.2019.02.009.
91 Lane, P. 1997. Regulation of T and B cell responses by modulating interactions between CD28/CTLA4 and their ligands, CD80 and CD86. *Ann. N. Y. Acad. Sci.* 815:392. doi:10.1111/j.1749-6632.1997.tb52090.x.
92 Mann, M. K., Maresz, K., Shriver, L. P *et al.* 2007. B cell regulation of CD4+CD25+ T regulatory cells and IL-10 via B7 is

essential for recovery from experimental autoimmune encephalomyelitis. *J. Immunol.* 178:3447.
93 Koch, M., Stronge, V. S., Shepherd, D. *et al.* 2005. The crystal structure of human CD1d with and without alpha-galactosylceramide. *Nat. Immunol.* 6:819.
94 Oleinika, K., Rosser, E. C., Matei, D. E. *et al.* 2018. CD1d-dependent immune suppression mediated by regulatory B cells through modulations of iNKT cells. *Nat. Commun.* 9:684. doi:10.1038/s41467-018-02911-y.
95 Palmer, V. L., Nganga, V. K., Rothermund, M. E. *et al.* 2015. Cd1d regulates B cell development but not B cell accumulation and IL10 production in mice with pathologic CD5(+) B cell expansion. *BMC Immunol.* 16:66.
96 Liu, Y., Chen, H., Chen, Z *et al.* 2021. Novel roles of the Tim family in immune regulation and autoimmune diseases. *Front. Immunol.* 12:748787. doi:10.3389/fimmu.2021.748787.
97 Kuchroo, V. K., Dardalhon, V., Xiao, S. *et al.* 2008. New roles for TIM family members in immune regulation. *Nat. Rev. Immunol.* 8:577.
98 Ding, Q., Yeung, M., Camirand, G. *et al.* 2011. Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. *J. Clin. Invest.* 121:3645.
99 Lee, K. M., Kim, J. I., Stott, R. *et al.* 2012. Anti-CD45RB/anti-TIM-1-induced tolerance requires regulatory B cells. *Am. J. Transplant.* 12:2072. doi:10.1111/j.1600-6143.2012.04055.x.
100 Xiao, S., Brooks, C. R., Sobel, R. A. *et al.* 2015. Tim-1 is essential for induction and maintenance of IL-10 in regulatory B cells and their regulation of tissue inflammation. *J. Immunol.* 194:1602.
101 Aravena, O., Ferrier, A., Menon, M. *et al.* 2017. TIM-1 defines a human regulatory B cell population that is altered in frequency and function in systemic sclerosis patients. *Arthritis Res. Ther.* 19:8.
102 Shankar, S., Stolp, J., Juvet, S. C. *et al.* 2022. Ex vivo-expanded human CD19(+)TIM-1(+) regulatory B cells suppress immune responses in vivo and are dependent upon the TIM-1/STAT3 axis. *Nat. Commun.* 13:3121. doi:10.1038/s41467-022-30613-z.
103 Shibuya, A. and Shibuya, K. 2021. DNAM-1 versus TIGIT: competitive roles in tumor immunity and inflammatory responses. *Int. Immunol.* 33:687. doi:10.1093/intimm/dxab085.
104 Xiao, S., Bod, L., Pochet, N. *et al.* 2020. Checkpoint receptor TIGIT expressed on Tim-1(+) B cells regulates tissue inflammation. *Cell Rep.* 32:107892.
105 Deaglio, S., Dwyer, K. M., Gao, W. *et al.* 2007. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J. Exp. Med.* 204:1257.
106 Saze, Z., Schuler, P. J., Hong, C. S. *et al.* 2013. Adenosine production by human B cells and B cell-mediated suppression of activated T cells. *Blood* 122:9.
107 Zacca, E. R., Amezcua Vesely, M. C., Ferrero, P. V. *et al.* 2021. B cells from patients with rheumatoid arthritis show conserved CD39-mediated regulatory function and increased CD39 expression after positive response to therapy. *J. Mol. Biol.* 433:166687. doi:10.1016/j.jmb.2020.10.021.
108 Workman, C. J. and Vignali, D. A. 2003. The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells. *Eur. J. Immunol.* 33:970.
109 Maruhashi, T., Okazaki, I. M., Sugiura, D. *et al.* 2018. LAG-3 inhibits the activation of CD4(+) T cells that recognize stable pMHCII through its conformation-dependent recognition of pMHCII. *Nat. Immunol.* 19:1415.
110 Won, W. J. and Kearney, J. F. 2002. CD9 is a unique marker for marginal zone B cells, B1 cells, and plasma cells in mice. *J. Immunol.* 168:5605.
111 Sun, J., Wang, J., Pefanis, E. *et al.* 2015. Transcriptomics identify CD9 as a marker of murine IL-10-competent regulatory B cells. *Cell Rep.* 13:1110.
112 Matsushita, T., Huu, D. L., Kobayashi, T. *et al.* 2016. A novel splenic B1 regulatory cell subset suppresses allergic disease through phosphatidylinositol 3-kinase–Akt pathway activation. *J. Allergy Clin. Immun.* 138:1170.
113 Cariappa, A., Shoham, T., Liu, H. *et al.* 2005. The CD9 tetraspanin is not required for the development of peripheral B cells or for humoral immunity. *J. Immunol.* 175:2925. doi:10.4049/jimmunol.175.5.2925.
114 Brosseau, C., Colas, L., Magnan, A. *et al.* 2018. CD9 Tetraspanin: a new pathway for the regulation of inflammation? *Front. Immunol.* 9:2316. doi:10.3389/fimmu.2018.02316.
115 Mavropoulos, A., Simopoulou, T., Varna, A. *et al.* 2016. Breg cells are numerically decreased and functionally impaired in patients with systemic sclerosis. *Arthritis Rheumatol.* 68:494.
116 Guo, S., Chen, Q., Liang, X. *et al.* 2018. Reduced peripheral blood regulatory B cell levels are not associated with the Expanded Disability Status Scale score in multiple sclerosis. *J. Int. Med. Res.* 46:3970. doi:10.1177/0300060518783083.
117 Kleffel, S., Vergani, A., Tezza, S. *et al.* 2015. Interleukin-10+ regulatory B cells arise within antigen-experienced CD40+ B cells to maintain tolerance to islet autoantigens. *Diabetes* 64:158.
118 Hayashi, M., Yanaba, K., Umezawa, Y. *et al.* 2016. IL-10-producing regulatory B cells are decreased in patients with psoriasis. *J. Dermatol. Sci.* 81:93. doi:10.1016/j.jdermsci.2015.11.003.
119 Yoshihara, Y., Ishiuji, Y., Yoshizaki, A. *et al.* 2019. IL-10-producing regulatory B cells are decreased in patients with atopic dermatitis. *J. Invest. Dermatol.* 139:475. doi:10.1016/j.jid.2018.08.016.
120 Das, A., Ellis, G., Pallant, C. *et al.* 2012. IL-10-producing regulatory B cells in the pathogenesis of chronic hepatitis B virus infection. *J. Immunol.* 189:3925.
121 Liu, Y., Cheng, L. S., Wu, S. D. *et al.* 2016. IL-10-producing regulatory B-cells suppressed effector T-cells but enhanced regulatory T-cells in chronic HBV infection. *Clin. Sci. (Lond).* 130:907.
122 van der Vlugt, L. E., Zinsou, J. F., Ozir-Fazalalikhan, A. *et al.* 2014. Interleukin 10 (IL-10)-producing CD1dhi regulatory B cells from Schistosoma haematobium-infected individuals induce IL-10-positive T cells and suppress effector T-cell cytokines. *J. Infect. Dis.* 210:1207.
123 Zhivaki, D., Lemoine, S., Lim, A. *et al.* 2017. Respiratory syncytial virus infects regulatory B cells in human neonates via chemokine receptor CX3CR1 and promotes lung disease severity. *Immunity* 46:301.
124 Wang, K., Liu, J. and Li, J. 2018. IL-35-producing B cells in gastric cancer patients. *Medicine (Baltimore)* 97:e0710.
125 Pallier, A., Hillion, S., Danger, R. *et al.* 2010. Patients with drug-free long-term graft function display increased numbers of peripheral B cells with a memory and inhibitory phenotype. *Kidney Int.* 78:503.
126 Chesneau, M., Michel, L., Dugast, E. *et al.* 2015. Tolerant kidney transplant patients produce B cells with regulatory properties. *J. Am. Soc. Nephrol.* 26:2588.
127 Chesneau, M., Pallier, A., Braza, F. *et al.* 2014. Unique B cell differentiation profile in tolerant kidney transplant patients. *Am. J. Transplant.* 14:144.
128 Bigot, J., Pilon, C., Matignon, M. *et al.* 2016. Transcriptomic signature of the CD24(hi) CD38(hi) Transitional B cells associated with an immunoregulatory phenotype in renal transplant recipients. *Am. J. Transplant.* 16:3430. doi:10.1111/ajt.13904.
129 Blair, P. A., Chavez-Rueda, K. A., Evans, J. G. *et al.* 2009. Selective targeting of B cells with agonistic anti-CD40 is an efficacious strategy for the generation of induced regulatory T2-like B cells and for the suppression of lupus in MRL/lpr mice. *J. Immunol.* 182:3492.
130 Teichmann, L. L., Kashgarian, M., Weaver, C. T. *et al.* 2012. B cell-derived IL-10 does not regulate spontaneous systemic autoimmunity in MRL.Fas(lpr) mice. *J. Immunol.* 188:678.
131 Matsushita, T., Hamaguchi, Y., Hasegawa, M. *et al.* 2016. Decreased levels of regulatory B cells in patients with systemic sclerosis: association with autoantibody production and disease activity. *Rheumatology (Oxford)* 55:263. doi:10.1093/rheumatology/kev331.