



Available online at
SciVerse ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en



Review

Regulatory B cells play a key role in immune system balance

Jean-Marie Berthelot^{a,*}, Christophe Jamin^b, Kahina Amrouche^b, Benoit Le Goff^a, Yves Maugars^a, Pierre Youinou^b

^a Service de rhumatologie, Hôtel-Dieu, CHU de Nantes, place Alexis-Ricordeau, 44093 Nantes cedex 01, France

^b EA 2216 "Immunology and Pathology", IFR 148 ScInBioS, European University of Brittany, University of Brest, hôpital Morvan, 29609 Brest cedex 01, France

ARTICLE INFO

Article history:
Accepted 18 April 2012
Available online xxx

Keywords:
B lymphocyte
Regulatory B cells
Br1
Br3
Foxp3
Autoimmunity
IL-10

ABSTRACT

Regulatory B cells (Bregs) may act earlier than regulatory T cells (Tregs) and may play as important a role in autoimmune and allergic diseases. Obstacles to the investigation of Bregs are the same as those encountered for Tregs: the regulatory effects are short-lived in some cases, there is no consistent phenotype (C5 expression is neither indispensable nor sufficient), differences exist across species (e.g., between humans and mice), and there are a number of suppression modalities (IL-10, TGF-beta, expression of proapoptotic membrane molecules) that vary across Breg subtypes. The Breg subtypes may be homologous to the Treg subtypes (Br1 cells expressing IL-10, Br3 cells expressing TGF-beta, and B-Foxp3 cells), although the Br1 subtype seems to predominate. Nevertheless, differences with Treg cells may exist: Breg activation may chiefly involve the toll-like receptors rather than the antigen receptor; and Bregs act earlier, facilitating the recruitment of Tregs then disappearing once the Tregs become operational. Bregs make a major contribution to autoimmune disorders associated with several forms of immune deficiency, as well as to the absence of transplant rejection when there is a strong B cell response. Breg deficiencies have been reported in lupus, and the disappointing effects in this disease of treatments designed to inhibit the B cell response may be related to further Breg impairment. In several animal models, Breg stimulation is effective in correcting a variety of autoimmune disorders, most notably those initiated in the mucous membranes. Research into the interactions between the gut microbiota and Bregs holds considerable promise.

© 2012 Published by Elsevier Masson SAS on behalf of the Société Française de Rhumatologie.

The identification of regulatory T cells (Tr1, Tr3, TCD4+CD25+FoxP3+) suggested the existence of regulatory macrophages (M2) and B cells (Bregs). T cell involvement in the immune response is delayed until the antigen is cleaved into peptides. In contrast, B cells have a membrane antibody (BCR) that rapidly captures the antigen in its native form. This feature suggested earlier involvement of Bregs compared to Tregs [1], providing a strong rationale for investigating Bregs. Furthermore, Breg impairment promotes the development of autoimmune and allergic diseases [2].

1. Emergence of the B regulatory cell (Breg) concept

An observation made in 1950 suggested an association between some forms of B cell deficiency and autoimmune manifestations (cytopenias) [3]. Subsequently, expansion of B cells carrying CD5 and having a longer half-life and an auto-reactive repertoire was

reported in several autoimmune diseases [4]. Data from experimental models confirmed that B cell depletion could paradoxically induce allergic diseases (asthma and atopic dermatitis) or autoimmune diseases (experimental autoimmune encephalomyelitis [EAE], inflammatory bowel disease, biliary cirrhosis, arthritis, diabetes), which resolved after the administration of syngeneic B cells [2]. Further impetus to investigations into the regulatory effects of specific B cell subsets came from reports that transplant recipients who can be rapidly weaned off immunosuppressants exhibited higher levels of B cell activation compared to those who required continued immunosuppressant therapy [5].

The limited effectiveness of the anti-CD20 antibody rituximab in lupus may be ascribable to counterbalancing of the beneficial effects of effector B cell depletion by concomitant depletion of Bregs [6], which are already decreased under basal conditions in lupus patients [7,8]. The same mechanism probably applies to inhibition of B cell-Activating Factor belonging to the TNF Family (BAFF), since this cytokine promotes the production of IL-10-expressing Bregs [9]. That anti-CD20 antibodies improve other autoimmune diseases, including most cases of rheumatoid arthritis (RA), may be related to the usually greater resistance of

* Corresponding author. Tel.: +33 02 40 08 48 22; fax: +33 02 40 08 48 30.
E-mail address: jeanmarie.berthelot@chu-nantes.fr (J.-M. Berthelot).

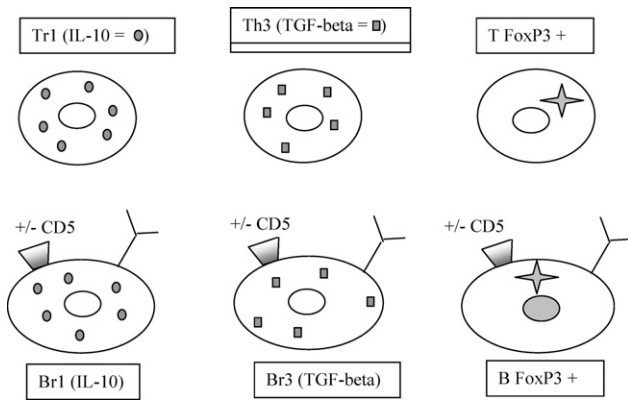


Fig. 1. The three types of regulatory T cells (Tr1, Th3, and Treg Foxp3+) have their counterparts among B cells: Br1 cells produce IL-10, similar to Tr1 cells; Br3 cells produce TGF-beta similar to Th3 cells; and B-Foxp3+ cells express FoxP3 and CD25++ similar to FoxP3+ Tregs. All three Breg types express CD19 and a membrane IgM; they often (but not always) express CD5.

Bregs to anti-CD20 antibodies in RA [10], and to the fact that CD5+ B cells are the first B cell subset to reappear after B cell depletion by anti-CD20 antibodies [11].

2. The different types of regulatory B cells (Bregs)

2.1. Borderline Bregs: naive or neonatal B cells

Some naive B cells, although not Bregs in the strict sense of the term, present their peptides in the absence of co-stimulation signals (expression of co-stimulation molecules, cytokines) and can promote the induction of iTregs [8,12,13] (Fig. 1). The same regulatory function is exhibited by allogeneic B cells, which promote transplant tolerance [13]. It is lost, however, when these B cells are activated.

Neonatal B cells (called B-1) are chiefly found in the pleural membrane, peritoneum, and spleen. They are capable of self-renewal outside the bone marrow and express polyreactive low-affinity IgM. The B-1 repertoire is fairly restricted and remains stable because, in contrast to B-2 cells from the bone marrow, B-1 cells have little contact with T cells. This archaic B cell population [12] promotes the clearance of autoantigens released during apoptosis, without generating a T cell response [14]. For instance, B-1 cells can recognize neo-epitopes expressed by apoptotic cells such as certain membrane lipids (phosphorylcholine) exposed at the atheroma plaque surface.

2.2. IL-10 producing B cells (known as Br1 cells, in analogy with Tr1 cells)

Br1 or “B10” cells [15] are defined by their ability to produce large amounts of IL-10, the cytokine with the strongest inhibitory effect on the immune response, most notably on antigen-presenting cells. IL-10 also inhibits the differentiation of Th0 cells to Th1 and Th2 cells; whereas Th17 and cytotoxic TCD8 cells are less sensitive to IL-10. IL-10 is an autocrine factor for most B cells, including Bregs, although exceptions have been reported, such as murine peritoneal B-1P Bregs [16].

2.2.1. Conditions required for IL-10 production by Br1 cells

The most important molecules for IL-10 production by murine Br1 cells are CD1d, CD19, and MyD88. In mice, aberrant CD40L (CD-154) expression on B cells correlates with IL-10 production [17], which is increased by binding to an antigen and by a higher level of membrane IgM expression [18]: engagement of the BCR activates

Stromal Interaction Molecule (STIM), which induces an influx of calcium into the cells [19]. Murine Br1 cells can also differentiate to plasma cells that release IgM initially then IgG [20].

In humans, in contrast, binding of an antigen to the membrane antibody may diminish the production of IL-10 by Br1 cells [6]. IL-10 production increases substantially when T cell CD40L (CD154) binds to B cell CD40 [8,13] and when the B cell toll-like receptors (TLR) are activated, most notably by microbial molecules. Other molecules may affect IL-10 production, such as CD24 [20] and CD38 [8] in humans and TIM-1 (T cell immunoglobulin and mucin domain 1) in mice [21].

CD5 is chiefly expressed on the T cell surface but is overexpressed on Bregs. CD5 has the original property of interacting with various membrane ligands (on hematopoietic and non-hematopoietic cells) and with conserved microbial structures such as some of the yeast β -glucans [22]. In humans, CD5 stimulation induces IL-10 synthesis by activating the NFAT2 pathway and, to a lesser extent, the STAT3 pathway [23]. However, the presence of CD5 is neither sufficient, nor indispensable to IL-10 synthesis in humans [6]. In cord blood, where CD5+ cells contribute 7% of the total cell population, less than 2% of CD5+ B cells have IL-10 in their cytoplasm. In lupus, the B cells that produce IL-10 are CD5- and CD40L+ [24].

CD5 may promote regulatory mechanisms other than IL-10 production, such as increased expression of granzyme, FAS-ligand, and PD-L2 (see below) [12]. The presence of CD5 may also be associated with improved autoantigen presentation, although this function may sometimes depend more heavily on CD1d than on class I or II HLA molecules, as the CD5+ B cell repertoire is skewed towards non-protein (auto)antigens (i.e., sugars, lipids, and glycolipids) [25].

2.2.2. Variety in Br1 phenotypes (subsets) and differences among species

The most suggestive phenotypes of murine Br1 cells are CD19+, CD5+, CD1d(high), and TIM-1 [21]. However, only 25% of Br1 cells exhibit this phenotype, and even within this subset some B cells do not produce IL-10. Whereas the level of CD19 expression correlates closely with IL-10 production in mice, the opposite seems true in humans, in whom IL-10 is chiefly produced by the BCD5+ subset [23] characterized by a lower level of CD19 expression [26]. Finally, the most suggestive Br1 phenotypes (which are, however, neither necessary nor sufficient [6]) in humans may be CD19+(low) CD5+CD1d(high) CD24(high) [15]; CD19+CD24(high) CD38+; and CD19+CD24(high) CD27+ [27]. CD5+ B cell subsets are heterogeneous: some cells express a distinctive CD5 molecule produced by alternate splicing of exon 1B [28].

2.2.3. Frequency of Br1 cells

In mice, Br1 cells are found chiefly in the spleen and peritoneal cavity, but may be detected in peripheral blood, where they contribute 1% to 3% of all B cells. In humans, 0.6% of peripheral blood B cells may have Br1 functions but 5% may be capable of transforming into Br1 cells, a property seen in particular with CD24(high) CD27(+) cells [29]. The percentage of CD19(low) CD5(+) Br1 cells may even increase to 15%, although these cells show a greater propensity for apoptosis in this situation (17% versus 4% for the other B cells [26]).

Another difficulty when investigating Bregs, including Br1 cells, is the absence of a strict correlation between cell counts and regulatory function. Mice that spontaneously develop autoimmune diseases (New Zealand black [NZB], non obese diabetic [NOD], Lymphoproliferative [lpr], and Murphy Roths Large [MRL]) usually have elevated peripheral blood Br1 counts as a result of compensatory but inadequate Breg stimulation [30]. In contrast, mice used in various models of induced autoimmunity based on their greater susceptibility to loss of tolerance have lower peripheral blood

Br1 counts compared to normal mice [15]. Therefore, defective regulation by B cells (including Br1 cells) cannot be excluded, despite the absence of phenotypic differences versus controls. In other words, investigation of IL-10 production by B cells should remain the reference criterion for identifying Br1 cells.

2.2.4. Br1 cells in animal models and human diseases

Br1 cells are involved in tolerance to food allergens [26]. Their preventive or curative effect has been convincingly demonstrated in several murine models of autoimmune diseases (inflammatory bowel disease, diabetes, lupus, arthritis, and encephalitis) [12]. The increase in Br1 counts with increasing age in mice may constitute a compensatory response to T cell repertoire contraction during aging [15], which increases the risk of autoimmunity via cross reactions. In humans, counts of Br1 cells (producing IL-10) are often elevated in patients with autoimmune diseases (RA, lupus, Sjögren's syndrome, multiple sclerosis, and bullous skin diseases) [29]. Few studies have addressed Br1 function in humans, but there is evidence of impaired Br1 function in patients with lupus [8].

2.3. Br3 or Bregs producing TGF-beta

Similar to the Th3 subsets of Treg cells, which produce TGF-beta, Br3 cells producing TGF-beta have been identified within both CD5- and CD5+ populations of B cells [2]. There is no evidence to date that these Br3 cells are different from B cells at rest. However, activated B cell subsets have been shown to produce large amounts of TGF-beta [6]. This Breg subset may be less important than the Br1 subset and it may serve only to induce transient amplification of the regulatory B cell effect by ensuring the transition between Br1 cells and Treg involvement. Whereas Br1 cells generate a positive autocrine loop (because IL-10 induces Br1 cells), Br3 cells induce a negative autocrine loop, as TGF-beta inhibits CD5+ B cells and forces them into apoptosis [2], while promoting the emergence of Tregs (Th3 or Treg Foxp3). Br3 cells may nevertheless play a role in some diseases. For instance, Br3 cell impairment may contribute to food allergies (most notably to dairy products) in humans [31].

2.4. B-Foxp3+

Expression of Foxp3, a transcription factor that is fairly specific of Tregs, was demonstrated using cytometry in a small proportion (8%) of peripheral blood CD19+CD5+ B cells from 6 healthy adolescents (15.3 ± 3.5 years) [31]. CD19+CD5+Foxp3+ B cells are characterized by a very high rate of apoptosis (63%) that is far greater than that of other B cell subsets [32]. The immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) related to an inactivating mutation in the *Foxp3* gene is characterized by severe food allergies despite normal CD5+ B cell counts, and the B cell phenotype resembles that of naive B cells [33]. Although the *Foxp3* mutation predominantly affects Treg function, the high prevalence of food allergies suggests concomitant Breg function impairment, since deficits in other Breg subsets (producing IL-10 or TGF-beta) are often associated with food allergies [31].

To date, no studies have evaluated whether the Foxp3+ Bregs constitute a subset of the CD20+CD25+ B cells, in analogy with Foxp3+ Tregs. In all likelihood, only a small percentage of CD25+ B cells exert regulatory functions in mice. In contrast to CD4+CD25+ T cells, CD20+CD25+ B cells proliferate in response to IL-2 [34]. As a result, they contribute up to 30% of all peripheral blood B cells in mice and can effectively present peptides to helper T cells. In humans, only B cells expressing high levels of CD25 (BCD25++) seem capable of acting as Bregs with abundant TGF-beta production [35]. No studies so far have assessed, in analogy with Foxp3+ Tregs [36], the existence of iBregsFoxp3+ (induced peripherally) or of an nBregsFoxp3+ subset ("natural" Bregs educated in the thymus).

It would nevertheless be of interest to determine whether the few B cells found in the thymus medullary exhibit a phenotype consistent with regulatory effects [37].

2.5. B cells with cytotoxicity for other immune response cells

Similar to activated T cells, activated Bregs express programmed death ligand-1 (PD-1) [11] and most CD5+ B cells also express increased levels of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (CD253) and Fas-ligand (CD178), most notably in the spleen and tonsils and to a lesser extent in the lymph nodes and thymus. These properties are consistent with the main Breg function, which consists in preventing an excessive immune response in the epithelia, at the interface between self and non-self, where commensal microorganisms must be allowed to survive while being closely supervised by the immune system. CD5+FasL+ B cells slow the progression of several experimental autoimmune diseases including collagen-induced arthritis, in which a negative correlation has been reported between the number of these cells and the severity of the clinical manifestations [38]. Apoptosis induction by Bregs via PD-1, Fas-L, or TRAIL varies with the nature of the target T cell: susceptibility may decrease from Th1 cells to Th2 cells then to Th17 cells and seems lowest for Tregs [12]. Differences exist also across tissues: increased expression of proapoptotic molecules by Bregs in some tissues, such as the eye or reproductive organs, contributes to explain the immune privilege of these sites [12]. It is worth pointing out that susceptibility to apoptosis is considerably greater for Bregs, including Br1, Br3, and B-Foxp3+ cells, compared to Tregs [2]. However, Breg apoptosis is compensated for in part by proliferation of the surviving Bregs [2].

3. The T/Treg and B/Breg foursome

At least in mice, Bregs activated by binding to their TLR inhibit not only T cells, but also dendritic cells [39]. In humans, Bregs activated via their CD40 molecules and binding to their TLR also impair the maturation of monocytes to immature dendritic cells then the differentiation of these last into mature cells and the ability of mature dendritic cells to stimulate T cells [40]. Interestingly, Bregs (which may be CD5+ or CD5-) probably originate in a B cell population that differs from the B cells involved in regulating T cell proliferation (which are effective only if they express CD5 [8]). Bregs, most notably those expressing CD1d, can also stimulate NKT cells, which can exert regulatory effects [12]. Finally, in several experimental models, Bregs block the switch from IgM to IgG [17], probably by inhibiting the effects of T helper cells.

The main role of Bregs seems to consist in controlling the effects of T cells. Bregs can fulfill this role either by releasing soluble factors (IL-10, TGF-beta) or via membrane-to-membrane contact (in particular, involving Bregs induced by CD40 stimulation [8]). Bregs can also force T cells to exhibit short-lived Treg behavior [41]. However, Bregs seem to control T cells chiefly via conventional Tregs [8] or via Tregs exhibiting unusual phenotypes [42]. Whether Foxp3+ Bregs selectively promote the appearance of Foxp3+ Tregs is unknown. Bregs promote Treg accumulation within target tissues via chemokine release [2], most notably in multiple sclerosis and arthritis models [43]. Human Bregs also increase Foxp3 and CTLA-4 expression at the surface of pre-existing Tregs (via direct cell contact and TGF-beta release, whereas the effect of IL-10 seems more incidental [44]). Treg inhibition by Bregs, in contrast, seems exceedingly rare [45], as Tregs are highly resistant to inhibitory signals from Bregs. Bregs seem to serve as scouts that prepare the field for the Tregs then disappear once the Tregs are operational, particularly during the induction of tolerance via the mucous membranes, after oral [46] or intranasal [47] antigen administration. Once

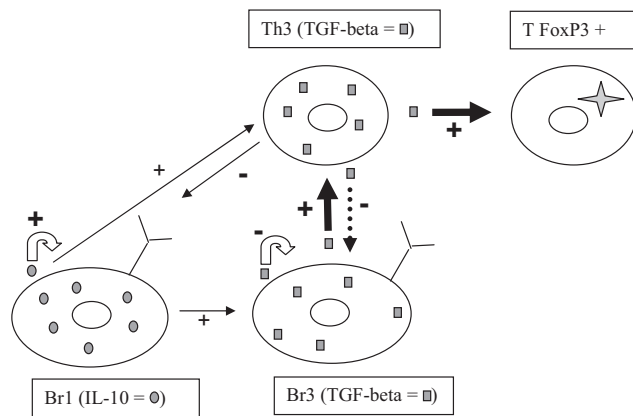


Fig. 2. IL-10 produced by Br1 cells has autocrine and paracrine effects on the other Bregs (including Br3 cells) and promotes Treg recruitment. In contrast, TGF-beta released by Br3 (and Th3) cells inhibits Bregs and stimulates Tregs. Some of the Bregs, whose involvement precedes that of the Tregs, seem to be “dismissed” by the Tregs once these are recruited.

activated, Tregs tend to inhibit Bregs, most notably via the release of TGF-beta, which exerts autocrine effects on Tregs (particularly Foxp3+ Tregs) [48] but inhibit Bregs [2] (Fig. 2).

4. Role for Bregs in disease and treatment

The promoting and/or exacerbating effect of Breg deficiency has been demonstrated, including in models characterized by excessive autoantibody production such as the murine K/BxN model, which resembles RA [49]. At least two groups of researchers have found impairments in B cells having Breg effects in human patients with lupus [7,8]. Impaired IL-10-independent control of T cell proliferation and impaired, partly IL-dependent, control of T cell differentiation promotes an excessive Th1 response [5,50], which, combined with activation of autoantibody production by IL-10, may contribute to the pathogenesis of lupus. As shown for Tregs, decreased susceptibility of specific target cells to the effects of Bregs may exacerbate a number of diseases. In lupus, for instance, the dendritic cells seem insensitive to the effects of Bregs [40].

The restoration of impaired Breg function may hold promise as a novel therapeutic strategy. In mice, Breg stimulation by monoclonal anti-TIM-1 antibodies results in IL-10 release and prevents the rejection of pancreatic allografts [21]. Several immunomodulating agents used empirically in humans such as glatiramer acetate (Copaxone®) for multiple sclerosis may act via Breg stimulation [51]. High-dose estrogens enhance the function of some Bregs, at least in mice and in EAE models. No studies to date have determined whether anti-CD3 or IL-2 treatments used in humans and mice to shift the Treg/T effector balance toward Tregs also exert favorable effects on Bregs. Such effects may exist with IL-2 and the CD5+CD25(high) B cell subset, as CD25 is the IL-2 receptor. One of the mechanisms of action of tocilizumab [52] may consist in enhancing the effect of Bregs (increased TGF-beta and CD25 expression at the B cell surface has been found in RA patients treated with tocilizumab [35]).

Bacterial molecules have been used to stimulate Bregs. However, the term “vaccination” should be qualified or avoided, as Breg stimulation occurs chiefly via some of the TLR, such as TLR9 in lupus [53], in keeping with the apparently opposite roles for TLR7 and TLR9 in this disease [54]. In mice, splenic B cells stimulated ex vivo by bacteria acquire the CD5+CD1d+ phenotype, characterized by high-level IL-10 expression and capable of markedly suppressing the activity of experimental inflammatory bowel disease. Similarly, lipopolysaccharide-induced activation of bowel CD5+ Bregs can prevent the induction of EAE [55], and this effect is transmissible

via the transfusion of B cells from treated mice. The protective subset (contributing 1% to 2% of all splenic B cells) is composed of CD5+CD1d(high) B cells activated via the TLR2/4 pathway by bacterial antigens in the gut flora [55]. Investigations into the influence of the gut microbiota on the balance between effector B cells and Bregs may open up new therapeutic possibilities, particularly in multiple sclerosis and in arthritis associated with chronic inflammatory bowel disease.

An excessively zealous Breg population may promote tumor cell growth, as one of the mechanisms used by tumor cells to escape from the immune response consists in activation of Bregs that produce TGF-beta [56]. Another adverse effect of excessive Breg stimulation may be the perpetuation of some forms of infection. In chronic salmonella infection, the organisms activate the gut Bregs via their TLR and these activated cells then produce IL-10, which inhibits Th1 cells [57]. Similarly, the hepatitis C virus eludes the host immune response by stimulating some of the Breg cells in the mucous membranes. Several parasites (schistosoma, trypanosomes) and viruses (including the HIV and the Epstein-Barr virus) protect themselves from the Th1 response by stimulating the Breg cells and forcing them to express proapoptotic molecules such as FasL [58]. The parasite molecules with the strongest Breg-stimulating effects are beginning to be identified. An example is lacto-N-fucopentaose (LNFP-III) in schistosoma eggs [59]. The ability of parasites to activate Bregs may contribute to explain the negative correlation between the rising incidence of autoimmune diseases and the near disappearance of parasitic infections in countries characterized by high levels of hygiene [60].

Research into the Bregs seems particularly promising as Breg impairment may be, if not a crucial abnormality, at least antecedent to Treg impairment in the pathogenesis of autoimmune diseases. When subjected to excessive solicitation, some “natural” Bregs may lose their regulatory phenotypes and gradually take on a B-helper behavior. This sequence may contribute to explain why autoantibody production antedates the first symptoms in several autoimmune diseases.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References

- [1] Mauri C, Ehrenstein MR. The “short” history of regulatory B cells. *Trends Immunol* 2007;29:34–40.
- [2] Noh G, Lee JH. Regulatory B cells and allergic diseases. *Allergy Asthma Immunol Res* 2011;3:168–77.
- [3] Cunningham-Rundles C. Autoimmunity in immune deficiency: taking lessons from our patients. *Clin Exp Immunol* 2011;164:6–11.
- [4] Youinou P, Mackenzie L, Broker BM, et al. The importance of CD5-positive B cells in non-organ-specific autoimmune diseases. *Scand J Rheumatol Suppl* 1988;76:243–9.
- [5] Newwell KA, Asare A, Kirk AD, et al. Identification of a B cell signature associated with renal transplant tolerance in humans. *J Clin Invest* 2010;120:1836–47.
- [6] Jamin C, Morva A, Lemoine S, et al. Regulatory B lymphocytes in humans. A potential role in autoimmunity. *Arthritis Rheum* 2008;58:1900–6.
- [7] Blair PA, Noreña LY, Flores-Borja F, et al. CD19+CD24hiCD38hi B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity* 2010;32:129–40.
- [8] Lemoine S, Morva A, Youinou P, et al. Human T cells induce their own regulation through activation of B cells. *J Autoimmun* 2011;36:228–38.
- [9] Yang M, Sun L, Wang S, et al. Novel function of B cell-activating factor in the induction of IL-10 producing regulatory B cells. *J Immunol* 2010;184:3321–5.
- [10] Hamaguchi Y. Molecular mechanisms of B lymphocyte depletion by CD20 immunotherapy. *Nihon Rinsho Meneki Gakkai Kaishi* 2009;32:29–34.
- [11] Pers JO, Daridon C, Bendaoud B, et al. B cell depletion and repopulation in autoimmune diseases. *Clin Rev Allergy Immunol* 2008;34:50–5.
- [12] Klinker MW, Lundy SK. Multiple mechanisms of immune suppression by B lymphocytes. *Mol Med* 2012;18:123–37.

- [13] Morlacchi S, Soldani C, Viola A, et al. Self-antigen presentation by mouse B cells results in regulatory T cell induction rather than energy or clonal deletion. *Blood* 2011;118:984–91.
- [14] Shaw PX, Horkko S, Chang MK, et al. Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. *J Clin Invest* 2000;105:1731–40.
- [15] Yanaba K, Bouaziz JD, Matsushita T. The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. *J Immunol* 2009;182:7459–72.
- [16] Sindhu V, Woodman ME, Stevenson B, et al. Interleukin-10 mediated autoregulation of murine B-1 B cells and its role in *Borrelia hermsii* infection. *PLoS One* 2010;5:e11445.
- [17] Poe JC, Smith SH, Haas KM, et al. Amplified B lymphocyte CD40 signaling drives regulatory B10 cell expansion in mice. *PLoS One* 2011;6:e22464.
- [18] Yanaba K, Bouaziz JD, Haas KM, et al. A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity* 2008;28:639–50.
- [19] Matsumoto M, Fujii Y, Baba A, et al. The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. *Immunity* 2011;34:703–14.
- [20] Maseda D, Smith SH, Dilillo DJ, et al. Regulatory b10 cells differentiate into antibody-secreting cells after transient IL-10 production in vivo. *J Immunol* 2012;188:1036–48.
- [21] Ding Q, Yeung M, Camirand G, et al. 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* 2011;121:3645–56.
- [22] Carnero-Montoro E, Bonet L, Engelken J, et al. Evolutionary and functional evidence for positive selection at the human CD5 immune receptor gene. *Mol Biol Evol* 2012;29:811–23.
- [23] Garaud S, Morva A, Lemoine S, et al. CD5 promotes IL-10 production in chronic lymphocytic leukemia B cells through STAT3 and NFAT2 activation. *J Immunol* 2011;186:4835–44.
- [24] Díaz-Alderete A, Crispin JC, Vargas-Rojas MI, et al. IL-10 production in B cells is confined to CD154+ cells in patients with systemic lupus erythematosus. *J Autoimmun* 2004;23:379–83.
- [25] Lundy SK. Killer B lymphocytes: the evidence and the potential. *Inflamm Res* 2009; doi:10.1007/s00011-009-0014-x.
- [26] Lee JH, Noh J, Noh G, et al. IL-10 is predominantly produced by CD19(low)CD5(+) regulatory B cell subpopulation: characterisation of CD19(high) and CD19(low) subpopulations of CD5(+) B cells. *Yonsei Med J* 2011;52:851–5.
- [27] Fujimoto M. Regulatory B cells in skin and connective tissue diseases. *J Dermatol Sci* 2010;60:1–7.
- [28] Garaud S, Le Dantec C, de Mendoza AR, et al. IL-10 production by B cells expressing CD5 with the alternative exon 1B. *Ann N Y Acad Sci* 2009;1173:280–5.
- [29] Iwata Y, Matsushita T, Horikawa M, et al. Characterization of a rare IL-10-competent B cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 2011;117:530–41.
- [30] Teichmann LL, Kashgarian M, Weaver CT, et al. B cell-derived IL-10 does not regulate spontaneous systemic autoimmunity in MRL.Fas^{lpr} mice. *J Immunol* 2012;188:678–85.
- [31] Lee JH, Noh J, Noh G, et al. Allergen-specific transforming growth factor- β -producing CD19+CD5+ regulatory B cell (Br3) responses in human late eczematous allergic reactions to cow's milk. *J Interferon Cytokine Res* 2011;31:441–9.
- [32] Noh J, Choi WS, Noh S, et al. Presence of Foxp3-expressing CD19(+)CD5(+) B cells in human peripheral blood mononuclear cells: human CD19(+)CD5(+)Foxp3(+) regulatory B cell (Breg). *Immune Netw* 2010;10:247–9.
- [33] Zennaro D, Scala E, Pomponi D, et al. Proteomics plus genomics approaches in primary immunodeficiency: the case of immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. *Clin Exp Immunol* 2012;167:120–8.
- [34] Brisslert M, Bokarewa M, Larsson P, et al. Phenotypic and functional characterization of human CD25+ B cells. *Immunology* 2006;117:548–57.
- [35] Snir A, Kessel A, Haj T, et al. Anti-IL-6 receptor antibody (tocilizumab): a B cell targeting therapy. *Clin Exp Rheumatol* 2011;29:697–700.
- [36] Biton J, Boissier MC, Bessis N. TNF- α : activator or inhibitor of regulatory T cells? *Joint Bone Spine* 2012;79:119–23.
- [37] Berthelot JM, Le Goff B, Maugars Y. Thymic Hassall's corpuscles, regulatory T cells, and rheumatoid arthritis. *Semin Arthritis Rheum* 2010;39:347–55.
- [38] Lundy SK, Fox DA. Reduced Fas-ligand expressing splenic CD5+ lymphocytes in severe collagen-induced arthritis. *Arthritis Res Ther* 2009;11:R-128.
- [39] Lo-Man R. Regulatory B cells control dendritic cell functions. *Immunotherapy* 2011;3:19–20.
- [40] Morva A, Lemoine S, Achour A, et al. Maturation and function of human dendritic cells are regulated by B lymphocytes. *Blood* 2012;119:106–14.
- [41] Reichardt P, Dornbach B, Rong S, et al. Naive B cells generate regulatory T cells in the presence of a mature immunologic synapse. *Blood* 2007;110:1519–29.
- [42] Wei B, Velazquez P, Turovskaya O, et al. cells centrally inhibit CD4+ T cell colitis through interaction with regulatory T cell subsets. *Proc Natl Acad Sci U S A* 2005;102:2010–5.
- [43] Carter NA, Vasconcellos R, Rosser EC, et al. 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* 2011;186:5569–79.
- [44] Kessel A, Haj T, Peri R, et al. Human CD19(+)CD25(high) B regulatory cells suppress proliferation of CD4(+) T cells and enhance Foxp3 and CTLA-4 expression in T regulatory cells. *Autoimmun Rev* 2012;11:670–7.
- [45] Yu S, Maiti PK, Dyson M, et al. B cell-deficient NOD.H-2h4 mice have CD4+CD25+T regulatory cells that inhibit the development of spontaneous autoimmune thyroiditis. *J Exp Med* 2006;203:349–58.
- [46] Sun JB, Flach CF, Czerkinsky C, et al. B lymphocytes promote expansion of regulatory T cells in oral tolerance: powerful induction by antigen coupled to cholera toxin B subunit. *J Immunol* 2008;181:8278–87.
- [47] Singh A, Carson WF, Secor Jr ER, et al. Regulatory role of B cells in a murine model of allergic airway disease. *J Immunol* 2008;180:7318–26.
- [48] Zhang L, Yi H, Xia XP, et al. Transforming growth factor- β : an important role in CD4(+)CD25(+) regulatory T cells and immune tolerance. *Autoimmunity* 2006;39:269–76.
- [49] Korganow AS, Ji H, Mangialaio S, et al. From systemic T cell self-reactivity to organ specific autoimmune disease via Ig. *Immunity* 1999;10:451–61.
- [50] Yin Z, Bahtiyar G, Zhang N, et al. IL-10 regulates murine lupus. *J Immunol* 2002;169:2148–55.
- [51] Kala M, Miravalle A, Vollmer T. Recent insights into the mechanism of action of glatiramer acetate. *J Neuroimmunol* 2011;235:9–17.
- [52] Assier E, Boissier MC, Dayer JM. Interleukin-6: from identification of the cytokine to development of targeted treatments. *Joint Bone Spine* 2010;77:532–6.
- [53] Ota SI, Niino H, Tsuzuki H, et al. Induction of regulatory B cells in healthy individuals and patients with systemic lupus erythematosus. *Arthritis Rheum* 2011;63:1766 [Abstract].
- [54] Richez C, Blanco P, Rifkin I, et al. Role for toll-like receptors in autoimmune disease: the example of systemic lupus erythematosus. *Joint Bone Spine* 2011;78:124–30.
- [55] Ochoa-Reparaz J, Mielcarz DW, Haque-Begum S, et al. Induction of a regulatory B cell population in experimental allergic encephalomyelitis by alteration of the gut commensal microflora. *Gut Microbes* 2010;1:103–8.
- [56] Olkhanud PB, Damsduren B, Bodogal M, et al. Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4+T cells to T regulatory cells. *Cancer Res* 2011;71:3505–15.
- [57] Fillatreau S. Novel regulatory functions for toll-like receptor-activated B cells during intracellular bacterial infection. *Immunol Rev* 2011;240:52–71.
- [58] Lundy SK, Boros DL. 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* 2002;70:812–9.
- [59] Harn DA, McDonald J, Atochina O, et al. Modulation of host immune responses by helminth glycans. *Immunol Rev* 2009;230:247–57.
- [60] Hussaerts L, van der Vlugt LE, Yazdanbakhsh M, et al. Regulatory B cell induction by helminths: implications for allergic disease. *J Allergy Clin Immunol* 2011;128:733–9.