The Many Faces of B Regulatory Cells

Zahava Vadasz MD and Elias Toubi MD

Division of Allergy and Clinical Immunology, Bnai Zion Medical Center, affiliated with Technion-Israel Institute of Technology, Haifa, Israel

KEY WORDS: B regulatory cells (Bregs), self-tolerance, autoimmunity, interleukin-10 (IL-10), transforming growth factor-beta (TGFβ)

IMAJ 2014; 16: 631–633

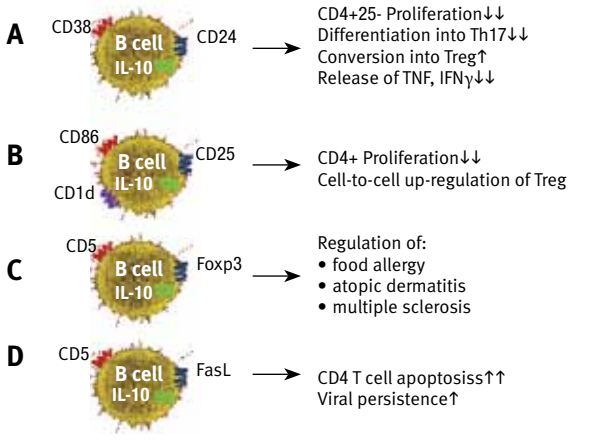
Autoimmune diseases develop when high titers of autoantibodies against self-antigens are continuously produced. More than 5% of the entire world population suffers from at least one of numerous autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), among others. The role of B cells in the development of autoimmune diseases has been growing persistently. Apart from being the source of autoantibodies, they are efficient antigen-presenting cells and producers of pro-inflammatory cytokines. Many studies focused on the complexity of B cell overactivity, namely, the overproduction of B cell-activating factor (BAFF), the escape of autoreactive B cells from apoptosis, and the unbalanced production of various inflammatory and protective cytokines. On the other hand, B cells are a source of inhibitory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGFβ). Depending on the signals that B cells receive, pro- or anti-inflammatory cytokines are produced, and the shift towards an inflammatory or a protective/suppressive response is induced.

B regulatory cells (Bregs) were first reported in a murine model of experimental autoimmune encephalomyelitis (EAE) showing that B cells are not required for the induction of EAE but they may contribute to immune regulation, resulting in complete recovery from acute EAE. In a later study, the stimulation of arthritogenic B cells with an agonistic anti-CD40 and collagen generated a subset of IL-10-producing B cells. The transfer of these B cells to syngeneic immunized mice prevented the induction of arthritis and ameliorated established disease by down-regulating Th1 cytokines [1]. During the last 5 years Bregs were intensively investigated in healthy humans and in many autoimmune diseases. Different phenotypic Bregs, inducing their regulatory functions via many different pathways and thereby influencing the course of viral infections, malignancies and autoimmune diseases, were identified.

HUMAN B REGULATORY CELLS

The question how to identify Bregs with membrane markers or transcription factors is yet unresolved. Also unresolved is how to better stimulate them in order to improve their regulatory properties. CD19+CD25+ B cells were the first subset of human B cells previously suggested to have a regulatory function. They were characterized as expressing high levels of immunoglobulins compared with CD19+CD25- B cells, but they lacked the ability to secrete them. They were defined as memory B cells (being CD27+) that secrete high levels of the inhibitory cytokine IL-10 compared with CD25- B cells [2]. Later, CD19+CD25high B cells were reported to be significantly higher in patients with anti-neutrophil cytoplasmic antibodies (ANCA)-related vasculitis when in remission, but much lower when disease was active. In an elegant study by Mauri et al [3], the presence of human Bregs, namely CD19+CD24highCD38high B cells, were identified as being able to suppress the differentiation of naïve T cells into T helper-1 (Th1) 1 and Th17 cells. They also converted CD4+CD25- T cells into regulatory T cells (Tregs) in a CD40-dependent way and through the production of IL-10 but not TGFβ. In healthy individuals, CD19+CD24highCD38high B cells suppressed CD4+CD25- T cell proliferation as well as the release of interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα) [Figure 1A]. This suppressive capacity was blocked by the addition of CD80 and CD86 monoclonal antibodies. When analyzed in SLE patients, these cells were refractory to further CD40 stimulation, produced less IL-10,

Figure 1. B regulatory cells are characterized by many membrane markers, whose role could be different in many diseases. They are reported as different subtypes: **[A]** CD38highCD24highIL-10high, **[B]** CD25highCD1dhighIL-10high, **[C]** CD5highFoxp3highIL-10high, **[D]** CD5highFasLhighIL-10high



and were incapable of suppressing Th1 proliferation compared to their ability in healthy individuals [3]. Later we characterized Breg cells as CD25highCD1dhighIL-10highTGF-βhigh. These were able to down-regulate CD4+ T cell proliferation when a co-culture, in a cell-to-cell-dependent way, suggesting that this regulatory function is CD86-dependent. Our other finding in this study was that Bregs were efficient in up-regulating autologous Treg cell properties, namely, enhancing FoxP3 and CTLA-4 expression in these Treg cells following Breg/Treg cell-to-cell co-culture [Figure 1B] [4].

Aiming to improve the characteristics of Bregs, we found that other regulatory markers were highly expressed on these cells. We showed that molecules such as semaphorin3A (a regulatory protein) and C72 (a regulatory B cell co-receptor) were mainly expressed on CD19CD25highIL-10high, suggesting CD19+CD25highCD72+sema3Ahigh cells to be a subset of Breg cells in humans; this needs further attention [5]. Trying to identify other markers for Bregs, IL-10 and TGFβ-producing B cells were found to highly express Foxp3 and CD5, suggesting CD19CD5highFoxP3highIL-10high to be a different subset of Breg cells. Looking into their possible involvement in inflammatory diseases, they were found to have a regulatory role in non-immunoglobulin E (IgE)-mediated food allergy and in atopic dermatitis [6]. In line with this, CD19+CD25+FoxP3+ cells were noticed to play a role in multiple sclerosis (MS). They were significantly higher in relapsing-remitting MS during relapse symptoms when compared to non-clinically active MS patients [Figure 1C]. Further evidence is required to establish the true presence of Foxp3+ B cells and to prove that these are indeed Bregs.

"KILLER" B REGULATORY CELLS

The FasLigand/Fas receptor axis has been studied extensively as a mechanism of killing CD4+ T cells and other immune cells, thereby preventing autoimmunity and cancer. Evidence has emerged that in addition to activated cytotoxic T cells (CTL) and natural killer (NK) cells, B lymphocytes were also found to express FasL, thus mediating cell death of many over-activated immune cells. Among B cell subsets, the expression of both FasL and IL-10 was highest on CD5+ B cells, suggesting this subset to be a unique one in the field of human Bregs. This subset of cells was found to be higher in aggressive forms of B cell lymphoma and during persistence of viral infections such as Epstein-Barr virus, suggesting their expansion to be one of the mechanisms by which tumor and infected cells may escape efficient immune responses. The above explains why this subset of Breg cells is defined as "killer"/regulatory cells, which serve to protect against the development of autoimmunity [Figure 1D] [7].

Granzym B (GzmB) represents a major component of the granules of NK cells and CTL. Classically, GzmB has been linked primarily to the induction of apoptosis in target cells after attack by CTL. Various autoimmune diseases have been linked to elevated levels of IL-21 and GzmB, which were shown to play an immunosuppressive and thereby protective role in the early phase of SLE. With this in mind, CD5high B cells were demonstrated to be IL-10 and GzmB producers playing an additional role in suppressing autoimmune responses. Aiming to assess the relationship between CD5+ B cells, IL-21 and GzmB in SLE patients, both IL-21 and GzmB serum levels were evaluated. Here, in vitro experiments showed that IL-21 directly induces GzmB expression and secretion by CD5+ B cells, suggesting again that CD5+GzmB+ are important disease-modifying players in the early phase of SLE [8].

IL-35 AND IL-21 AND B REGULATORY CELLS

In addition to their ability to produce IL-10 and TGFβ, Bregs were reported to produce IL-35, which is essential for their suppressive/regulatory function. In one study, IL-35 increased the ability of Bregs to suppress experimental autoimmune uveitis. Moreover, recombinant IL-35 inhibited lipopolysaccharide-driven B cell activation while inducing IL-10 production, thus suggesting IL-10high B cells to be IL-35high [9]. To identify signals that regulate IL-10 producing B cells in vivo, purified B cells were cultured with cytokines known to influence B cell function. Stimulation with IL-21, but not IL-4, IL-6, IL-12 and IL-23 induced 4.4 to 5.3-fold more IL-10 secretion at 48 and 72 hours respectively. IL-21 also induced a threefold increase in IL-10+ B cells within the spleen CD1dhighCD5high B cell subset, but did not induce IL-10+ B cells among the CD5 subset. To verify that T cell-derived IL-21 and CD40 signals drive B10 cell expansion and IL-10 production, B cells were cultured with anti-CD40 antibodies and B lymphocyte stimulator (BLyS) in the presence of IL-4. B cells were then cultured with exogenous IL-21 for 5 days, which was essential to optimally expand IL-10+ B cells and induce IL-10 production. The transfer of CD5+ B cells markedly reduced EAE disease severity in wild-type mice, explaining in part why EAE is exacerbated in the absence of IL-21 [10].

CONCLUSIONS

A better characterization of Bregs – their membrane markers, cytokine profile and contribution to self-tolerance – is needed. How to achieve their maximal regulatory effect should also be the subject of future studies.

Correspondence
Dr. E. Toubi
Division of Allergy & Clinical Immunology, Bnai Zion Medical Center, Haifa 33394, Israel
Phone: (972-4) 835-9659
email: elias.toubi@b-zion.org.il

References

1. Fillatreau S, Sweenie C, McGeachy M, Gray D, Anderson S. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol* 2002; 3: 944-50.
2. Amu S, Tarkowski A, Dorner T, Bokarewa M, Brisslert M. The human immunomodulatory CD25+ B cell population belongs to the memory B cell pool. *Scand J Immunol* 2007; 66: 77-86.
3. Blair PA, Norena LY, Flores-Borja F, et al. CD19+CD24hiCD38hi B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus. *Immunity* 2010; 32: 129-40.
4. Kessel A, Haj T, Peri R, Snir A, Melamed D, Sabo E, Toubi E. Human CD19+CD25hi 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.
5. Vadasz Z, Haj T, Balbir A, et al. A regulatory role for CD72 expression on B cells in systemic lupus erythematosus. *Semin Arthritis Rheum* 2014; 43: 767-71.
6. Noh J, Noh G. Allergen-specific responses of CD19hi and CD19low B cells in Non-IgE-mediated food allergy of late eczematous reactions in atopic dermatitis: presence of IL-17 and IL-32-producing regulatory B cells. *Inflamm Allergy Drug Targets* 2012; 11: 320-9.
7. Lundy S. Killer B lymphocytes: the evidence and potential. *Inflamm Res* 2009; 58: 345-57.
8. Hagn M, Ebel V, Sontheirmer K, et al. CD5+ B cells from individuals with systemic lupus erythematosus expresses granzyme B. *Eur J Immunol* 2010; 40: 2060-9.
9. Tedder T, Leonard W. Regulatory B cells – IL-35 and IL-21 regulate the regulators. *Nat Rev Rheumatol* 2014; 10: 452-3.
10. Yoshizaki A, Miyagaki T, DiLillo D, et al. Regulatory B cells control T-cell autoimmunity through IL-21 dependent cognate interactions. *Nature* 2012; 491: 264-8.