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Review

Regulatory B cells: Phenotype, function and role in transplantation

CM Wortel [1,2], S Heidt *

Department of Immunohaematology and Blood Transfusion, Leiden University Medical Center, The Netherlands

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ABSTRACT

While B cells are traditionally known for their roles in antibody production, antigen presentation and cytokine production, recent studies have highlighted the existence of B cells with regulatory properties, which have been termed Bregs, analogous to regulatory T cells (Tregs). Bregs have been found to play a role in autoimmune disease, malignancies, infections, and may also be involved in solid organ transplantation. Their main mechanism of action is by promoting the development of Tregs while suppressing effector $CD4^+$ and $CD8^+$ T cells, primarily by IL-10 secretion. In the field of transplantation evidence for an active role of Bregs is scarce. While the presence of Bregs has been associated with improved graft survival and operational tolerance in kidney transplant recipients, these findings are not without controversy. Since the majority of fundamental research on Bregs has been performed in the fields in autoimmunity and infectious diseases, we will first focus on what these fields taught us on basic Breg biology, after which the relevance for the transplant setting is discussed.

Contents

Abbreviations: AA, allergic asthma; AAD, allergic airway disease; AIA, antigen induced arthritis; APC, antigen presenting cell; APRIL, A proliferation inducing ligand; B10pro, B10 progenitor cell; BCR, B cell receptor; Br1, B regulatory 1 (Br1) cells; Bregs, regulatory B cell; CIA, collagen induced arthritis; CHS, contact hypersensitivity; DC, dendritic cell; EAE, experimental autoimmune encephalitis; EAMG, experimental autoimmune myasthenia gravis; GD, Graves' disease; GzmB, Granzyme B; HBV, Hepatitis B virus; HIV, human immunodeficiency virus; iNKT, inducible natural killer T cell; ITP, autoimmune thrombocytopenia; MS, multiple sclerosis; MG, myasthenia gravis; MZ, marginal zone; NK, natural killer cell; OVA, ovalbumin; PD-L1, programmed death-ligand 1; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T2-MPZ, transitional 2 marginal zone precursor; Tim-1, T cell immunoglobulin and mucin-domain containing protein 1; TLR, toll-like receptor; TCR, T cell receptor; Tregs, regulatory T cells; UC, ulcerative colitis.

* Corresponding author at: Leiden University Medical Center, Dept. of Immunohaematology and Blood Transfusion, Albinusdreef 2, 2333 ZA Leiden, The Netherlands.
E-mail address: s.heidt@lumc.nl (S. Heidt).
[1] Current working address: Department of Rheumatology, Leiden University Medical Center.
[2] CW and SH: wrote the article.

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1. Introduction

For many years B cells are known to play an important role in immune responses by producing antibodies, presenting antigens to T cells, and secreting cytokines. However, in recent years, an increasing body of work has been published which identified B cell subsets with regulatory functions, the so-called regulatory B cells (Bregs). Due to their regulatory effects, Bregs are an interesting cell population in diseases characterized by an unbalance in the immune system, such as autoimmune diseases, chronic infections, cancer and graft rejection. Indeed, in murine studies Bregs have been shown to be involved in dampening inflammation [1–3], were effective in ameliorating disease in several models of autoimmune disorders [4–8], and were involved in the induction of transplantation tolerance [9–11]. Analogous to the murine setting, human B cells with immunoregulatory capacity have been described. The existence of regulatory B cells is implied by patients showing exacerbated autoimmunity upon treatment with the B cell-depleting antibody rituximab [12,13].

Despite the vast number of publications on Bregs in the last decade many questions still remain unanswered. In this review, information will be provided on the discovery of regulatory B cells and what is known so far about their phenotype and function culminating in the description on their possible role in the transplantation setting.

2. Identification of Bregs

Although the term regulatory B cell originates from just 15 years ago [14,15], the first mentioning of B cells with anti-inflammatory properties dates back to the 1970s. In two independent studies, Katz et al. and Neta et al. observed an increase in severity and duration of contact hypersensitivity (CHS) responses in guinea pigs upon selective B cell depletion [16,17]. Already in that era, Neta et al. hypothesized an inhibitory effect of B cells on T cell activation [17]. In the decades thereafter only a few papers were published on the topic, possibly due to the fact that 'suppressor T cells' became rather unfashionable due to irreproducible results. In 1996, a renewed interest in Bregs emerged after Wolf and colleagues showed that B cell deficiency in mice caused a lack of spontaneous recovery from experimental autoimmune encephalitis (EAE), a mouse model of multiple sclerosis (MS) [18]. A few years later, several studies expanded on these findings and described that B cells exerted their regulatory effects via IL-10 in this model, but also in experimental ulcerative colitis (UC) and collagen-induced arthritis (CIA) [15,19]. Since then, many publications have described different mechanisms of immune regulation by various subsets of B cells.

3. Phenotypical characterization of Bregs

Since several B cell subsets with regulatory capacity have been described, there is no consensus about the exact phenotype of Bregs. This poses a major challenge in fully understanding their origin and function. In general, Bregs are identified functionally by their ability to produce the regulatory cytokine IL-10.

3.1. Bregs defined in murine studies

In mice, a multitude of Breg subsets with similarities in function and surface markers have been described, such as $CD19^+CD1d^{hi}CD5^+$ (B10) cells [1,7,20–26], $CD19^+CD1d^{hi}CD21^{hi}CD23^{hi}CD24^{hi}$ transitional 2 marginal zone precursor (T2-MZP) B cells [5,27,28], $CD19^+CD5^+CD21^{+-}CD23^-$ marginal zone (MZ) B cells [2,29,30], and even $CD138^+CD44^{hi}$ plasmablasts [8].

The most widely investigated subset of murine Bregs are the B10 cells, which are characterized by IL-10 production and have been shown to play a role in various animal models of autoimmunity [6,7,21,23,26], healthy pregnancy [20], and *Listeria* infections [1]. IL-10-producing B10 cells represent approximately 1–2% of cells in the murine spleen and are enriched in the subpopulation of splenic $CD1d^{hi}CD5^+$ B cells (10–20%) [21,22].

As the majority of $IL\text{-}10^+$ B cells fall outside of the $CD1d^{hi}CD5^+$ B cell population, the search for an inclusive murine Breg marker is still ongoing. T cell immunoglobulin and mucin-domain containing protein (Tim-1) has so far been the most inclusive marker of IL-10-secreting B cells. While Tim-1 (also known as Kidney Injury Molecule, KIM-1) was first identified as a marker of acute kidney injury [31], it became subsequently known as a T cell associated molecule, involved in the Th1/Th2 balance [32]. Ding et al. successively demonstrated that Tim-1 is predominantly expressed on B rather than T cells and that 70% of IL-10-producing B cells are Tim-1 positive [9]. $Tim\text{-}1^+$ B cells were found across several B cell subsets, such as $CD19^+CD1d^{hi}CD5^+$ cells, MZ B cells and B1 B cells, suggesting that B cell regulation is not limited to a single B cell subset. Tim-1 appears to be functionally involved in regulation, since mice lacking the Tim-1 mucin domain display spontaneous autoimmunity [9,33–35]. Ligation of Tim-1 with an agonistic Tim-1 antibody induces Breg expansion and cytokine production. Interestingly, Tim-1 appears to be important in the balance between regulatory and inflammatory B cell properties, since B cells with a Tim-1 defect produce less IL-10 but more IL-6, IL-1β, and IL-12 than wild type B cells [34].

It is important to keep in mind that $IL\text{-}10^+$ cells still represent a minority within these enriched subsets (5% in $Tim\text{-}1^+$ B cells, 10–20% in B10 cells) [9,21,22]. Moreover, IL-10-producing B cells are often detected in other B cell subsets as well [36]. Furthermore, limiting the definition of Bregs to cells capable of IL-10 production might exclude B cells with regulatory function, expressing for example IL-35 or TGF-β [37,38]. This notion is supported by experiments in which blocking of either IL-10 or the IL-10 receptor does not completely abolish the regulatory activity [39–41]. Furthermore, not all IL-10-producing B cells may contribute equally to immune suppression [36].

3.2. Bregs defined in human studies

Research on human Bregs is complicated by the limited access to the human spleen, the main site of Bregs in mice. Therefore, in humans, several Breg phenotypes have been identified in the peripheral blood only, ranging from early immature B cells up to fully differentiated plasmablasts. These include transitional B cells ($CD19^+CD24^{hi}CD38^{hi}$) [36,42–50], plasmablasts ($CD19^+CD27^{int}CD38^+$) [8] and B regulatory 1 (Br1) cells ($CD19^+CD25^+CD71^+CD73^-$) [39]. Furthermore, a human equivalent of B10 cells with a $CD19^+CD24^{hi}CD27^+$ phenotype was described by Iwata et al. [51–53] Recently, human $Tim\text{-}1^+$ Bregs have also been reported, and were preferentially present in transitional B cells [54]. An overview of the different subsets that have been published in studies on mice and humans is shown in Table 1 and Fig. 1.

Similar to the murine setting, no specific set of markers has been found to effectively and exclusively identify Bregs, and the stability of the regulatory capacity remains unknown. IL-10 production may be a temporal feature, as many Breg subsets display an immature phenotype, characteristic for cells in transition. Furthermore, the lack of consensus on gating strategies further diminishes the comparability of studies, increasing the difficulty in establishing a single Breg phenotype. These problems could be circumvented by the discovery of a Breg-specific transcription factor, equivalent to Foxp3 in Tregs. A few studies were recently published, appointing Foxp3 as a potential marker for Bregs as well, but remained inconclusive [55–57]. This apparent lack of a general Breg transcription factor supports the idea that the occurrence of IL-10-producing B cells may not be restricted to a specific lineage, but rather may be a hallmark of an inflammatory micro-environment, as will be discussed below.

4. Breg induction and development

Observations showing that Breg numbers increase during several types of infection and in models of autoimmunity support the notion