Review

B regulatory cells in cancer

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B regulatory cells are a newly described subpopulation of B cells that appear to play important roles in autoimmunity and more recently, in cancer. In this review we summarize our current knowledge of B regulatory cells, as well as the body of evidence pointing towards a role for B cells in general, and B regulatory cells in particular, in promoting tumor growth.

Regulatory B cells

B lymphocytes play important roles in adaptive immunity, fighting invading pathogens. Historically, the ability of B cells to differentiate into antibody secreting cells (ASCs) has been well investigated, less so their capacity to produce cytokines and the effect of these cytokines on other immune cells. In recent years, however, a distinct subset of B cells has been described that exerts significant immunoregulatory functions through the production of the immunosuppressive cytokine interleukin (IL)-10 [1].

The first evidence that B cells could exert a regulatory role came from murine studies of chronic intestinal inflammation [2] and experimental autoimmune encephalomyelitis (EAE) [3]. Both studies showed that these inflammatory conditions could give rise to IL-10-producing B cells. In the absence of this B cell subset, inflammation worsened. In the ensuing years since these initial studies were published, a considerable body of evidence has been collected, especially from autoimmune mouse models and patients with autoimmune diseases, which reinforces the notion of B cells as potential regulatory cells. These cells are variously called B regulatory cells, Bregs, or B10; in this article we refer to them as Bregs. Despite the extensive body of evidence gathered so far, some controversy over the role and phenotype of Bregs persists. The main problem encountered by researchers studying these cells is the paucity of markers that can unequivocally identify them. Different groups have described different sets of markers. For mouse Bregs, the two most widely accepted are Claudia Mauri's definition of Bregs as a subgroup of the transitional 2 (T2) B cells, defined as $CD19^{+}CD21^{hi}CD23^{hi}IgM^{hi}CD24^{hi}$ [4] and Thomas Tedder's description of B10 cells as $CD19^{+}CD5^{+}CD1d^{hi}$ [5] (Table 1). As mentioned earlier, none of these phenotyping strategies uniquely identifies Bregs, nonetheless Bregs are contained within these subgroups. There is still some controversy over whether these markers overlap in the identification of mouse Bregs.

The identification of Bregs in humans is even more controversial. Blair and coworkers have described human Bregs as $CD19^{+}CD24^{hi}CD38^{hi}$, a phenotype that normally defines human transitional B cells [6]. Furthermore, they showed that there was overlap with cells identified by the markers $CD5^{+}CD1d^{hi}$ (Table 1). This correlation, however, was not supported by recent evidence showing human Bregs, identified through IL-10 intracellular staining, to have similar CD5 and CD1d expression to non-IL-10-producing cells and being contained within the $CD24^{hi}CD27^{+}$ B cell subset [7]. Therefore, the evidence on how to identify human Bregs phenotypically is even more limited than for mouse Bregs. An additional controversial point is what impairment Bregs show in patients with autoimmune diseases. Blair *et al.* reported a higher percentage of Bregs within the peripheral blood mononuclear cell (PBMC) population but equivalent absolute numbers compared to healthy controls, however, Bregs from systemic lupus erythematosus (SLE) patients had a reduced capacity to produce IL-10 and impaired suppressive activity on T cell production of tumor necrosis factor (TNF)α and interferon (IFN)γ [6]. Iwata and coworkers, however, described a trend for an increased percentage of IL-10 producing B cells in patients with autoimmune diseases, including SLE [7]. Nonetheless, this study did not investigate the role played by Bregs from autoimmune disease patients on T cells. It remains to be clarified whether these discrepancies are simply due to the different strategies used to identify Bregs (membrane markers in one case and IL-10 intracellular staining in the second) or additional, still unidentified, reasons. A more recent publication has highlighted a role for IL-10-producing B cells in regulating suppression of T helper (Th)1 and Th17 responses in a model of collagen-induced arthritis, indicating that Bregs might be involved in the pathogenesis of additional inflammatory conditions [8].

The immune modulatory function of Bregs is mediated by their production of IL-10, an inhibitory cytokine utilized also by T regulatory cells or Tregs; unlike Tregs, however, Bregs do not seem to rely also on transforming growth factor (TGF)β to exert their inhibitory role and do not require cytotoxic T lymphocyte-associated antigen (CTLA)-4 for cell-contact-mediated inhibition [6]. By contrast, they appear to depend on CD80 and CD86, the co-stimulatory molecules for T cells expressed by B cells and other antigen-presenting cells, because blocking CD80/CD86 can reduce Breg-mediated inhibition of T cell function (Table 1) [6]. Furthermore, myeloid cells, as well as T cells, can be regulated by Bregs, producing lower levels of TNFα in co-culture [7].

Given their clear functional identity (i.e., IL-10 production) but elusive phenotype, many researchers have questioned whether Bregs exist as a true separate B-cell

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Table 1. Bregs populations in humans and mice

Species	Name	Specific markers	Immunoregulatory mechanisms	Refs
Human	Bregs	$CD19^{+}CD24^{hi}CD38^{hi}CD5^{+}CD1d^{hi}$	IL-10 – CD80/86	[6]
	Bregs	$CD24^{hi}CD27^{+}$	IL-10	[7]
Mouse	Bregs	$CD19^{+}CD21^{hi}CD23^{hi}IgM^{hi}CD24^{hi}$	IL-10	[4]
	B10	$CD19^{+}CD5^{+}CD1d^{hi}$	IL-10	[5]

subpopulation [9]. More recent evidence would support the view that IL-10 production by B cells is a transient function exerted by a subpopulation of B cells destined to differentiate into ASCs [10]. Murine Bregs and the ensuing ASCs were observed *in vivo* after lipopolysaccharide (LPS) treatment, which can also induce IL-10 production by Bregs *in vitro*. It is interesting to note that when *in vitro* stimulation with another Toll-like receptor (TLR) agonist, CpG, is combined with B cell receptor (BCR) stimulation, the number of IL-10-producing cells appears reduced compared to CpG stimulation alone [7]. This suggests that IL-10 production is a default phenotype of at least a subgroup of B cells: when they receive bystander stimulation through TLRs or CD40 in the absence of BCR activation, perhaps they automatically secrete IL-10 to limit excessive inflammation and tissue damage.

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Whether Bregs exist as a separate subpopulation or whether they are a transient state of a larger proportion of B cells, maybe a byproduct of B cell activation, their production of IL-10 and the ensuing immune regulation remains a well-defined characteristic. Until recently, however, Bregs have only been investigated in their role in autoimmune diseases or chronic inflammatory conditions. Nonetheless, a new wave of research is beginning to shed light on the possible roles of Bregs in cancer. A recent study even suggests that chronic lymphocytic leukemia (CLL) cells resemble Bregs in their phenotype and IL-10 secretion [11]; a first indication that Bregs might incur malignant expansion.

The T cell counterparts of Bregs, Tregs, have been described in many types of cancer and their immunosuppressive activity often promotes tumor growth, mainly by inhibiting the cytotoxic activity of $Th1/CD8^{+}$ cell responses [12,13]. Although a high number of Tregs does not always correlate with a worse prognosis in human and experimental cancers [14], it is clear that Tregs can exert inhibitory functions on other immune cells in the tumor microenvironment. Whether Bregs have a similar role is currently being investigated and some evidence to support this notion is beginning to emerge.

A first indication that B cells and antibodies could be tumor promoting came from studies conducted almost 60 years ago. In these early works, transfer of tumor-specific antibodies increased growth of transplanted tumor cells and chemically induced tumors [15,16], whereas absence of B cells limited tumor formation [17]. Subsequent studies have confirmed this tumor-promoting role and have offered more mechanistic insight. Coussens and coworkers showed how B cells supported tumor growth in a mouse model of skin carcinogenesis [18,19]. These studies highlighted how B cell deficiency in mice reduced infiltration of immune cells in premalignant lesions, leading to inhibition of skin carcinoma development. Despite the low B cell infiltration in the premalignant lesions, B cells exert a distal effect on carcinoma development by secreting antibodies that are deposited at tumor sites in the form of immune complexes (ICs). Through Fcγ-activating receptors binding to ICs, myeloid cells are recruited to tumor sites and macrophages primed to become tumor promoting, secreting proangiogenic factors and immunoregulatory cytokines.

A similar role for B cells was reported in a syngeneic mammary cancer mouse model, in which tumor growth was reduced after partial deletion of B cells by treatment with anti-mouse IgM/IgG antibodies [20]. Moreover, B cell deficiency in mice (Box 1) was associated with an enhanced T cell antitumor immune response to a syngeneic adenocarcinoma tumor [21]. A more recent study confirmed these results and also suggested that B cells exerted their suppressive activity towards T cells partly via the co-stimulatory protein CD40 [22]. Subsequently, Inoue *et al*. confirmed these results in additional tumor models; they went on to illustrate that T cell inhibition by B cells was mediated by their IL-10 production [23], possibly providing the first hint that Bregs could be involved in cancer. Finally, Ammirante *et al*. showed how B cell-derived lymphotoxin is also implicated in promoting tumor development in castration-resistant prostate cancer in mice [24]. Following castration, the authors showed a transient increase in leukocyte infiltration that included B cells within the regressing tumors. In this context, production of lymphotoxin α/β by B cells was responsible for the androgen-independent activation of inhibitor of κB α-kinase (IKKα) and signal transducer and activator of transcription (STAT)3 in tumor cells, which favored relapse of the

Box 1. B cell deficient genetic mouse models

- **Rag1 and Rag2 KO mice ($Rag1^{-/-}$ or $Rag2^{-/-}$)**
  Deficient in both B cells and T cells. Loss of recombination-activating gene 1 or 2 results in the inability to initiate VDJ recombination and therefore leads to the absence of the BCR and TCR, which are essential for the differentiation of mature B cells and T cells [35,36].
- **μMT mice**
  A stop cassette is inserted in the μ chain of IgM. Loss of IgM expression results in loss of mature B cells [37].
- **Igβ KO mice**
  Igα and Igβ are the signaling components that associate with the immunoglobulin protein to form the BCR. Igβ loss also causes disruption of BCR membrane expression, which results in almost complete disruption of the mature B cell compartment [38].
- **J_H mice**
  Lack immunoglobulin assembly due to a targeted disruption of the gene coding for the heavy chain J loci (J_H). Mice are deficient in mature B cells and unable to secrete immunoglobulins of any subclass [39].