Perspective

The Emerging Field of Regulatory B cell Immunometabolism

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ABSTRACT

B cells are well-known as critical mediators of humoral immune responses via the production of antibodies. However, numerous studies have also identified populations of B cells that are characterized by their anti-inflammatory properties. These "regulatory B cells" restrain excessive inflammatory responses in a wide range of health conditions. A significant knowledge gap remains concerning the nature of the signals that determine whether a B cell exerts a pro-inflammatory or anti-inflammatory function. In this perspective, we explore the concept that in addition to the cytokine microenvironment, intracellular and extracellular metabolic signals play a pivotal role in controlling the balance between regulatory and antibody producing B cell subsets. Determining the metabolites and tissue-specific signals that influence B cell fate could establish novel therapeutic targets for the treatment of diseases where abnormal B cell responses contribute to pathogenesis.

MAIN TEXT

Introduction

B cells play a pleiotropic role in the immune system. Most recognized for their ability to produce antibody, B cells can also present peptide and lipid antigens, and secrete a wide range of pro- and anti-inflammatory cytokines (Mauri and Bosma, 2012). The

production of immunosuppressive cytokines is attributed to a specialized subset of B cells known as regulatory B cells or ‘Bregs’, which via the provision of cytokines such as IL-10, IL-35 and TGFβ restrain the differentiation of pro-inflammatory T cells and myeloid cells in a wide-range of inflammatory environments (Mauri and Bosma, 2012, Rosser and Mauri, 2015). Both the production of antibodies and immunosuppressive cytokines are dependent upon the activation of B cells via Thymus (T)-dependent, T-independent antigens and/or co-stimulatory signals such as CD40 ligand (CD40L and B cell activating receptor (BAFF-R) (Mauri and Bosma, 2012).

In the resting state, B cells are naturally quiescent. However, following activation of the B cell receptor (BCR), Toll-like receptors (TLR) and/or cell-surface co-stimulation molecules, B cells undergo an intense period of proliferation and rapid upregulation in biomass, which is fundamental for their ability to produce both antibodies and cytokines. Although understudied when compared to T cell metabolism, new studies are starting to clarify the requirements that allow these highly energy-dependent processes to take place. TLR-signaling and engagement of the BCR increases oxygen consumption by B cells and a marked upregulation in glucose and amino acid transport (Caro-Maldonado et al., 2014, Cantor et al., 2009). Whereas in T cells increased glucose uptake is associated with ‘aerobic glycolysis’, in B cells this glucose is prevalently utilized in the pentose phosphate pathway (PPP) to produce nicotinamide adenine dinucleotide phosphate (NADPH) and ribose 5-phosphate, supporting the generation of the new ribosomes, or riboneogenesis, which is fundamental for supporting antibody production (Waters et al., 2018). Key transcriptional regulators that have been shown to control metabolic rewiring following B cell activation include the phosphoinositide 3-kinase (PI3K) signaling cascade downstream of BCR, TLR and BAFF-R signaling and activation of mTOR (Saxton and Sabatini, 2017, Jellusova and Rickert, 2016, Boothby and Rickert, 2017). These processes in the context of the humoral immune response and germinal centre reaction have been expertly reviewed elsewhere (Boothby and Rickert, 2017, Choi and Morel, 2020).

However, as similar signals (BCR-activation, TLR-signaling) induce Breg differentiation, it is important to now clarify whether humoral immune responses and immunoregulatory responses within the B cell compartment are dictated by different

metabolic stimuli. Notably, it has been previously reported that manipulation of metabolic pathways (e.g. inhibition of mTOR with rapamycin) can tip the balance between regulatory and effector T cells (Sun et al., 2017). Thus, further exploration of how metabolic pathways respond to “environmental changes” and how this affects B cell differentiation and maturation could lead to the discovery of novel therapeutics that skew B cell in favour of a regulatory phenotype. These type of therapies could then be given stand-alone or in conjunction with B cell depletion therapies (e.g. Rituximab) to promote the repopulation of Bregs in patients with autoimmune disease, where the balance between antibody-producing and regulatory subsets of B cells is thought to be key determinant in disease severity and response to therapy (Flores-Borja et al., 2013b, Bosma et al., 2012b). In this perspective, we explore how intracellular and extracellular metabolic pathways may be influencing the immunoregulatory phenotype of B cells, to enhance the discussion of how metabolic stimuli may be shaping the B cell compartment - a topic that remains underexplored in the field of immunometabolism. To do this, we start by giving a brief overview of what is currently known about Breg biology before addressing the questions*: What intracellular bioenergetic pathways control Breg function? How do extracellular metabolites impact Breg differentiation? Can Bregs be found in metabolically active tissue sites?* This allows us to carry out an in-depth discussion of new studies that describe a pivotal role for metabolic rewiring and nutrient availability in controlling the immunoregulatory function of B cells.

Regulatory B cells: origin, phenotype and function.

The principal functional feature of a Breg is a potent immune-suppressive capacity (Mauri and Bosma, 2012). Originally thought to act primarily via the production of IL-10, numerous studies have now demonstrated that Bregs can also suppress inflammatory responses via IL-10 independent mechanisms (Figure 1A-F). By employing these co-operative mechanisms of immunosuppression, Bregs limit tissue damage during immune-mediated inflammatory disorders and suppress immune surveillance mechanisms that prevent cancer and chronic viral infections (Mauri and Bosma, 2012). More recently, the participation of Bregs in limiting inflammatory responses have been extended to pathologies where low-grade inflammation has been implicated in disease pathogenesis including depression (Ahmetspahic et al., 2018) and atherosclerosis (Strom et al., 2015). A greater understanding of Breg

phenotype, induction and function could facilitate the identification of novel therapeutic strategies that could be applied to different pathological settings.

Heterogenous extracellular markers have been used to define Breg subsets in mouse and humans *in vivo* (Table 1), with a substantial overlap in the markers used to designate IL-10 producing Bregs and non-IL-10 dependent Bregs. However, as no specific transcriptional marker has been identified that exclusively defines 'the Breg phenotype' in mouse or humans, many questions remain concerning whether Bregs are a defined lineage of cells and whether their immunoregulatory phenotype remains stable within different inflammatory environments. Despite this, there is evidence that certain subsets of B cells are predisposed to exert immunoregulatory functions and, in particular, are poised to produce IL-10. Subsets that are enriched for IL-10 producing B cells include populations that contain a high number of autoreactive B cells such as splenic marginal zone (MZ) B cells (Miles et al., 2012), peritoneal B-1 B cells (O'Garra et al., 1992, Geherin et al., 2016, Wu et al., 2019) and antibody-producing B cell subsets in mice (Matsumoto et al., 2014, Lino et al., 2018), as well as antibody-producing and immature B cells in humans (Blair et al., 2010, Liu et al., 2016, Matsumoto et al., 2014). The overlap between B cell subsets that control regulatory and autoreactive inflammatory responses suggests that there is a subtle balance between the signals that control B cell fate decisions. The increased propensity to produce IL-10 within each subset is likely to be defined epigenetically, with the chromatin landscape guiding the transcriptional signature needed to produce IL-10 conserved prior to, and following, IL-10 production. Murine IL-10^{neg}CD21^{hi}CD24^{hi} splenic B cells, which are enriched for precursors for IL-10 producing B cell subsets, have increased chromatin accessibility at the transcription start site and upstream of the *Il10* locus when compared to IL-10^{neg}follicular B cells, which produce low levels of IL-10 (Piper et al., 2019), whilst murine B cells with the capacity to produce IL-10 can be defined by a unique methylation status and in particular, by hypomethylation around the *Il10* locus (Lino et al., 2018, Tonon et al., 2019). These data are not dissimilar from studies demonstrating that epigenetic modifications around the *foxp3* locus are important for Treg stability (Lal et al., 2009).

The enrichment of IL-10 producing B cells within autoreactive and antibody producing B cell subsets has led to questions concerning what microenvironmental signals

decide upon whether a B cell exerts a regulatory or inflammatory function. Well-defined signals that induce IL-10 production by B cells in both mouse and humans include toll-like receptor (TLR) agonists, such as lipopolysaccharide (LPS) and CpG, and molecules upregulated on activated T cells and type 3 innate lymphoid cells, such as CD40-ligand (CD40L) (Mauri and Bosma, 2012, Komlosi et al., 2018). More recently, there has been an interest in understanding how soluble microenvironmental signals, and in particular how cytokines, influence the polarization of naïve B cells into Bregs. Cytokines, such as IL-21, IL-6, IFNα, IL-1β, IL-33, IL-35 and the synthetically produced GIFT-15, a fusokine created by fusing GM-CSF and IL-15 together, have all been shown to induce Breg differentiation (Dambuza et al., 2017, Rosser et al., 2014, Menon et al., 2016a, Yoshizaki et al., 2012, Sattler et al., 2014, Rafei et al., 2009, Wang et al., 2014) (Figure 2A, B). B cell helper cytokines BAFF and APRIL that control metabolic rewiring of naïve B cell subsets have also been shown to induce IL-10 production by B cells. In mice, BAFF induces Bregs by engaging its receptor TACI, and upregulating the BAFF-induced transcription factor AP-1, which binds directly to the *Il10* locus (Yang et al., 2010) (Figure 2C), while in humans APRIL induces IL-10 production by, and PDL1 upregulation on, B cells by upregulating STAT3 phosphorylation (Hua et al., 2016, Fehres et al., 2019) (Figure 2D).

As well as inducing IL-10, many of these signals also induce antibody-producing B cell differentiation. In mice and humans, TLR-stimulation and interactions with CD40L-expressing T cells are well known to increase antibody production by B cells. Exposure of B cells to IL-6, IL-21 and IFNα also increases the expression of IRF4 and Blimp-1, key transcriptional regulators that control plasma cell differentiation, and have now been shown to bind to, and induce transcription of, the *Il10* locus (Matsumoto et al., 2014, Bibby et al., 2020). In addition, increased levels of BAFF in patients with autoimmunity, has also been associated with a breakdown in B cell tolerance and autoantibody production (Mackay et al., 2007). There remains a limited understanding of how similar signals can induce both antibody and/or IL-10 production by B cells. Previous reports from our laboratory have suggested that the induction of regulatory versus antibody-producing B cell responses may depend upon the concentration of cytokines within the immediate microenvironment (Menon et al., 2016a). However, more recent data has suggested that activation of particular intracellular bioenergetic

pathways (e.g. glycolysis) and the concentration of microbiota-derived or dietary metabolites (e.g. short-chain fatty acids, vitamin D) may also play an important role in controlling this fine balance within the B cell compartment.

What intracellular bioenergetic pathways control Breg function?

As described above, CD40 activation, TLR-agonists, BAFF-R signaling and engagement of the B cell receptor (BCR) all lead to metabolic rewiring in resting B cells, controlling both class switch recombination and plasma cell differentiation (Boothby and Rickert, 2017), suggesting that these signals also activate intracellular bioenergetic pathways in Bregs. Very few studies have been conducted that directly unravel the bioenergetic needs of Bregs compared to other B cell subsets. New evidence suggest that activation of Bregs, similarly to other B cell responses as described above, is highly dependent upon an increase in glycolytic activity. As well evidence garnered from studies that demonstrate that stimulation of naïve B cells with anti-CD40, anti-IgM, and/or LPS, signals that have been shown to induce IL-10 production by B cells and increase glycolytic flux (Waters et al., 2018), there are a handful of new studies that directly investigate Breg metabolism. Hypoxia-inducible factor (HIF)-1α, a transcription factor that is known to respond to decreases in available oxygen in the extracellular environment, has been recently shown to regulate IL-10 transcription in, and expansion of, $CD5^{+}CD1d^{hi}$ IL-10 producing B cells in mice (Meng et al., 2018) (Figure 2E). Notably, HIF1α is widely expressed within the immune system and can be detected in virtually all innate and adaptive immune cell subsets. In $CD5^{+}CD1d^{hi}$ B cells, HIF1α regulates glycolysis and the expression of glycolytic genes (Meng et al., 2018), with $CD5^{+}CD1d^{hi}$ B cells displaying increased glucose uptake compared to $CD5^{-}CD1d^{lo}$ B cells (Meng et al., 2018). HIF-1α, in conjunction with pSTAT3, also transcriptionally regulates IL-10 production in B cells by binding to putative hypoxia-responsive elements (Meng et al., 2018). These data suggest that low oxygen levels in cancerous or inflammatory tissues could be a driving factor in Breg development by supporting glycolysis – a hypothesis supported by data demonstrating that in humans Bregs can be found infiltrating certain tumors, and, importantly, that in breast cancer their presence is associated with poor disease prognosis (Ishigami et al., 2019).

As well as the utilization of glucose by murine B10 cells, a recent study by Bibby and collaborators has suggested that changes to intracellular cholesterol metabolism contributes to signaling pathways that control IL-10 production by human B cells (Bibby et al., 2020). Inhibition of cholesterol metabolism with atorvastatin, an HMG-CoA reductase inhibitor, suppressed IL-10 production and the ability of B cells to suppress effector T cell differentiation following stimulation of TLR9 with CpG (Bibby et al., 2020). More specifically, isoprenylation, a post-translational modification dependent upon cholesterol, was found to control signaling downstream of TLR9-activation (Bibby et al., 2020). Whether different IL-10-inducing stimuli activate different metabolic pathways, or whether there is some synergy concerning common adaptor molecules in these pathways is yet to be identified. The paucity of data that has been gathered regarding the intracellular metabolic pathways that control Breg function leaves a significant knowledge gap, with many potential exciting opportunities for future research.

How do extracellular metabolites impact Breg differentiation?

Further to the cytokine stimuli, new evidence suggests that exposure to microbiota derived and dietary extracellular metabolites within the immediate microenvironment may play an important role in determining the balance between antibody producing and Breg. As well the discovery that oxygen-sensing HIF1α controls IL-10 transcription in B cells (Meng et al., 2018), we have recently identified that the aryl-hydrocarbon receptor (AhR), a ligand activated transcription factor that senses dietary, microbial and metabolic cues, binds to the *Il10* locus in murine B cells (Piper et al., 2019). AhR is highly expressed in splenic $CD19^{+}CD21^{hi}CD24^{hi}$ Bregs and controls IL-10 production by these cells in response to certain stimuli (Piper et al., 2019, Rosser et al., 2020). Importantly, AhR acts to repress a proinflammatory transcriptional programme in B cells, thus preserving Breg stability under Breg-polarising conditions (Piper et al., 2019). In the search to identify stimuli that activate AhR in B cells, we have found that supplementation with the microbially-derived short-chain fatty acid (SCFA) butyrate, which is produced when components of the gut-microbiota metabolize complex carbohydrates from the diet, supports Breg function by amplifying AhR-activation. Amplifying AhR activation by butyrate supplementation suppresses the expression of transcriptional factors in $CD19^{+}CD21^{hi}CD24^{hi}$ Bregs that control GC

B cell and plasmablast differentiation, while enhancing Breg suppressive activity. This leads to a suppression in experimental arthritis (Rosser et al., 2020). Butyrate does not directly activate AhR-dependent gene transcription but rather alters the components of the microbiota, changing the balance between tryptophan-derived metabolites, increasing the availability of the serotonin-derived metabolite 5-Hydroxyindole-3-acetic acid (5-HIAA) and dampening the production of kynurenic acid (Rosser et al., 2020) (Figure 2F). These data suggest that microbe-derived metabolites may uncouple the need for inflammatory stimuli in the induction of Bregs by activating pathways that halt the transcription of genes required for GC and plasmablast differentiation whilst favouring the transcription of IL-10. Importantly, we also found that in humans there is a correlation between butyrate levels and IL-10^{+}Bregs in patients with rheumatoid arthritis, suggesting that butyrate may support Breg function in both mouse and humans (Rosser et al., 2020).

It has also been demonstrated that other SCFAs pentanoate and acetate can support IL-10 production by B cells subsets (Daien et al., 2021, Luu et al., 2019). Pentanoate, also known a valerate (C5), potentiates IL-10 production in CpG-stimulated murine B cells by increasing glycolysis, glycolytic activity and mTOR activation in B cells (Luu et al., 2019) (Figure 2G). Acetate promotes IL-10 production by B1a cells in mice and $CD19^{+}CD24^{hi}CD27^{+}$ B cells in humans by increasing the production of acetyl coenzyme A, fueling the TCA cycle and posttranslational lysine acetylation (Daien et al., 2021) (Figure 2H). In this study, the authors demonstrate that short-term dietary fibre intervention with FibreMax increases both acetate levels in the blood and B10 differentiation in human (Daien et al., 2021). Of note, metabolites derived from essential vitamins have also been shown to act as ‘Breg-supporting’ metabolites with the bioactive form of Vitamin D_3, Calcitriol, promoting IL-10 production by human B cells (Heine et al., 2008). Taken in conjunction with our work, these studies highlight that dietary modulation or supplementation could be a novel safe and effective way to modulate B cell responses *in vivo.*

Historical data have also demonstrated that Bregs can act to modulate the extracellular metabolic environment themselves. Bregs suppress both T and B cell proliferation by modulating extracellular levels of the metabolites adenosine triphosphate (ATP), adenosine diphosphate (ADP) adenosine 5’monophosphate

(AMP), and adenosine (ADO) through expression of the ecto-nucleotidases CD39 and CD73 in both mice and humans: CD39 catalyses ATP to ADP and AMP, whilst CD73 catalyses AMP to ADO (Kaku et al., 2014, Saze et al., 2013) (Figure 1E). Although these studies were carried out before the advent of 'immunometabolism' as a field, they demonstrate a reciprocal relationship between extracellular metabolite availability and Breg functionality. Future research is needed to address this relationship in more detail, and specifically whether Breg induction and function is altered within different healthy and diseased tissue-sites depending upon the quantity of different nutrients and metabolites within the immediate microenvironment.

Can Bregs be found in metabolically active tissue sites?

The majority of studies in human and mouse have focused upon the phenotypical and functional assessment of Bregs in the peripheral blood or secondary lymphoid tissue, respectively. However, an emerging concept is that Bregs can be found directly within peripheral tissues to regulate immune responses *in situ*. In particular, there is evidence that Bregs are found within metabolically active tissue including the white adipose tissue, gut and liver. In a seminal study by Nishimura and collaborators, Bregs were found to be present within the adipose fat, with deletion of IL-10 producing Bregs enhancing adipose inflammation and insulin resistance in mice fed with a high fat diet (Nishimura et al., 2013). In secondary lymphoid tissues, IL-10 production by Bregs is induced in response to extracellular signals such as pro-inflammatory cytokines or cellular signals such as activated $CD4^+$ T cells. However, within this study, adipose Bregs were found to constitutively express IL-10, demonstrating a role for adipose chemokine and metabolic tissue-specific signals, such as CXCL12 and free fatty acids, in supporting Breg function and IL-10 production. These data provide further evidence that particular metabolites within the immediate microenvironment may support Breg induction over certain inflammatory B cell responses. Of interest, adipose Bregs have a distinct phenotype compared to other peripheral Breg subsets suggesting possible adaptations to the tissue site (Nishimura et al., 2013). These results have recently been validated in humans, with data demonstrating that there is a reduction in Breg numbers in the adipose fat from obese patients compared to healthy controls (Garcia-Hernandez et al., 2018). Bregs have also been found in pericardial fat, where they regulate the outcome of acute myocardial infarctions in mice (Wu et al., 2019). IL-10 producing B cells within the pericardial fat were found to have

a phenotype reminiscent of B1 lineage cells, characterized by the expression of CD5. Thus, similarly to numerous Breg subsets described in the peripheral blood and secondary lymphoid tissues, tissue-residing IL-10 producing B cells may display heterogenous phenotypes based on the site and inflammatory environments in which they are identified. Characterising how B cell targeting therapeutic agents (e.g. Rituximab) influence the homeostasis of white adipose tissue, and the impact of B cell depletion therapies on associated metabolic pathologies such as type 2 diabetes and cardiovascular disease, could give further novel insights into the function of Bregs in adipose tissue deposits.

Considering the intimate connection between microbially-derived metabolites such as SCFAs and Breg function, it is perhaps unsurprising that another important reservoir of Bregs is within SCFA-rich sites such as the gut and liver (Cummings et al., 1987). IL-10 producing B cells are found within healthy gastric and hepatic tissue in humans (Liu et al., 2016, Murakami et al., 2019) and in situations where suppression of immune surveillance is an important prognostic factor for disease outcomes, Bregs are expanded within these sites. For instance, Bregs are expanded in patients with gastric cancer, and similarly to breast cancer, their presence and frequency within tumors are associated with worse long-term outcomes (Murakami et al., 2019). Bregs have also been shown to be expanded within the liver of patients with chronic hepatitis B infection, where they act to suppress local anti-viral $CD8^+$ T cell responses (Liu et al., 2016, Das et al., 2012). Preliminary studies from mice have suggested that migration of immune-suppressive B cells into colorectal cancers is controlled by micro-RNA 15A and 16-1 (Liu et al., 2018) suggesting that Bregs within the liver and gut may be recruited rather than naturally-occuring tissue-resident cells. However, future work is needed to establish whether there is also an unappreciated role for potential Breg-inducing metabolic signals such as SCFA or low oxygen levels at supporting Breg differentiation *in situ* at particular tissue sites.

Concluding remarks

In this perspective we have highlighted new studies that pinpoint a new emerging role for metabolic signals, both intracellular and extracellular, in controlling Breg function. These new data provide novel insights into Breg biology but also further highlight the intimate connection between the differentiation of regulatory versus antibody

producing B cell subsets. In recent years, similar cytokine microenvironmental signals have been identified that control both these subsets of effector B cells, but the new studies discussed in this perspective have begun to identify divergent pathways that control different outcomes in B cell fate. In particular, by demonstrating that certain extracellular metabolites (e.g. SCFAs, 5-HIAA, free fatty acids) may preferentially support Breg responses over antibody production. Although the way in which nutrient/metabolite availability controls B cell fate decisions remains a relatively unexplored phenomenon, considering the cornucopia of metabolites that have been shown to regulate T cell function, it is likely that a similarly large array of metabolites will also regulate B cell and therefore Breg responses. There is also an unmet need for better definition of the cellular metabolic pathways that regulate Breg bioenergetic demands. Technical difficulties in carrying out metabolic assays due to their cell "hungry" nature may have impeded this study of Breg cellular immunometabolism. However, new techniques such as "SCENITH", a flow based method that allows single cell resolution of metabolic pathways in multiple subsets simultaneously *ex vivo*, should allow better resolution of metabolic requirements for smaller population of immune cells such as IL-10 producing Bregs (Arguello et al., 2020). Identifying the metabolites, bioenergetic pathways, and tissue-specific signals may open possibilities where repurposing of drugs used for metabolic conditions could be used to drive B cell fate decisions following B cell depletion. Considering that the balance between antibody producing B cells and IL-10 producing B cells within the tissue-site is likely to be a key determinant of disease outcomes in both autoimmunity and cancer, greater resolution of the signals that tips this balance in either direction is an important consideration for future research.

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FIGURE LEGENDS

Figure 1. Mechanisms of regulatory B cell-mediated suppression. A, Regulatory B cells (Bregs) suppress inflammatory leukocyte function through the production of immunosuppressive cytokines such as IL-10, TGFβ and IL-35 (Shen et al., 2014, Tian et al., 2001, Parekh et al., 2003, Mauri and Bosma, 2012). **B**, Bregs induce tolerogenic dendritic cells (DCs) through the production of the enzyme thrombospondin-1 (TSP1), which converts latent TGFβ into active TGFβ. Bregs produce Granzyme B (GNZM-B), which degrades T cell receptors (TCR) on $CD4^+$ T cells (Lindner et al., 2013, Zhang et al., 2013). **C**, Bregs skew T cell and iNKT cell differentiation in favor of a regulatory phenotype through antigen-presentation of protein antigens in MHCII molecules and lipid antigens in CD1d molecules, respectively (Oleinika et al., 2018, Yoshizaki et al., 2012). **D**, PD-$L1^+$ Bregs suppress pro-inflammatory T cell function by engaging PD-1 (Khan et al., 2015). **E**, Bregs express the ecto-5-nucleotidase CD73 and CD39, which hydrolyze exogenous adenosine triphosphate (ATP) to 5′-adenosine monophosphate (AMP) and adenosine (ADO). AMP/ADO have been previously shown to suppress inflammatory T cell function (Saze et al., 2013, Kaku et al., 2014). **F**, $FasL^+$ B cells suppress pro-inflammatory T cell differentiation by inducing target cell death by engaging Fas (Lundy, 2009).

Figure 2. Microenvironmental stimuli that induce regulatory B cell differentiation. A, The cytokines IL-21, IFNα, IL-33, IL-35, the IL-35 subunit IL-12p35, and the fusokine GIFT-15 (formed of GM-CSF and IL-15) induce IL-10 production by B cells (Dambuza et al., 2017, Rosser et al., 2014, Menon et al., 2016b, Yoshizaki et al., 2012, Sattler et al., 2014, Rafei et al., 2009, Wang et al., 2014). **B**, IL-6 and IL-1β induce IL-10 production by B cells by increasing phosphorylation of STAT3 and NFκB (Rosser et al., 2014). **C,** APRIL induces IL-10 production by B cells by increasing phosphorylation of STAT3 (Hua et al., 2016, Fehres et al., 2019) and **D,** BAFF induces IL-10 production by B cells by inducing AP-1, which binds to the *Il10* locus (Yang et al., 2010). **E,** Low oxygen levels and activation of hypoxia inducible factor 1 alpha (HIF-1α) supports Breg function by increasing glucose uptake and Breg expansion. pSTAT3 and HIF1α also bind to the *Il10* locus and induce transcription (Meng et al., 2018). **F,** The short-chain fatty acid Butyrate increases the availability of the tryptophan derived metabolite 5-Hydroxyindole-3-acetic acid (5-HIAA), which in turn, activates the

ligand-activated transcription factor aryl-hydrocarbon receptor (AhR), increasing IL-10 transcription in B cells (Rosser et al., 2020). **G,** The short-chain fatty acid pentanoate upregulates IL-10 production by B cells by increasing glycolytic activity and the expression of hexokinase-2 (HSK2) and by increasing mTOR activation (Luu et al., 2019). **H,** Acetate promotes IL-10 production by B1a cells in mice and $CD19^{+}CD24^{hi}CD27^{+}$ B cells in humans by increasing the production of acetyl coenzyme A, fueling the TCA cycle and posttranslational lysine acetylation (Daien et al., 2021).

Type of Breg	Mouse	Human	Tissue Site	Suppressive mechanism	Suggested references

T2-MZP	$CD19^{+}CD21^{hi}$ $CD23^{hi}CD24^{hi}$ $CD1d^{hi}$	-	Spleen, mesenteric lymph-nodes	IL-10, CD1d-lipid presentation	(Evans et al., 2007, Blair et al., 2009, Rosser et al., 2014, Carter et al., 2011, Oleinika et al., 2018, Schioppa et al., 2011)
AhR^{+}Bregs	$CD19^{+}CD21^{hi}$ $CD24^{hi}AhR^{+}$	-	Spleen	IL-10	(Piper et al., 2019, Rosser et al., 2020)
Immature B cell	-	$CD19^{+}CD24^{hi}$ $CD38^{hi}CD1d^{hi}$	Peripheral blood, liver	IL-10, CD80/86	(Blair et al., 2010, Liu et al., 2016, Flores-Borja et al., 2013a, Menon et al., 2016a, Bosma et al., 2012a)
MZ	$CD19^{+}CD21^{hi}$ $CD23^{-}$	-	Spleen	IL-10	(Miles et al., 2012, Gray et al., 2007, Bankoti et al., 2012)
B10	$CD19^{+}CD5^{+}$ $CD1d^{hi}$	$CD19^{+}CD24^{hi}$ $CD27^{hi}$	Spleen, peripheral blood, gastric tissue, gastric cancer	IL-10	(Yanaba et al., 2008, Iwata et al., 2011, Murakami et al., 2019, Daien et al., 2021, Meng et al., 2018)
Adipose Breg	$CD19^{+}CD1d^{lo}$ $CD5^{lo}CD11b^{lo}$ $CD21^{lo}CD23^{lo}$ $CD25^{+}CD69^{+}$ $CD72^{hi}$ $CD185^{-}IgM^{+}$ $CD196^{+}IgD^{+}$	$CD19^{+}CD27^{+}$ $CD38^{hi}$	Adipose tissue	IL-10	(Nishimura et al., 2013, Garcia-Hernandez et al., 2018)
B1a	$CD19^{+}CD5^{+}$	-	Peritoneum, pericardial fat, skin	IL-10, IgM	(O'Garra et al., 1992, Geherin et al., 2016, Wu et al., 2019)
Natural Regulatory Plasma cell	$B220^{+}Lag3^{+}CD138^{+}MHC\text{-}11^{lo}$	-	Spleen	IL-10, IL-35	(Lino et al., 2018, Neves et al., 2010, Shen et al., 2014)
Plasmablast	$CD138^{+}$ $CD44^{hi}$	$CD19^{+}CD24^{hi}$ $CD27^{int}$	Draining LN, peripheral blood, spleen	IL-10	(Matsumoto et al., 2014)
Br1	-	$CD19^{+}CD25^{+}$ $CD71^{hi}CD73^{lo}$	Peripheral blood	IL-10, IgG_4	(van de Veen et al., 2013)
$Tim\text{-}1^{+}$Breg	$CD19^{+}Tim\text{-}1^{+}$	$CD19^{+}Tim\text{-}1^{+}$	Spleen, peripheral blood	IL-10	(Aravena et al., 2017, Ding et al., 2011, Xiao et al., 2012)
$CD9^{+}$ B cell	$CD19^{+}CD9^{+}$ $CD1d^{hi}CD5^{+}$	$CD9^{+}CD19^{+}$	Spleen, peripheral blood	IL-10	(Sun et al., 2015, Brosseau et al., 2018)
$PD\text{-}L1^{hi}$ B cells	$CD19^{+}PD\text{-}L1^{hi}$	$CD19^{+}PD\text{-}L1^{hi}$	Spleen, Solid tumors	PD-L1, IgA, IL-10	(Khan et al., 2015, Fehres et al., 2019) and various tumor phenotypes reviewed in (Sun et al., 2019)
Killer B cell	$FasL^{+}CD19^{+}$	-	Spleen	FasL-induced cell death	(Lundy et al., 2001, Klinker et al., 2013, Lundy, 2009)
Granzyme B^{+} B cell	-	$CD19^{+}CD38^{+}$ $CD1d^{+}$	Peripheral blood, solid tumors	Granzyme B	(Lindner et al., 2013)

		IgM^+CD147^+			
CD73-expressing B cells	$B220^+CD73^+$ $CD23^+$/ $B220^+CD73^+$ $CD5^+CD1d^{hi}$	$CD19^+CD39^+$ $CD73^+$	Spleen, peripheral blood	CD39/CD73-production of AMP/ADO	(Kaku et al., 2014, Saze et al., 2013)

TABLE 1. Heterogeneity within described regulatory B cell subsets in mouse and man. This table shows the heterogeneity in the phenotype and function of Breg subsets described in mouse and man. Abbreviations are as follows: T2-MZP, transitional 2 marginal zone precursor; AhR, Aryl hydrocarbon receptor; MZ, marginal zone precursor cells; Br1, B regulatory 1; IgG4, Immunoglobulin G4, PD-L1, programmed death-ligand 1; FasL, Fas-ligand; AMP, adenosine monophosphate; ADO, adenosine.

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