Immunity
Perspective
CellPress

# Regulatory B Cells: Origin, Phenotype, and Function

Elizabeth C. Rosser[1] and Claudia Mauri[1,*]
[1]Centre for Rheumatology Research, Division of Medicine, University College London, 5 University Street, London WC1E 6JF, UK
*Correspondence: c.mauri@ucl.ac.uk
http://dx.doi.org/10.1016/j.immuni.2015.04.005

Regulatory B (Breg) cells are immunosuppressive cells that support immunological tolerance. Through the production of interleukin-10 (IL-10), IL-35, and transforming growth factor β (TGF-β), Breg cells suppress immunopathology by prohibiting the expansion of pathogenic T cells and other pro-inflammatory lymphocytes. Recent work has shown that different inflammatory environments induce distinct Breg cell populations. Although these findings highlight the relevance of inflammatory signals in the differentiation of Breg cells, they also raise other questions about Breg cell biology and phenotype. For example, what are the functional properties and phenotype of Breg cells? Can a Breg cell arise at every stage in B cell development? Is inflammation the primary requisite for Breg cell differentiation? Here, we use these questions to discuss the advances in understanding Breg cell biology, with a particular emphasis on their ontogeny; we propose that multiple Breg cell subsets can be induced in response to inflammation at different stages in development.

## Introduction

The hallmark of an effective immune response is inflammation. After infection, the inflammatory response is critical for clearing pathogens and initiating protein cascades that control wound healing (Medzhitov, 2008). If unresolved, this inflammatory response causes injury to host tissues, which can lead to the development of a wide variety of immune-mediated pathologies (Medzhitov, 2008). In the healthy individual, inflammation is self-limiting, and resolution is controlled by the release of anti-inflammatory mediators and cytokines, such as interleukin-10 (IL-10), produced by cells that have been termed "suppressive" or "regulatory" (Nathan and Ding, 2010). Conversely, in individuals with chronic inflammation, the immune system is persistently activated, often characterized by a deficiency in the number and function of these suppressor cells in circulation and at the site of inflammation (Nathan and Ding, 2010). Over the past decade, a population of suppressor B cells, collectively known as regulatory B (Breg) cells, have been associated with the inhibition of excessive inflammation (Mauri and Bosma, 2012). The use of genetically altered mice that lack B cells (Wolf et al., 1996), and more specifically IL-10-producing B cells (Fillatreau et al., 2002), has shown that defective Breg cell development and function result in chronic inflammation. This suggests that these cells could be targeted therapeutically for alleviating a wide variety of immune-mediated inflammatory conditions. For Breg cells to be useful therapeutically, greater clarity regarding the phenotype, induction, and stability of these cells in vivo is needed. Here, we will discuss the principal advances made in our understanding of the function, phenotype, and developmental origin of Breg cells. In particular, we will focus on newly emerging evidence demonstrating the importance of inflammation in the differentiation of Breg cells.

## What Are the Functional Properties of Breg Cells?

A suppressive function for B cells was first postulated in the 1970s after the observation that B-cell-depleted splenocytes were unable to suppress delayed-type hypersensitivity in guinea pigs on adoptive transfer (Katz et al., 1974; Neta and Salvin, 1974). However, the molecular or biochemical mechanism responsible for these initial observations was never characterized, and the field of "suppressor" B cells was abandoned. The present revival of the study of B cell suppression can be traced back to the observation that B-cell-deficient mice were unable to recover from experimental autoimmune encephalitis (EAE) (Wolf et al., 1996). After this, three studies showing that B cells could suppress inflammation by the provision of IL-10 in models of colitis (Mizoguchi et al., 2002), EAE (Fillatreau et al., 2002), and arthritis (Mauri et al., 2003) were published. Over the last decade, much progress has been made to characterize immunosuppressive B cells, or "Breg cells," leading to more rigorous study of the multiple mechanisms they employ to suppress pro-inflammatory responses in vivo. Primarily, Breg cells function by skewing T cell differentiation in favor of a regulatory phenotype in both mice (Carter et al., 2011) and humans (Flores-Borja et al., 2013). The importance of B cells in the maintenance of the regulatory T (Treg) cell compartment can be derived from early studies showing that Treg cells are reduced in B-cell-deficient μMT mice (Sun et al., 2008; Tadmor et al., 2011). Later studies have shown that mice harboring a B-cell-specific deletion of IL-10 also display a Treg cell deficiency, which is associated with an outgrowth of pro-inflammatory T cells after the induction of autoimmunity (Carter et al., 2012; Carter et al., 2011). Directly, cognate interactions between Breg cells and T cells are thought to control Treg cell induction, given that B cells deficient in major histocompatibility complex class II (Yoshizaki et al., 2012) and B7 (Mann et al., 2007) do not exhibit regulatory function (Rosser et al., 2014a). Indirectly, Breg cells suppress the differentiation of T helper 1 (Th1) and Th17 cells by suppressing pro-inflammatory cytokine production by dendritic cells (Matsumoto et al., 2014; Sun et al., 2005). In addition to expressing IL-10, Breg cells express other immune-regulatory cytokines, including transforming growth factor β (TGF-β) and IL-35. Through the production of TGF-β, lipopolysaccharide (LPS)-activated B cells can induce both apoptosis of $CD4^+$ (Tian et al., 2001) and anergy in $CD8^+$ (Parekh et al., 2003) effector T cells. The identification of IL-35 as a key

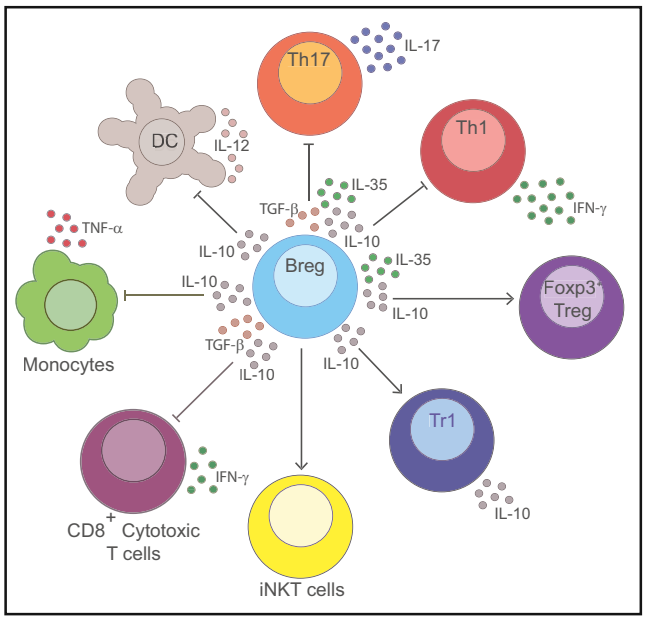


**Figure 1. Functional Properties of Breg Cells**
Through the production of IL-10, TGF-β, and IL-35, Breg cells can suppress the differentiation of pro-inflammatory lymphocytes, such as tumor necrosis factor α (TNF-α)-producing monocytes, IL-12-producing dendritic cells, Th17 cells, Th1 cells, and cytotoxic $CD8^+$ T cells. Breg cells can also induce the differentiation of immunosuppressive T cells, $Foxp3^+$ T cells, and T regulatory 1 (Tr1) cells. Breg cells also support the maintenance of iNKT cells.

immunoregulatory cytokine produced by Breg cells is a relatively recent breakthrough in the field. Chimeric mice lacking expression of IL-35 subunits, either p35 or EBi3, in B cells alone develop exacerbated EAE and are provided with greater protection against *Salmonella*-induced sepsis. In the *Salmonella* model, lack of IL-35 expression by B cells resulted in enhanced Th1 cell responses and an increase in the number of macrophages in the spleen (Shen et al., 2014). Another independent study has shown that IL-35-stimulated B cells produce IL-35 and are able to inhibit experimental uveitis on adoptive transfer (Wang et al., 2014). It has also been proposed that Breg cells are critical in maintaining invariant natural killer (iNKT) cell homeostasis in humans (Bosma et al., 2012). These examples also show the advancement in the understanding of the pleiotropic role of Breg cells in the suppression of immune responses, given that Breg cells have the capacity to target many immune-system cells to exert suppression (Figure 1).

## What Is the Phenotype of Breg Cells? Is There a Breg-Cell-Specific Transcription Factor?

Although a partial consensus regarding the effector function of Breg cells has been reached, the field has yet to produce a unified view concerning their phenotype. To date, multiple Breg cell subsets with many similarities in phenotype and effector functions have been described. Whether the differences observed are due to the existence of distinct Breg cell lineages or to changes dependent upon the immunological environment has yet to be elucidated. In mice, multiple subsets of IL-10-producing Breg cells have been described; these include transitional 2 marginal-zone precursor (T2-MZP) cells (Evans et al., 2007; Rosser et al., 2014b), $CD5^+CD1d^{hi}$ B (B10) cells (Yanaba et al., 2008; Yoshizaki et al., 2012), marginal-zone (MZ) B cells (Gray et al., 2007), Tim-$1^+$ B cells (Ding et al., 2011), $CD138^+$ plasma cells (Neves et al., 2010; Shen et al., 2014), and plasmablasts (Matsumoto et al., 2014). In humans, both $CD19^+CD24^{hi}CD38^{hi}CD1d^{hi}$ (Blair et al., 2010; Flores-Borja et al., 2013) and $CD19^+CD24^{hi}CD27^+$ (Iwata et al., 2011) Breg cells have been identified. The phenotypes of published Breg cell subsets and a summary of their associated effector functions can be found in Table 1. At present, it is unknown whether and how subsets of Breg cells are developmentally linked.

Although the expression of IL-10 has been useful in defining populations of suppressive B cells in mice and humans, many surface markers that have been used for identifying Breg cells are up- or downregulated during immune activation, leading to inherent problems in the definition of different Breg cell subsets among different experimental settings, which possibly accounts for some of the discrepancies in described Breg cell subsets. Thus, as a result of the heterogeneity of Breg cell subsets, a principal challenge of Breg cell research has been the identification of a Breg-cell-specific transcription factor, similar to Foxp3 in Treg cells (Rudensky, 2011). The identification of such a molecule would allow some resolution regarding the phenotype of Breg cells and would help answer the question of whether these cells represent a distinct lineage. To date, two models of Breg cell development can be suggested. The first is that Breg cells, similar to thymus-derived Treg cells, are a dedicated lineage of B cells where a specific factor controls the expression of genes responsible for their suppressive nature. The second is that in response to certain stimuli, B cells take on a regulatory phenotype to suppress local inflammation. Despite considerable effort, no study that has performed gene arrays on Breg cells in both mice (Shen et al., 2014) and humans (van de Veen et al., 2013) has conclusively identified a lineage-specific marker equivalent to Foxp3. The inability to identify a unique transcription factor, together with the heterogeneity of the phenotype of Breg cells, supports the idea that suppressor B cells are not lineage specific but rather are "reactive." Thus, unlike natural Treg cells, any B cell might potentially differentiate into a "Breg" cell in response to the right environmental stimuli.

## Can Breg Cells Arise at Every Stage of B cell Development?

A recent publication demonstrating that, in addition to previously described Breg cell subsets, plasmablasts can also suppress inflammatory responses supports the proposal that any B cell has the potential to differentiate into a Breg cell; mice whose B cells are deficient in *Irf4* and *Prdm1*, genes necessary for plasma cell differentiation, develop exacerbated EAE (Matsumoto et al., 2014). This is not the first time that antibody-producing B cells have been attributed with regulatory function: $CD138^+$ plasma cells that produce IL-10 and IL-35 suppress pro-inflammatory responses during EAE and *Salmonella* infection (Shen et al., 2014). Furthermore, an earlier report suggested that splenic B10 cells have the propensity to differentiate into antibody-producing plasmablasts after stimulation in vivo and in vitro (Maseda et al., 2012). Matsumoto et al. also suggest a developmental link between $CD19^+CD24^{hi}CD38^{hi}$ B cells, previously

**Table 1. Different Breg Cell Subsets**

| Type of Breg Cell | Mouse | Human | Key Features | Reference |
|---|---|---|---|---|
| T2-MZP cells | $CD19^+CD21^{hi}$ $CD23^{hi}CD24^{hi}$ | – | found in spleen, produce IL-10, induce Treg cells, and suppress effector $CD4^+$ and $CD8^+$ T cells | Blair et al., 2009; Carter et al., 2011; Evans et al., 2007; Schioppa et al., 2011 |
| MZ cells | $CD19^+CD21^{hi}$ $CD23^-$ | – | found in spleen, produce IL-10, induce Treg cells, and suppress effector $CD4^+$ and $CD8^+$ T cells | Bankoti et al., 2012; Gray et al., 2007; Miles et al., 2012 |
| B10 cells | $CD5^+CD1d^{hi}$ | $CD24^{hi}CD27^+$ | found in spleen (mice) and blood (humans), produce IL-10, and suppress effector $CD4^+$ T cells, monocytes, and DCs | Horikawa et al., 2013; Iwata et al., 2011; Matsushita et al., 2010; Yanaba et al., 2008 |
| Plasma cells | $CD138^+$MHC-$11^{lo}$ $B220^+$ | – | found in spleen, produce IL-10 and IL-35, and suppress NK cells, neutrophils, and effector $CD4^+$ T cells | Neves et al., 2010; Shen et al., 2014 |
| Tim-$1^+$ B cells | Tim-$1^+CD19^+$ | – | found in spleen (mice), produce IL-10, and suppress effector $CD4^+$ T cells | Ding et al., 2011; Xiao et al., 2012 |
| Plasmablasts | $CD138^+CD44^{hi}$ | $CD19^+CD24^{hi}$ $CD27^{int}$ | found in dLNs (mice) and blood (humans), produce IL-10, and suppress DCs and effector $CD4^+$ T cells | Matsumoto et al., 2014 |
| Immature cells | – | $CD19^+CD24^{hi}$ $CD38^{hi}$ | found in blood and at site of inflammation, produce IL-10, induce Treg cells, suppress Th1 and Th17 cells, suppress virus-specific $CD8^+$ T cell responses, are defective in patients with SLE and RA, and support iNKT cell homeostasis | Blair et al., 2010; Bosma et al., 2012; Das et al., 2012; Flores-Borja et al., 2013 |
| Br1 cells | – | $CD19^+CD25^{hi}$ $CD71^{hi}$ | found in blood and produce IL-10 and IgG4 | van de Veen et al., 2013 |

This table shows currently described subsets of Breg cells in mice and humans. Abbreviations are as follows: Br1, B regulatory 1; DC, dendritic cell; dLN, draining lymph node; IgG4, immunoglobulin G4; MHC, major histocompatibility complex; MZ, marginal zone; NK, natural killer; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T2-MZP, transitional 2 marginal-zone precursor.

ascribed with a regulatory phenotype (Blair et al., 2010), and IL-10-producing plasmablasts in humans. This suggests that a similar fate—the development into plasma cells—exists for Breg cells in both mice and humans (Matsumoto et al., 2014). The idea that antibody-producing B cells are also regulators of immune responses is hard to reconcile with current knowledge that plasma cells drive inflammatory responses through the production of antibody, which is often pathogenic in the context of autoimmunity or allergy. Thus, it might be possible that a subset of plasmablasts maintain the ability to regulate inflammatory responses while producing antibody. This is supported by data showing that the lack of *Bcl6* has no effect on regulatory plasmablast generation (Matsumoto et al., 2014), which is known to be important for the expansion of class-switched cells through B cell proliferation in germinal centers (Dent et al., 1997), suggesting that regulatory plasmablasts are contained within an immunoglobulin-M-positive subset (Matsumoto et al., 2014).

Taking into account these latest studies, it has now been demonstrated that immature B cells, mature B cells, and plasmablasts all have the capacity to differentiate into IL-10-producing Breg cells in both mice and humans. This supports the concept that the primary requisite for Breg cell differentiation is not the expression of a Breg-cell-specific lineage factor but rather the environment in which a B cell finds itself. With this in mind, the identification of stimuli necessary to induce B cells to become regulatory is an important consideration in the assessment of the origin of Breg cells. Toll-like receptor (TLR) and/or CD40 activation is the most well-characterized signal known to induce their differentiation (reviewed in Mauri and Bosma, 2012). However, a spate of recent publications has revealed that pro-inflammatory cytokines can also drive the induction of IL-10-producing Breg cells.

## Is Inflammation the Primary Requisite for the Differentiation of Breg Cells?

There is strong evidence that the number and suppressive ability of Breg cells increase in response to inflammation. For example, although they are present in naive mice, Breg cells increase in number during the inflammatory phase of several autoimmune disorders (Evans et al., 2007; Mizoguchi et al., 2002). Moreover, it is known that Breg cells are functionally suppressive in the inflammatory phase of autoimmunity, given that in their absence, mice develop exacerbated arthritis or unremitting EAE (Carter et al., 2012; Carter et al., 2011; Fillatreau et al., 2002). This suggests that Breg cells are activated in response to the same inflammatory signals that drive autoimmune disease and thus limit damaging inflammation that would otherwise develop. Recently, it was demonstrated that Breg cells arise in response to IL-1β and IL-6, pro-inflammatory cytokines that are produced after the induction of antigen-induced arthritis (Rosser et al., 2014b). The production of these cytokines in arthritic mice is controlled by the community of bacteria in the gut, collectively known as the microbiota, a pathway that has been previously shown to induce the differentiation of pro-arthritogenic Th17

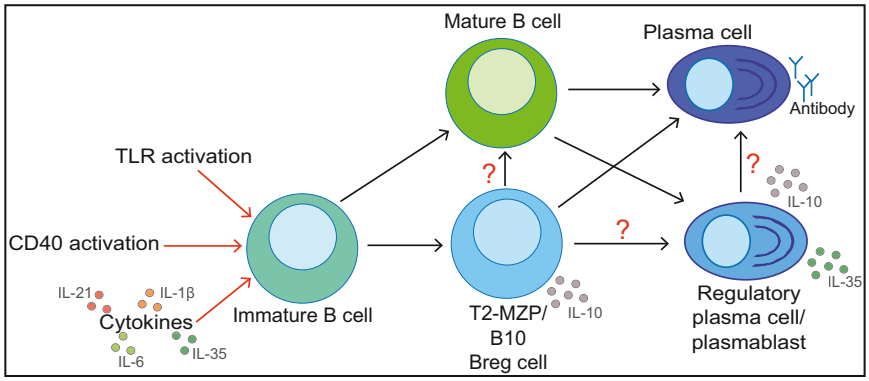


**Figure 2. Proposed Developmental Pathways for Breg Cell Differentiation**
After CD40 activation, TLR activation, or activation with cytokines, immature B cells can differentiate into B10 cells, IL-10-producing T2-MZP cells, and mature B cells. It is possible that while B10 and T2-MZP cells are differentiating into plasmablasts and/or plasma cells, they retain their ability to produce IL-10 and/or IL-35. B10 and T2-MZP cells can also differentiate into mature B cells. IL-10- and/or IL-35-producing plasmablasts and/or plasma cells can also develop directly from mature B cells. All Breg cell types can terminally differentiate into antibody-producing plasma cells.

cells (Wu et al., 2010). In the absence of IL-1R1 or IL-6R expression on B cells, mice housed in non-sterile conditions develop exacerbated arthritis (Rosser et al., 2014b). Thus, perhaps Breg cells are expanded in response to IL-1β and IL-6 in order to keep the immune system in check, preventing the uncontrolled expansion of pro-inflammatory lymphocytes such as Th17 cells. Other inflammatory cytokines that are known to be critical for Th17 cell differentiation, such as IL-21 (Yoshizaki et al., 2012) and granulocyte macrophage colony-stimulating factor (GM-CSF, in combination with IL-15) (Rafei et al., 2009), have also been shown to be important in Breg cell differentiation. Importantly, different cellular sources of the cytokines that can induce IL-10 production by B cells have been identified. Myeloid-derived cells producing IL-6 (in the mesenteric lymph nodes) and both IL-6 and IL-1β (in the spleen) are responsible for Breg cell induction in arthritis (Rosser et al., 2014b), whereas IL-21-producing $CD4^+$ T cells located in the spleen are responsible for Breg cell induction in EAE (Yoshizaki et al., 2012). Conversely, it has been reported that treatment of mice with the anti-inflammatory cytokine IL-35 induces a population of IL-10- and IL-35-expressing B cells and thus suppresses the development of uveitis (Wang et al., 2014). This suggests that anti-inflammatory cytokines might also have a role in Breg cell differentiation. However, there is evidence suggesting that IL-35 is not constitutively expressed but is induced in response to inflammation (Li et al., 2012).

Although non-cognate inflammatory stimuli, e.g., IL-1β, IL-6, and IL-21, are clearly important in the generation of Breg cells, it should not be forgotten that evidence suggests that B cell receptor (BCR) recognition is important in Breg cell induction. In MD4 mice, where the BCR is fixed for an irrelevant antigen, Breg cell activation is impaired; bone marrow chimeras that have MD4 B cells are unable to resolve EAE (Fillatreau et al., 2002), and MD4 mice produce less B-cell-derived IL-10 in response to TLR-9 activation (Miles et al., 2012) and have fewer B10 cells than do wild-type mice (Yanaba et al., 2009). Further evidence of the importance of BCR recognition in Breg cell function has been provided by experiments utilizing mice with a B-cell-specific deletion of stromal interaction molecule 1 (STIM-1) and STIM-2. STIM-1 and STIM-2 are important for mediating the influx of calcium into the B cell cytosol from outside the cell after antigen recognition of the BCR. Mice lacking STIM-1 and STIM-2 exclusively on B cells produce less IL-10 after stimulation with the auto-antigen MOG and anti-CD40 (Matsumoto et al., 2011). Taken together, these data show that antigen-specific recognition by the BCR is important for Breg cell function and development, but it is still not clear whether Breg cells are reactive to the auto-antigens or putative endogenous ligands. Thus, in response to BCR recognition and inflammation, B cells might differentiate into both regulatory and antibody-producing cells.

The importance of inflammation in the differentiation of Breg cells calls into question the location of their maturation. To date, most studies have characterized splenic populations of B cells. However, other publications have reported that Breg cells are found in the lymph node draining the site of inflammation after the development of colitis (Mizoguchi et al., 2002) and EAE (Matsumoto et al., 2014). Importantly, the study in EAE by Matsumoto et al. (2014) demonstrates that Breg cells can develop and acquire their suppressive capabilities outside the spleen, in the draining lymph node, given that splenectomy has no effect on their generation. This supports the idea that the induction of Breg cells is dictated by inflammatory environment but is at odds with previously published data characterizing the spleen as the primary location for Breg cell development.

## Breg Cells: What Next?

Although it cannot be ruled out that a transcriptional regulator that defines Breg cell function might be discovered in the future, at present there is no evidence to support this model. Thus, on the basis of the idea that Breg cells are not lineage specific but rather are expanded in response to inflammation, different models can be suggested for their development. These include the proposal that Breg cells are short-lived effector cells that are expanded in response to inflammation or, alternatively, that Breg cells are an inflammation-inducible subset that enters a further differentiation pathway after the resolution of an inflammatory responses, such as the maturation of immature B cells into plasmablasts. There is evidence for both hypotheses, yet without the development of a fate reporter mouse that allows the identification of the historical expression of IL-10, it is currently not possible to be certain exactly what happens to a Breg cell after the cessation of an inflammatory response. Thus, at the moment, more questions have been raised than answered. For example, is the acquisition of regulatory function tissue dependent? Do certain stimuli direct B cells to traffic to the site of inflammation? Can we separate the stimuli necessary to induce the differentiation of antibody-producing and regulatory B cells? We believe that it is these questions that need to be

addressed before we can understand how to harness these cells therapeutically.

## Concluding Remarks

Experimental evidence concerning the role of Breg cells in the suppression of inflammatory responses has been confused by the description of multiple Breg cell subsets. We propose that immunosuppression is not the purview of a devoted Breg cell lineage with a specific phenotype but rather is the outcome of the dynamic balance between multiple B cell subsets and other cells of the immune system. This hypothesis is supported by recent data underlining the importance of inflammatory cytokines in the induction of Breg cells, suggesting that Breg cells arise in response to inflammation, when immunosuppression is most needed. More recent publications suggest a developmental link between B cell subsets previously ascribed a regulatory phenotype and antibody-producing B cells, suggesting that although Breg cells might be inducible from multiple developmental stages, they might all share the capacity to become terminally differentiated plasma cells (Figure 2). In conclusion, although the Breg cell field has in the last year made many advances regarding the biological processes that control Breg cell differentiation, more resolution is needed before we can fully understand what happens during the life cycle of a Breg cell in vivo.

### ACKNOWLEDGMENTS

This work was funded by a program grant (MP/17707 to C.M.) and PhD studentship (MP/19314 to C.M.) from Arthritis Research UK. We would like to thank Dr. Kiran Nistala and Dr. Paul Blair for their constructive criticism of the manuscript.

### REFERENCES

Bankoti, R., Gupta, K., Levchenko, A., and Stäger, S. (2012). Marginal zone B cells regulate antigen-specific T cell responses during infection. J. Immunol. *188*, 3961–3971.

Blair, P.A., Chavez-Rueda, K.A., Evans, J.G., Shlomchik, M.J., Eddaoudi, A., Isenberg, D.A., Ehrenstein, M.R., and Mauri, C. (2009). Selective targeting of B cells with agonistic anti-CD40 is an efficacious strategy for the generation of induced regulatory T2-like B cells and for the suppression of lupus in MRL/lpr mice. J. Immunol. *182*, 3492–3502.

Blair, P.A., Noreña, L.Y., Flores-Borja, F., Rawlings, D.J., Isenberg, D.A., Ehrenstein, M.R., and Mauri, C. (2010). CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. Immunity *32*, 129–140.

Bosma, A., Abdel-Gadir, A., Isenberg, D.A., Jury, E.C., and Mauri, C. (2012). Lipid-antigen presentation by CD1d(+) B cells is essential for the maintenance of invariant natural killer T cells. Immunity *36*, 477–490.

Carter, N.A., Vasconcellos, R., Rosser, E.C., Tulone, C., Muñoz-Suano, A., Kamanaka, M., Ehrenstein, M.R., Flavell, R.A., and Mauri, C. (2011). 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. *186*, 5569–5579.

Carter, N.A., Rosser, E.C., and Mauri, C. (2012). Interleukin-10 produced by B cells is crucial for the suppression of Th17/Th1 responses, induction of T regulatory type 1 cells and reduction of collagen-induced arthritis. Arthritis Res. Ther. *14*, R32.

Das, A., Ellis, G., Pallant, C., Lopes, A.R., Khanna, P., Peppa, D., Chen, A., Blair, P., Dusheiko, G., Gill, U., et al. (2012). IL-10-producing regulatory B cells in the pathogenesis of chronic hepatitis B virus infection. J. Immunol. *189*, 3925–3935.

Dent, A.L., Shaffer, A.L., Yu, X., Allman, D., and Staudt, L.M. (1997). Control of inflammation, cytokine expression, and germinal center formation by BCL-6. Science *276*, 589–592.

Ding, Q., Yeung, M., Camirand, G., Zeng, Q., Akiba, H., Yagita, H., Chalasani, G., Sayegh, M.H., Najafian, N., and Rothstein, D.M. (2011). 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. *121*, 3645–3656.

Evans, J.G., Chavez-Rueda, K.A., Eddaoudi, A., Meyer-Bahlburg, A., Rawlings, D.J., Ehrenstein, M.R., and Mauri, C. (2007). Novel suppressive function of transitional 2 B cells in experimental arthritis. J. Immunol. *178*, 7868–7878.

Fillatreau, S., Sweenie, C.H., McGeachy, M.J., Gray, D., and Anderton, S.M. (2002). B cells regulate autoimmunity by provision of IL-10. Nat. Immunol. *3*, 944–950.

Flores-Borja, F., Bosma, A., Ng, D., Reddy, V., Ehrenstein, M.R., Isenberg, D.A., and Mauri, C. (2013). CD19+CD24hiCD38hi B cells maintain regulatory T cells while limiting TH1 and TH17 differentiation. Sci. Transl. Med. *5*, 73ra23.

Gray, M., Miles, K., Salter, D., Gray, D., and Savill, J. (2007). Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. Proc. Natl. Acad. Sci. USA *104*, 14080–14085.

Horikawa, M., Weimer, E.T., DiLillo, D.J., Venturi, G.M., Spolski, R., Leonard, W.J., Heise, M.T., and Tedder, T.F. (2013). Regulatory B cell (B10 Cell) expansion during Listeria infection governs innate and cellular immune responses in mice. J. Immunol. *190*, 1158–1168.

Iwata, Y., Matsushita, T., Horikawa, M., Dilillo, D.J., Yanaba, K., Venturi, G.M., Szabolcs, P.M., Bernstein, S.H., Magro, C.M., Williams, A.D., et al. (2011). Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. Blood *117*, 530–541.

Katz, S.I., Parker, D., and Turk, J.L. (1974). B-cell suppression of delayed hypersensitivity reactions. Nature *251*, 550–551.

Li, X., Mai, J., Virtue, A., Yin, Y., Gong, R., Sha, X., Gutchigian, S., Frisch, A., Hodge, I., Jiang, X., et al. (2012). IL-35 is a novel responsive anti-inflammatory cytokine—a new system of categorizing anti-inflammatory cytokines. PLoS ONE *7*, e33628.

Mann, M.K., Maresz, K., Shriver, L.P., Tan, Y., and Dittel, B.N. (2007). B cell regulation of CD4+CD25+ T regulatory cells and IL-10 via B7 is essential for recovery from experimental autoimmune encephalomyelitis. J. Immunol. *178*, 3447–3456.

Maseda, D., Smith, S.H., DiLillo, D.J., Bryant, J.M., Candando, K.M., Weaver, C.T., and Tedder, T.F. (2012). Regulatory B10 cells differentiate into antibody-secreting cells after transient IL-10 production in vivo. J. Immunol. *188*, 1036–1048.

Matsumoto, M., Fujii, Y., Baba, A., Hikida, M., Kurosaki, T., and Baba, Y. (2011). The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. Immunity *34*, 703–714.

Matsumoto, M., Baba, A., Yokota, T., Nishikawa, H., Ohkawa, Y., Kayama, H., Kallies, A., Nutt, S.L., Sakaguchi, S., Takeda, K., et al. (2014). Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation. Immunity *41*, 1040–1051.

Matsushita, T., Horikawa, M., Iwata, Y., and Tedder, T.F. (2010). Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune encephalomyelitis initiation and late-phase immunopathogenesis. J. Immunol. *185*, 2240–2252.

Mauri, C., and Bosma, A. (2012). Immune regulatory function of B cells. Annu. Rev. Immunol. *30*, 221–241.

Mauri, C., Gray, D., Mushtaq, N., and Londei, M. (2003). Prevention of arthritis by interleukin 10-producing B cells. J. Exp. Med. *197*, 489–501.

Medzhitov, R. (2008). Origin and physiological roles of inflammation. Nature *454*, 428–435.

Miles, K., Heaney, J., Sibinska, Z., Salter, D., Savill, J., Gray, D., and Gray, M. (2012). A tolerogenic role for Toll-like receptor 9 is revealed by B-cell interaction with DNA complexes expressed on apoptotic cells. Proc. Natl. Acad. Sci. USA *109*, 887–892.

Mizoguchi, A., Mizoguchi, E., Takedatsu, H., Blumberg, R.S., and Bhan, A.K. (2002). Chronic intestinal inflammatory condition generates IL-10-producing

regulatory B cell subset characterized by CD1d upregulation. Immunity *16*, 219–230.

Nathan, C., and Ding, A. (2010). Nonresolving inflammation. Cell *140*, 871–882.

Neta, R., and Salvin, S.B. (1974). Specific suppression of delayed hypersensitivity: the possible presence of a suppressor B cell in the regulation of delayed hypersensitivity. J. Immunol. *113*, 1716–1725.

Neves, P., Lampropoulou, V., Calderon-Gomez, E., Roch, T., Stervbo, U., Shen, P., Kühl, A.A., Loddenkemper, C., Haury, M., Nedospasov, S.A., et al. (2010). Signaling via the MyD88 adaptor protein in B cells suppresses protective immunity during Salmonella typhimurium infection. Immunity *33*, 777–790.

Parekh, V.V., Prasad, D.V., Banerjee, P.P., Joshi, B.N., Kumar, A., and Mishra, G.C. (2003). B cells activated by lipopolysaccharide, but not by anti-Ig and anti-CD40 antibody, induce anergy in CD8+ T cells: role of TGF-beta 1. J. Immunol. *170*, 5897–5911.

Rafei, M., Hsieh, J., Zehntner, S., Li, M., Forner, K., Birman, E., Boivin, M.N., Young, Y.K., Perreault, C., and Galipeau, J. (2009). A granulocyte-macrophage colony-stimulating factor and interleukin-15 fusokine induces a regulatory B cell population with immune suppressive properties. Nat. Med. *15*, 1038–1045.

Rosser, E.C., Blair, P.A., and Mauri, C. (2014a). Cellular targets of regulatory B cell-mediated suppression. Mol. Immunol. *62*, 296–304.

Rosser, E.C., Oleinika, K., Tonon, S., Doyle, R., Bosma, A., Carter, N.A., Harris, K.A., Jones, S.A., Klein, N., and Mauri, C. (2014b). Regulatory B cells are induced by gut microbiota-driven interleukin-1β and interleukin-6 production. Nat. Med. *20*, 1334–1339.

Rudensky, A.Y. (2011). Regulatory T cells and Foxp3. Immunol. Rev. *241*, 260–268.

Schioppa, T., Moore, R., Thompson, R.G., Rosser, E.C., Kulbe, H., Nedospasov, S., Mauri, C., Coussens, L.M., and Balkwill, F.R. (2011). B regulatory cells and the tumor-promoting actions of TNF-α during squamous carcinogenesis. Proc. Natl. Acad. Sci. USA *108*, 10662–10667.

Shen, P., Roch, T., Lampropoulou, V., O'Connor, R.A., Stervbo, U., Hilgenberg, E., Ries, S., Dang, V.D., Jaimes, Y., Daridon, C., et al. (2014). IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. Nature *507*, 366–370.

Sun, C.M., Deriaud, E., Leclerc, C., and Lo-Man, R. (2005). Upon TLR9 signaling, CD5+ B cells control the IL-12-dependent Th1-priming capacity of neonatal DCs. Immunity *22*, 467–477.

Sun, J.B., Flach, C.F., Czerkinsky, C., and Holmgren, J. (2008). B lymphocytes promote expansion of regulatory T cells in oral tolerance: powerful induction by antigen coupled to cholera toxin B subunit. J. Immunol. *181*, 8278–8287.

Tadmor, T., Zhang, Y., Cho, H.M., Podack, E.R., and Rosenblatt, J.D. (2011). The absence of B lymphocytes reduces the number and function of T-regulatory cells and enhances the anti-tumor response in a murine tumor model. Cancer Immunol. Immunother. *60*, 609–619.

Tian, J., Zekzer, D., Hanssen, L., Lu, Y., Olcott, A., and Kaufman, D.L. (2001). Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. J. Immunol. *167*, 1081–1089.

van de Veen, W., Stanic, B., Yaman, G., Wawrzyniak, M., Söllner, S., Akdis, D.G., Rückert, B., Akdis, C.A., and Akdis, M. (2013). IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. J. Allergy Clin. Immunol. *131*, 1204–1212.

Wang, R.X., Yu, C.R., Dambuza, I.M., Mahdi, R.M., Dolinska, M.B., Sergeev, Y.V., Wingfield, P.T., Kim, S.H., and Egwuagu, C.E. (2014). Interleukin-35 induces regulatory B cells that suppress autoimmune disease. Nat. Med. *20*, 633–641.

Wolf, S.D., Dittel, B.N., Hardardottir, F., and Janeway, C.A., Jr. (1996). Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. J. Exp. Med. *184*, 2271–2278.

Wu, H.J., Ivanov, I.I., Darce, J., Hattori, K., Shima, T., Umesaki, Y., Littman, D.R., Benoist, C., and Mathis, D. (2010). Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity *32*, 815–827.

Xiao, S., Brooks, C.R., Zhu, C., Wu, C., Sweere, J.M., Petecka, S., Yeste, A., Quintana, F.J., Ichimura, T., Sobel, R.A., et al. (2012). Defect in regulatory B-cell function and development of systemic autoimmunity in T-cell Ig mucin 1 (Tim-1) mucin domain-mutant mice. Proc. Natl. Acad. Sci. USA *109*, 12105–12110.

Yanaba, K., Bouaziz, J.D., Haas, K.M., Poe, J.C., Fujimoto, M., and Tedder, T.F. (2008). A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. Immunity *28*, 639–650.

Yanaba, K., Bouaziz, J.D., Matsushita, T., Tsubata, T., and Tedder, T.F. (2009). The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. J. Immunol. *182*, 7459–7472.

Yoshizaki, A., Miyagaki, T., DiLillo, D.J., Matsushita, T., Horikawa, M., Kountikov, E.I., Spolski, R., Poe, J.C., Leonard, W.J., and Tedder, T.F. (2012). Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. Nature *491*, 264–268.