# Regulatory roles of B cells in infectious diseases

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**ABSTRACT**

*B lymphocytes provide essential mechanisms of protection against infectious diseases. The secretion of specific antibodies by long-lived plasma cells is thought to account for the improved resistance afforded by most successful vaccines against pathogens. Accordingly, a goal in vaccine development is to induce potent B cell responses in order to drive the efficient formation of long-lived antibody-secreting cells. However, the roles of activated B cells are complex in infectious diseases. It was recently observed that activated B cells could also negatively regulate host defence mechanisms, both during primary infection and, after vaccination, upon secondary challenge, via mechanisms involving their production of the anti-inflammatory cytokines interleukin (IL)-10 and IL-35. Remarkably, the B cells expressing IL-10 and IL-35 in vivo were distinct subsets of $IgM^{hi}CD19^{+}CD138^{hi}$ antibody-secreting cells. A better understanding of the diverse roles of these distinct antibody-secreting cell subsets in immunity and immunological memory, as well as of the signals controlling their generation, might help the rational development of better prophylactic and therapeutic vaccines.*

## Introduction

Infectious diseases exert a formidable selection pressure on living organisms (1). The defence against these diseases is largely mediated by the adaptive immune system, as illustrated by the state of severe immunodeficiency of patients bearing loss of function mutations in genes controlling the development or function of lymphocytes (2). Children affected by monogenic deficiencies causing an arrest of B cell development at the pre-B cell stage display markedly reduced numbers of mature B cells as well as severe agammaglobulinaemia, and are recurrently affected by life-threatening infections (3, 4). A unique role of B cells in host defence is the production of protective antibodies, a function they acquire during their differentiation into plasmablasts and plasma cells, which are collectively referred to as antibody-secreting cells (ASC). Terminally differentiated plasma cells can accumulate in specific niches in the bone marrow where they can reside for a lifetime and stably produce protective antibodies (5). These long-lived memory plasma cells are thought to account for the protective effects of most effective vaccines against infectious diseases (6). A major goal in vaccinology is therefore to identify the optimal strategies to induce most efficiently the generation of such long-lived effector memory cells. The capacity of the adaptive immune system to potently combat microbial pathogens in a specific manner contrasts sharply with its ability to spare self-tissues (7). The establishment and maintenance of immune tolerance relies on central and peripheral mechanisms including suppressive so-called regulatory lymphocytes. Strikingly, regulatory lymphocytes do not only suppress autoimmune reactions but can also markedly attenuate immunity against pathogens. Some regulatory $CD4^{+}Foxp3^{+}$ T cells recognise antigens expressed by pathogens (8), and some microbe-reactive effector $CD4^{+}$ T cells such as $T_H1$ cells can gain the expression of cytokines with anti-inflammatory properties such as IL-10 during the course of the disease (9, 10). The latter can result in the inhibition of pathogen clearance and chronic infection (9), which is usually deleterious, or in the prevention of an overt inflammatory reaction that would otherwise cause excessive immunopathology (10), and thus be beneficial. B cells can also contribute to the limitation of autoimmune responses, and remarkably, infections can trigger the appearance of ASC that negatively impact on anti-microbial immunity. The induction

of polyclonal antibody responses was proposed as a strategy allowing some microbes to suppress the development of protective specific primary and secondary immune responses (11). Noteworthy, pathogens can induce ASC that produce IL-10 or IL-35, and inhibit anti-microbial immunity during both primary infection and secondary challenge after vaccination (12, 13). Thus, B cell responses are not homogeneously beneficial during infections. These conceptual advances have underlined the importance of identifying the distinctive characteristics of the various beneficial *versus* deleterious ASC subsets that are generated after immune challenge. In this context, this minireview discusses our current knowledge on the formation and function of these cytokine-producing "regulatory plasma cells" (Breg), in particular during infection by the Gram-negative bacterial pathogen *Salmonella typhimurium* in mice, which is commonly used as a model for the human disease typhoid fever. Typhoid fever is caused by *Salmonella typhi*, a foodborne pathogen that affects about 16 million people, and causes 500,000 to 600,000 deaths each year (14).

## Suppressive IL-10-producing Breg cells

Systemic *Salmonella* infection induces in spleen an enormous extrafollicular ASC response that is largely independent of Toll-like receptor (TLR) 2 and TLR4, as well as of the signalling adaptor protein MyD88, which is important for signalling via all TLR except TLR3 (15). This extrafollicular response initially involves B cells carrying receptors for antigen (BCR) of low affinity towards *Salmonella*-derived antigens (15). In fact, on day 7 post-challenge only about 1% of these ASC express BCR that show detectable reactivity towards *Salmonella* antigens in classical assays such as ELISPOT or ELISA (15). The affinity of the response for *Salmonella* antigens then progressively increases with time via an atypical process during which immunoglobulin genes undergo somatic hypermutation and high affinity variants are selected in these extrafollicular foci (15). It is so far unknown whether these early secreted antibodies of an overall low affinity contribute to the protection from the disease. B cells contribute to resistance against *Salmonella* infection via antibody-dependent and antibody-independent mechanisms, the latter being related at least in part to their capacity to act as antigen-presenting cells (APC) for protective $T_H1$ responses (16, 17).

Although TLR2 and TLR4 are not strictly required for this massive extrafollicular response, there is evidence that TLR signalling modulates the functional properties of these ASC. *Salmonella* directly stimulates IL-10 production by B cells via a mechanisms dependent *in vitro* on TLR2 and TLR4, as well as MyD88 (12). This pathway is relevant to the disease *in vivo* since mice with an *Il-10* or a *Myd88* deficiency restricted to B cells display a prolonged survival upon challenge with virulent *Salmonella* compared to mice with wild-type B cells, both during primary infection and after vaccination (12).

A detailed monitoring of the immune response elicited by *Salmonella* in mice with wild-type or *Myd88*-deficient B cells revealed largely similar humoral responses to the pathogen in these two groups (12). Memory $CD4^+$ and $CD8^+$ T cells also developed and underwent reactivation similarly upon re-challenge regardless of the status of MyD88 expression in B cells, implying that the improved survival of mice lacking IL-10 expression in B cells was not due to an increased adaptive response to the pathogen. In contrast, a lack of *Il-10* or *Myd88* in B cells resulted in a stronger activation of the innate immune system, and particularly of neutrophils, which accumulated in higher numbers in spleen and liver after challenge compared to controls (12). A stronger neutrophil response was also observed in B cell-deficient mice after challenge with other types of intracellular bacterial pathogens. For instance, intraperitoneal vaccination of mice with Bacillus Calmette-Guérin (BCG) led to a higher local accumulation of neutrophils in the peritoneal cavity of mice with a deficiency in the Bruton tyrosine kinase (Btk) gene, which lacked B1a cells (18). In this case, the increased neutrophilia was associated with a reduced T cell response, and an impaired vaccine performance, which was proposed to reflect an increased uptake of antigen by neutrophils, so that the latter was no longer available for APC subsets important for memory T cell responses. Accordingly, presentation of BCG-derived antigens by macrophages was reduced in *Btk*-deficient mice (18). The adoptive transfer of wild-type B cells into *Btk*-deficient mice erased these defects, restoring a normal neutrophilia, and an efficient T cell priming (18). A suppressive effect of B cells on neutrophils was also observed when wild-type and B cell-deficient mice were infected with *Mycobacterium tuberculosis* via the intranasal route (19). In this case, B cell deficient-mice displayed a higher accumulation of neutrophils in lungs even though their pathogen load was similar to wild-type mice (19). An adoptive transfer of B cells normalised the lung neutrophilia. The transferred B cells were only found in spleen, but not in lung, of the recipient B cell-deficient mice, suggesting that B cells regulated lung inflammation from the spleen (19). Is this B cell-neutrophil axis relevant in human tuberculosis, a pathology in which active disease is associated with an uncontrolled neutrophil response in comparison to latently infected individuals (20)? B cells and neutrophils can functionally interact in other lymphoid tissues than the spleen. A direct physical interaction between B cells and neutrophils has been documented in the lymph nodes of mice challenged with *Staphylococcus aureus* (21). These two cell types might also interact in bone marrow since some patients treated with the B cell depleting anti-CD20 antibody rituximab developed late-onset neutropenia at the time of B cell recovery (22, 23). It is worth noticing that the interaction between neutrophils and B cells is bi-directional, and can have different functional outcomes, for instance facilitating humoral immunity (24, 25).

The phenotype of the B cells producing IL-10 in the spleen of mice infected with *Salmonella* was characterised using a IL-10-eGFP reporter mouse strain

(12). According to eGFP expression, IL-10-producing B cells appeared in spleen within 24 hours after infection, before the development of classical B cell responses such as the extra-follicular plasmablast or the follicular germinal centre reactions. The kinetic of IL-10 expression in B cells therefore matched the manifestation of the inhibitory impact of B cell-derived IL-10 on neutrophils, which became distinguishably noticeable on day two post-challenge (12). Remarkably, at that time point all the B cells expressing the fluorescent protein eGFP carried high levels of CD138 expression on their surface, a typical feature of ASC (12), implicating ASC in the cytokine-mediated suppressive functions of B cells. Noteworthy, all splenic ASC were located at the interface between the red pulp and the white pulp, suggesting that this locale was the microenvironment where B cell-mediated regulation took place (13). A direct interaction between ASC and neutrophils was also observed in human spleen (25). In this case, the human splenic neutrophils stimulated B cells to secrete antibody via a mechanism involving their provision of the cytokines BAFF, APRIL, and IL-21 (25). It is possible that IL-10-producing ASC, which emerge very rapidly after challenge, control the subsequent capacity of splenic neutrophils to stimulate the extrafollicular plasmablast response against *Salmonella*. A role for regulatory lymphocytes in quality control of the adaptive immune response to foreign antigens has already been described for regulatory $CD4^+Foxp3^+$ T cells, which increased the avidity of a primary $CD8^+$ T cell response to a nonself antigen and promoted their differentiation into memory cells (26). Besides their role in limiting autoimmune responses, regulatory lymphocytes, including Treg and Breg cells, might therefore contribute to controlling the quality of adaptive B and T cell responses against nonself antigens including pathogens, with possible impact on immunological memory formation.

These functions of B cells might be relevant in other infectious diseases. A suppressive role for IL-10 production by B cells has also been identified during *Listeria monocytogenes* infection (27). Early after infection B cells are a major source of IL-10 in spleen, which has a suppressive role in this disease (27, 28). Accordingly, B cell-deficient mice better control *Listeria* than wild-type mice at early time points after infection (29), which correlates with higher amounts of IFN-γ and lower levels of IL-10 in spleen (27). Marginal zone B cells are implicated in this suppressive effect, but not follicular B cells (27). It is tempting to speculate that marginal zone B cells rapidly develop into ASC producing IL-10 upon *Listeria* infection.

**Suppressive IL-35-producing Breg cells**

Several studies documented that B cells could suppress immunity in an IL-10-independent manner (30, 31), prompting a search for additional mechanisms of B cell-mediated immune regulation, in particular of additional suppressive cytokines secreted by B cells. In secondary lymphoid tissues of naïve mice, B cells constitutively express and are the major source of *p35* mRNA (12). They robustly up-regulate Epstein-Barr virus-induced gene 3 (*Ebi3*) mRNA upon stimulation via CD40, BCR, and TLR4 (12). EBi3 and p35 can physically associate to form the heterodimer IL-35 (32), a cytokine implicated in the inhibitory functions of $CD4^+Foxp3^+$ T regulatory cells (33, 34). Activated B cells can secrete IL-35, and IL-35 expression contributes to their suppressive functions during *Salmonella* infection because mice with a deficiency in either *p35* or *EBi3* specifically in B cells were more resistant to *Salmonella* infection compared to control mice with wild-type B cells or with *p40*-deficient B cells (12). The inhibitory effect of B cell-derived IL-35 on host resistance to the disease correlated with a reduction of the number of inflammatory monocytes in spleens, and of *Salmonella*-reactive T cell in bone marrow of challenged mice (12). Thus, IL-35-producing B cells complement the function of IL-10-expressing ASC to regulate host defence during *Salmonella* infection.

The suppressive activities mediated by both B cell-derived IL-10 and IL-35 during *Salmonella* infection poses the question of the relationship between these two inhibitory axes. *Ebi3* mRNA, as well as EBi3 and p35 protein expression are selectively up-regulated in $CD19^+CD138^{hi}$ ASC, but not in $CD19^+CD138^-$ B cells in the spleen during *Salmonella* infection, emphasising the implication of ASC in immune regulation since ASC are also the major source of B cell-derived IL-10 (12). A few days after infection, all ASC in spleen of *Salmonella* infected mice display a uniform phenotype that is surface $IgM^+CD19^+CD80^+CD86^+CD40^+MHC\text{-}II^{hi}CD43^{hi}TACI^+CXCR4^+Tim1^{int}CD1d^{int}$ (12). These cells are therefore potentially equipped to capture antigen via their BCR and present it to $CD4^+$ T cells. They are however a heterogeneous population. Indeed, three ASC subsets can be distinguished according to their expression level of CD22 and CD138: $CD138^{int}CD22^+$, $CD138^{hi}CD22^+$, and $CD138^{hi}CD22^-$ cells (12). These subsets differ by their capacity to produce antibodies, and by their expression of distinct amounts of mRNA coding for transcription factors involved in ASC development (*Blimp1*, *Irf4*), or in the maintenance of B cell identity (*Pax5*). Single cell PCR analyses revealed that the two IL-35 subunits *Ebi3* and *p35* mRNA were predominantly expressed in intermediate $CD138^{hi}CD22^+$ and most mature $CD138^{hi}CD22^-$ cells, while none of the less advanced $CD22^+CD138^{int}$ cells co-expressed *Ebi3* and *p35* mRNA (12). Importantly, IL-10- and IL-35 producing ASC correspond to largely distinct cell subsets since only very rare cells co-expressed *Il10*, *Ebi3*, and *p35* mRNA in these single cell PCR analyses (12).

Distinct observations in experimental models of autoimmunity and cancer are consistent with the notion that IL-10- and IL-35-producing B cells provide two independent layers of immune regulation. In experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis, mice in which B cells could not produce IL-35 developed a markedly exacerbated

disease compared to mice with wild-type B cells (12). A lack of IL-35 provision by B cells resulted in increased autoreactive $CD4^+$ T cell responses of $T_H1$ and $T_H17$ types, and a higher accumulation of $CD4^+$ T cells as well as $CD11b^+LyC^+$ inflammatory monocytes in the central nervous system (12). B cells also contribute to protection of mice from this disease through the provision of IL-10 (35). To assess whether a B cell must express both IL-10 and IL-35 to exert a beneficial effect, mice were created in which half of the B cells could produce IL-35 but not IL-10, and the other half could express IL-10 but not IL-35, while no B cells were capable of expressing these two cytokines. Remarkably, these mice developed a disease comparable to control mice with wild-type B cells, demonstrating that B cells did not have to co-express both IL-10 and IL-35 to be suppressive (35). This concept is further supported by recent data obtained in a model of pancreatic ductal adenocarcinoma (36). Mice lacking B cells displayed an improved control of pancreatic tumour cells in comparison to control mice, and this difference was erased upon reconstitution of the B cell compartment through B cell adoptive transfer (36). In particular, $CD1d^{hi}CD5^+$ B cells isolated from the spleen of donor wild-type mice could recapitulate this B cell-mediated pro-tumourigenic effect upon adoptive transfer into recipient B cell-deficient mice, while $CD1d^{lo}$ B cells had no effect (36). This effect was independent of IL-10 but required their p35 expression, implicating IL-35 as the responsible factor (36). These observations corroborate the concept that IL-10 and IL-35 provide two largely separate suppressive pathways. In this tumour model, $CD1d^{hi}CD5^+$ B cells from the pancreatic lesions expressed both *Ebi3* and *p35* mRNA while intratumoural $CD1d^{lo}CD5^-$ B cells did not. ASC were not found in the pancreas, but were present in increased numbers in the spleen. The B cell subset expressing and secreting IL-35 protein, and the mechanism through which B cell-derived IL-35 influenced the tumour growth, remain to be determined. ASC derived from $CD1d^{hi}$ B cells might be a relevant source of B cell-derived IL-35. A pathogenic role for IL-10 production by ASC was previously described in a model of prostate cancer (37).

## Conclusion and perspectives

There is now clear evidence that B cells contribute both negatively and positively in autoimmune and infectious diseases as well as cancer (38). The suppressive activities of B cells depend on their production of the cytokines IL-10 and IL-35, which provide two inhibitory axes that can operate independently of each other. Remarkably, during *Salmonella* infection, Breg cells appear in spleen within 24 hours after challenge, a time preceding the classical extrafollicular plasmablast and follicular germinal centre responses. The fact that B cell-mediated regulation starts when innate cells orchestrate the immune response emphasises the importance of B cells in the reciprocal dialogue that occurs early after challenge between adaptive and innate immune cells and that controls the subsequent quality and magnitude of immunity. The rapidity of this response asks for a better understanding of its antigen specificity. The BCR repertoire of the B cells implicated in this regulatory response is a critical issue that remains to be clarified. Do Breg cells exist in human? The existence of "regulatory plasma cells" might explain the severe exacerbation of disease observed in patients with multiple sclerosis who were treated with atacicept, a drug that neutralises key survival factors for ASC, which led to an immediate arrest of that clinical trial (39). The existence and characterisation of human Breg cells is therefore an important issue because these cells might influence strongly the clinical effects of novel therapeutic strategies targeting B cells. Moreover, identifying approaches to distinctively target or induce these various deleterious *versus* protective ASC subsets, for instance Breg cells *versus* long-lived ASC producing protective antibodies, might provide novel opportunities for rational vaccine development, and conversely for immunotherapy of disease implicating undesirable immune responses.

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