

From the regulatory functions of B cells to the identification of cytokine-producing plasma cell subsets

Van Duc Dang, Ellen Hilgenberg, Stefanie Ries, Ping Shen and Simon Fillatreau

B lymphocytes have a unique role as antibody-producing cells. Antibodies are key mediators of humoral immunity against infections, and are thought to account for the protection afforded by successful vaccines. B cells can also secrete cytokines and subsequently regulate immune responses mediated by T and innate cells. Remarkably, recent studies identified plasma blasts/plasma cells as the main types of activated B cells producing the cytokines interleukin (IL)-10, IL-35, tumor necrosis factor (TNF)- α , IL-17, and GM-CSF in various contexts in mice. Here, we discuss these observations, which suggest the existence of various subsets of plasma blast/plasma cells distinguishable through their cytokine expression pattern.

Addresses

Deutsches Rheuma-Forschungszentrum, a Leibniz Institute, Chariteplatz 1, Berlin 10117, Germany

Corresponding author: Fillatreau, Simon (fillatreau@drfz.de, simonfillatreau@googlemail.com)

Current Opinion in Immunology 2014, 28:77–83

This review comes from a themed issue on **Lymphocyte activation and effector functions**

Edited by **Claude-Agnès Reynaud** and **Stuart Tangye**

Available online XXX

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<http://dx.doi.org/10.1016/j.coi.2014.02.009>

Introduction

B lymphocytes are the only cell type in the organism capable of producing antibodies. During their terminal differentiation into antibody-secreting cells (ASC) B cells undergo a dramatic expansion of their cytoplasm associated with an accumulation of organelles implicated in protein expression such as endoplasmic reticulum and polyribosomes [1[•]]. The development of these unique ultrastructural features correlates at the functional level with the acquisition of a remarkable capacity to synthesize and secrete proteins: plasma cells can produce several thousands antibody molecules per second and few plasma cells (they generally represent 0.1–1% of cells in lymphoid organs) are sufficient to maintain global antibody levels [1[•],2,3]. Plasma cells are professional antibody

factories, and, as a correlate of their extreme specialization, it has been assumed that they are devoid of any other immune function.

In addition to producing antibodies, B cells can contribute to immunity in multiple ways including presentation of antigen to T cells or secretion of cytokines. For instance, they can act as drivers or regulators of autoimmune diseases through production of cytokines such as interleukin (IL)-6, or IL-10, respectively [4[•],5]. The phenotypic features characterizing cytokine-producing B cells have remained elusive. Although several B cell subsets including transitional-2-like B cells or CD1d^{hi}CD5⁺ could be isolated from mice and provided IL-10-dependent regulatory function in recipient mice upon adoptive transfer [6,7], the phenotype of the B cells actually secreting IL-10 in a suppressive manner *in vivo* in these experiments was not defined. The identification of the phenotypic features distinguishing proinflammatory or anti-inflammatory cytokine-producing B cells might be of great significance for the development of new strategies aiming at the therapeutic targeting of B cells in the clinic. For instance, such knowledge could help to develop novel tools to selectively deplete plasma cells, which are a possible source of pathogenic autoantibodies, without affecting IL-10-producing B cells, which can play a protective role, during autoimmune diseases.

Here, we review recent studies that characterized the phenotypes of cytokine-producing B cells *in vivo* in different disease contexts. Remarkably, plasma blasts/plasma cells were recurrently identified as the major source of the B cell-derived cytokines studied in these investigations. These findings challenge the dogma that plasma blasts/plasma cells are solely living to produce antibodies, and uncover an unsuspected diversity of functional plasma cell subsets. In this review, we do not distinguish between plasma blasts and plasma cells, and collectively refer to these cells as plasma cells, because the distinction between the two has rarely been analyzed in the mentioned studies.

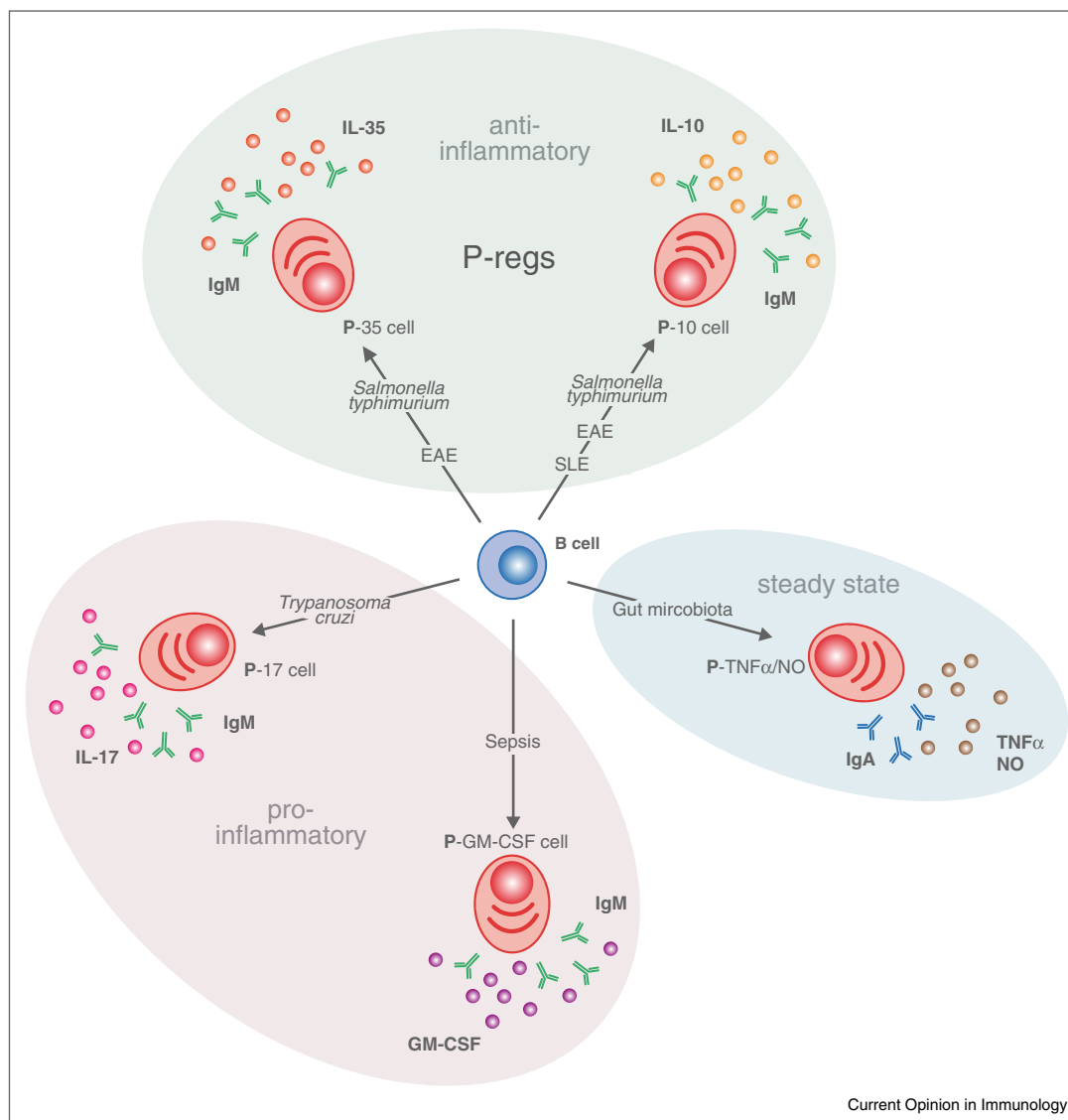
Plasma cells are a major source of immunosuppressive IL-10 *in vivo*

The phenotypic characterization of the B cells expressing IL-10 in a suppressive manner *in vivo* was performed in a mouse model of infection by the Gram-negative bacterial pathogen *Salmonella typhimurium* [8^{••}]. Upon infection with this pathogen, mice with a deficiency in IL-10

restricted to B cells displayed an improved resistance to the disease, manifested by a prolonged survival and a better control of the bacterial replication, compared to control mice [8^{••}]. Using an IL-10 reporter mouse strain, in which the sequence coding for the enhanced Green Fluorescent Protein (eGFP) was inserted in the *Il-10* locus, it was found that IL-10-expressing B cells appeared in spleen within 24 hours after challenge, while they were absent from naïve mice [8^{••}]. The development of IL-10-expressing B cells is therefore a rapid response that precedes the formation of germinal centers, which usually appear within 9–12 days after challenge, and the extra-follicular plasma cell response, which classically emerges around 3 days after exposure to the immune trigger. The

determination of the phenotype of these IL-10-expressing B cells at the single cell level by flow cytometry indicated that they all expressed the receptor CD138, which is a signature marker for plasma cells [8^{••}]. Noteworthy, the suppressive effect imposed by IL-10 from B cells on host defense mechanisms became noticeable on day 2 after challenge in this disease, and involved an inhibition of NK cells and neutrophils. Collectively, these data demonstrated that a subset of plasma cells rapidly up-regulated IL-10 expression after infection, and subsequently attenuated the protective anti-microbial innate response. Thus, plasma cells can regulate immunity through the production of cytokine, evoking the existence of regulatory plasma cells (Preg) (Figure 1).

Figure 1



Schematic representation of presently described subsets of cytokine-producing plasma cells in mice. Plasma cells have been identified that express IL-10 (P-10 cells), IL-35 (P-35 cells), iNOS and TNF- α (P-TNF/NO cells), IL-17 (P-17 cells), and GM-CSF (P-GM-CSF cells) in the indicated models in mice.

Noteworthy, plasma cells were also the major source of B cell-derived IL-10 in a mouse model of systemic lupus erythematosus [9], indicating that such link between plasma cell differentiation and induction of IL-10 expression in B cells might be common. These results were recently extended in a following study [10]. To further precise the characteristics of the CD138⁺ cells expressing IL-10 during *Salmonella* infection, these cells were separated into three distinct subpopulations according to their expression of CD138 and CD22. The three isolated fractions (CD138^{int}CD22⁺, CD138^{hi}CD22⁺, CD138^{hi}CD22⁻ cells) expressed unequal amounts of the transcription factors IRF4 and BLIMP-1, which instruct plasma cell differentiation [11], and Pax5, which maintains B cell identity [12], and correspondingly differed by their secretion of antibodies, indicating that they were at different stages in the plasma cell differentiation process. Remarkably, the expression of *Il-10* mRNA was highest in the most differentiated CD138^{hi}CD22⁻ plasma cell sub-population, which also had the highest content in *Irf4* and *Blimp-1* mRNA, and produced antibodies most efficiently [10]. About 10% of these CD138^{hi} plasma cells expressed *Il-10* mRNA, and all the cells producing *Il-10* mRNA co-transcribed *Blimp-1* in single cell PCR analyses, as expected for plasma cells. The fact that plasma cells were the main subtype of B cells expressing IL-10 might seem intriguing, but is not entirely surprising considering that the transcription factors controlling B cell differentiation into ASC are also involved in the induction of IL-10 expression in suppressive T cells. Indeed, besides their key roles in plasma cell differentiation [13,14], IRF4 and BLIMP-1 also contribute to IL-10 expression by regulatory CD4⁺ T cells (Treg), at least in part *via* direct action on the *Il-10* gene [15–17]. For instance, BLIMP-1 facilitates the local deposition of active chromatin marks on the *Il-10* locus [18]. Noteworthy, BLIMP-1-deficient Treg failed to block the development of colitis in mice, unlike control cells [19], and mice with a lack in BLIMP-1 restricted to T cells spontaneously developed severe immunopathology in intestinal mucosa and lungs [19,20]. IRF4 was recently found to directly control induction of IL-10 expression in B cells [21]. These data suggest that IRF4 and BLIMP-1 might be part of a shared transcription module controlling the suppressive activities of both Treg and Preg.

Plasma cells are a major source of immunosuppressive IL-35 *in vivo*

B cells can mediate regulatory effects independently of IL-10 [22,23]. To identify other secreted factor(s) involved in the inhibitory functions of B cells, genome-wide transcriptome analyses were performed on B cells activated *via* TLR4 and CD40, which are two receptors with key roles in the suppressive functions of B cells *in vivo* [4,5,24]. These analyses revealed that B

cells stimulated *via* TLR4 plus CD40 displayed a unique upregulation of Epstein Barr-induced gene 3 (EBI3), a protein that has no known biological activity on its own but can form IL-27 or IL-35 upon heterodimerization with p28 or p35, respectively [10]. Naïve B cells constitutively expressed *p35* mRNA, and secreted IL-35 upon activation *via* CD40 and TLR4 *in vitro* [10]. IL-35 is a cytokine typically associated with immune suppression as it was discovered in trophoblast extracts from human placenta [25], and was later found to be expressed by Treg and to be critical for their suppressive function [26,27]. IL-35 is also essential for the suppressive functions of B cells because mice lacking expression of either p35 or EBI3 selectively in B cells developed an exacerbated experimental autoimmune encephalomyelitis (EAE) compared to control mice. These mice also displayed an increased resistance to *Salmonella typhimurium* infection both during primary infection, and upon re-challenge in previously vaccinated mice [10]. In this case, the B cell-mediated suppressive effect was apparent in spleen already within one week after challenge, suggesting that this disease triggered the appearance of IL-35-producing B cells in this tissue within a few days. An analysis of *Ebi3* mRNA expression in CD19⁺CD138⁻ B cells and CD138^{hi} plasma cells indicated that, alike *Il-10*, in spleen *Ebi3* was selectively induced in plasma cells during the first week after challenge [10]. The possibility that plasma cells were the major source of B cell-derived IL-35 in this disease was further examined by measuring the co-expression of *p35* and *Ebi3* mRNA in distinct plasma cell subsets isolated according to their differentiation status, based on expression levels of CD22 and CD138. Remarkably, cells co-expressing *p35* and *Ebi3* mRNA were, in single cell PCR analyses, only found within the most mature CD138^{hi}CD22⁺ and CD138^{hi}CD22⁻ cells, which expressed higher levels of *Blimp-1* and *Irf4* mRNA and were the most efficient antibody producers, while none of the less mature CD138^{int}CD22⁺ cells co-expressed these two transcripts. Interestingly, nearly all the CD138^{hi} cells that co-expressed *p35* and *Ebi3* also transcribed *Blimp-1*, as expected for plasma cells. Moreover, expression of EBI3 and p35 proteins was found only in CD138⁺ plasma cells, but not in CD19⁺CD138⁻ B cells, in mice infected with *Salmonella* or during EAE. These data indicated that plasma cells were the major B cell source of IL-10 and IL-35 *in vivo* (Figure 1). Noteworthy, only very few CD138^{hi} plasma cells co-transcribed *Il-10*, *p35*, and *Ebi3* in spleen from mice infected by *Salmonella*, indicating that distinct sets of plasma cells provided IL-10 and IL-35, respectively. It is so far not known whether this differential expression of IL-35 subunits and IL-10 is due to instructive or stochastic mechanisms. Intriguingly, BLIMP-1 controls the transcription of *Ebi3* in Tregs [18], suggesting that it might provide a molecular link between the expression of IL-10, IL-35, and plasma cell differentiation.

Production of the pro-inflammatory factors iNOS and TNF- α by plasma cells in the intestinal mucosa

IgA accounts for more than 70% of produced immunoglobulin at steady state, and is the most abundant immunoglobulin isotype in the body [28]. It is essential for ensuring a balanced homeostasis between the host and its associated commensal microbiota, particularly at the level of the gut, which contains about 10^{12} bacteria per gram of luminal content [28,29]. The production of IgA is strongly dependent on iNOS, the inducible nitric oxide synthase involved in NO production, and TNF- α [30,31^{••}]. Dendritic cells might be an important source of iNOS for IgA expression [30]. However, mice constitutively lacking dendritic cells from birth due to expression of a diphtheria toxin transgene in CD11c⁺ cells displayed slightly increased levels of IgA in serum compared to controls [32], indicating that other cell types were also relevant sources of iNOS and TNF- α . An independent characterization of the cells expressing these two factors in gut indicated that plasma cells were a major source of iNOS and TNF- α in the small intestine lamina propria (Figure 1) [31^{••}]. In agreement with this, mice with a B cell restricted deficiency in iNOS and TNF- α displayed a marked reduction in serum IgA titers, and numbers of IgA plasma cells in lamina propria of the small intestine [31^{••}]. Thus, it seems that plasma cells can modulate immunoglobulin isotype switching through production of soluble mediators. This effect could operate *via* an autocrine loop, offering a way for plasma cells to regulate their own process of isotype switching, or involve an instructive influence of some plasma cells on isotype switching in neighboring cells. Further studies will be required to precise the temporal and geographical details of this circuit, as well as to examine the possibility that iNOS expression distinguishes a specific subset of IgA plasma cells, as suggested by the fact that not all IgA plasma cells expressed iNOS and TNF- α . The expression of iNOS and TNF- α in plasma cells depended on the gut microbiota because iNOS was not expressed in the few IgA plasma cells present in the lamina propria of germ-free mice [31^{••}], and this defect could be reversed upon reconstitution of germ-free mice with a limited microbiota — even a single bacteria specie. This effect of the microbiota on the plasma cells was not direct but as an intermediate involved the gut stroma, which triggered the expression of iNOS in IgA plasma cells *in vitro*. In a feedback fashion, the production of iNOS and TNF- α by plasma cells influenced the composition of the commensal flora, with an increase in *Clostridium leptum* and *Bacillus*, as well as an almost absence of segmented filamentous bacteria in mice lacking iNOS- and TNF- α -expression by B cells [31^{••}]. Of note, mice deficient in IgA on the opposite displayed an increased expansion of segmented filamentous bacteria in gut compared to controls [33]. In addition, iNOS and TNF- α intestinal plasma cells might contribute to intestinal anti-microbial immunity because

mice with a B cell-restricted deficiency in these two molecules displayed a reduced resistance to an orally transmitted *Citrobacter rodentium* infection compared to controls, leading to increased pathogen translocation to the spleen, weight loss, and exacerbated intestinal pathology [31^{••}]. In line with this, B cell-deficient mice, but not IgA-deficient mice failed to control *Citrobacter rodentium* infection [34]. Collectively, these data indicate that gut plasma cells can regulate humoral immunity, interaction between the host and its commensal microbiota, and local host defense, *via* antibody-independent mechanisms involving soluble factors.

Production of IL-17 by plasma cells during *Trypanosoma cruzi* infection

As observed with *Salmonella*, infection with *Trypanosoma cruzi*, the aetiological agent of Chagas' disease, also results in mice in an abundant polyclonal plasma cell response [35], which in this case produces mostly non-parasite specific antibodies [36,37], and can persist until the late stages of the infection beyond the control of the parasitemia [38]. Such polyclonal B cell response is observed with several infectious agents, and its significance is not fully understood, although it has been proposed to disfavor the optimal activation of specific lymphocytes, and to subsequently hamper the development of protective adaptive immune responses [39]. Indeed, this appears to be the case in *Salmonella* infection, as mice with a B cell specific deficiency in the adaptor MyD88, which is critical for signaling *via* all TLR except TLR3, displayed a transiently reduced plasma cell response in spleen, and developed stronger inflammatory *Salmonella*-reactive T cell responses compared to controls, suggesting a negative link between the extra-follicular B cell reaction, and the protective T cell response [8^{••}]. Do polyclonal B cell responses carry identical functions during distinct infections? The control of *Trypanosoma cruzi* infection is based on different mechanisms than for *Salmonella*, for instance being dependent on IL-17 [40], which has little role in *Salmonella* infection [41]. An investigation of the cell types expressing IL-17 in *Trypanosoma cruzi* infection revealed that CD19⁺CD138⁺ plasma cells were the main source of this cytokine in spleen at peak of parasitemia (Figure 1) [42^{••}]. At the peak of the response, on day 10 after challenge, about 10% of the CD19⁺CD138⁺ cells expressed IL-17. The impact of IL-17 production by B cells in this disease was investigated using B cell-deficient mice reconstituted with either wild-type or IL-17-deficient B cells. Mice with IL-17-deficient B cells displayed an enhanced production of IFN- γ and TNF- α , exacerbated tissue pathology, and accelerated death compared to controls [42^{••}]. Noteworthy, expression of IL-17 in B cells could be induced directly *in vitro* by adding live *Trypanosoma cruzi* trypomastigotes to the culture. This process was independent of IL-6, IL-23, AhR, RoR γ t, and ROR α , which are required for IL-17 production by T or innate lymphoid cells, and MyD88 or CD40, which

are major pathways of B cell activation [42^{••}]. These results point to existence of TLR-independent pathways of polyclonal B cell activation. In this case, it was found that the parasite-derived trans-sialidase was responsible for this B cell activation *via* a mechanism involving the modification of the glycosylation of molecules at the B cell surface [42^{••}]. Considering that *Salmonella* induced IL-10 and IL-35-producing plasma cells [8^{••},10^{••}], these results show that the extra-follicular plasma cell response can adopt different antibody-independent functions in various infectious diseases, according to the direct effect of microbial-derived compounds on B cells. It will be relevant to test whether IRF4, which can transactivate expression of the *IL-17* gene in CD4⁺ T cells [43,44], also contributes to IL-17 production by plasma cells.

Production of GM-CSF by plasma cells in microbial sepsis

The notion that B cells can produce cytokines upon polyclonal activation, under the instruction of microbial signals, strongly suggest that B cells could play important roles in sepsis, which is typically caused by disseminated infections by Gram-negative or Gram-positive bacteria or fungi [45]. Sepsis usually starts by a phase of overwhelming inflammation, which can lead to fatal septic shock [46], and is then followed by a phase of prolonged immune suppression, when most patients die due to their high susceptibility to nosocomial infections [47]. The role of B cells was recently examined in this disease using models of sepsis induced by administration of *Escherichia coli*, lipopolysaccharides (LPS), or cecal ligation puncture (CLP) in mice [48,49^{••}]. These treatments resulted in a rapid appearance of granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing B cells in spleen, where they accounted for most (about 75%) of the GM-CSF-expressing cells [49^{••}]. This response depended on intrinsic signaling *via* TLR4 and MyD88 [49^{••}], in line with the notion that it was directly triggered in B cells by microbial products. Remarkably, these GM-CSF-expressing B cells were IgM⁺CD138⁺, and secreted IgM, identifying them as plasma cells (Figure 1) [49^{••}]. Mice with a lack of GM-CSF production by B cells displayed an earlier death from the disease, compared to controls, which was associated with an increased bacteria burden, neutrophilia, and elevated levels of IL-1 β , IL-6, and TNF- α [49^{••}]. Thus, plasma cells can contribute to the control of microbial dissemination, subsequently limiting the severity of pathogenic inflammation through GM-CSF production in sepsis.

Perspectives and conclusions

The production of antibodies has been regarded as the only function performed by plasma cells [1[•]]. However, several studies highlighted plasma cells as a relevant source of cytokines. Their important capacity to synthesize and secrete proteins opens the possibility that they might be prominent sources of such molecules *in vivo*.

Intriguingly, distinct plasma cells seem to be involved in the production of different cytokines, suggesting the existence of diverse cytokine-producing plasma cell subsets, as identified for helper T cells. Accordingly, it might be useful to establish a similar nomenclature to classify plasma cell subsets, and provide a framework for their comparative analysis. The diagram drawn in Figure 1 attempts to provide such a classification, based on the limited numbers of studies presently available on cytokine-producing plasma cells (Figure 1).

The finding that plasma cells can secrete cytokines asks for a re-appraisal of their role(s) in immunity, including in autoimmune diseases, because in this way they can modulate immunity irrespectively of their antigen specificity. This might be relevant in multiple sclerosis (MS), which is associated with an accumulation of plasma cells, including cells of irrelevant antigen-specificity, in central nervous system [50,51]. Similarly, in the bleeding disorder idiopathic thrombocytopenia patients only a minor fraction of the plasma cells found in spleen secreted autoantibodies reactive towards the major platelet auto-antigen glycoprotein IIb/IIIa [52]. Could plasma cells secreting antibodies of irrelevant antigen-specificity contribute to these diseases through the production of cytokines or other soluble factors? The fact that MS patients treated with Atacicept, which can deplete short-lived and long-lived plasma cells [53,54], displayed a marked aggravation of disease leading to interruption of this clinical trial [55] might be consistent with this possibility. Indeed, it is possible that this treatment resulted in elimination of anti-inflammatory PC10 and/or PC35 cells. The production of cytokine by plasma cells might also be relevant in the context of viral infections, for instance during re-activation of gammaherpes virus residing inside B cells, which is associated with the differentiation of the latter into plasma cells [56]. Indeed, the virus could use the fact that such plasma cells might secrete IL-10- or/and IL-35 to suppress immune host defense mechanisms. Future investigations will be required to explore these hypotheses.

It will be important to identify the molecular and cellular mechanisms controlling the expression of cytokine genes in plasma cells. It is likely that IRF4 and BLIMP-1 play important roles because they are known to control cytokine expression in T cells. Moreover, IRF4 expression in Foxp3⁺ Treg cells is required for the latter to suppress Th2 cells efficiently [57]. However, these two molecules are insufficient to explain the expression of cytokine genes in plasma cells because not all plasma cells express cytokines, and they can express different cytokines. A fundamental parameter for the induction of cytokine expression by plasma cells appears to be the provision of signals from microbial derived molecules, which can act *via* innate receptors or by modulating receptors present at the surface of the B cells. The cellular microenvironment supporting

the B cell response might also be important. For instance, intestinal stroma cells contributed to the expression of iNOS in IgA-expressing plasma cells. Stroma cells are also thought to control the survival of long-lived plasma cells in bone marrow niches [58], suggesting a more general connection between stroma and plasma cells. As a conclusion, we would like to emphasize that future work will be important to precise and extend the concepts outlined in this review, which are presently based on a handful of studies in mouse.

Acknowledgements

SF is supported by grants from the Deutsche Forschungsgemeinschaft (SFB-650, TRR-36, TRR-130, FI-1238/02), Hertie Stiftung, and an advanced grant from the Merieux Institute.

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