[Frontiers in Bioscience, Elite, 5, 78-86, January 1, 2013]

Intrinsic toll-like receptor signalling drives regulatory function in B cells

Ping Shen[1], Vicky Lampropoulou[1], Ulrik Stervbo[1], Ellen Hilgenberg[1], Stefanie Ries[1], Aurelie Mecqinion[1], Simon Fillatreau[1]

[1]*Deutsche Rheuma-Forschungszentrum, a Leibniz institute, Chariteplatz 1, 10117 Berlin, Germany*

TABLE OF CONTENTS

1. ABSTRACT

B cells can contribute to immunity through production of antibodies, presentation of antigen to T cells, and secretion of cytokines. B cell activation can result in various outcomes for the host. In general B cell responses are beneficial during infections, and deleterious during autoimmune diseases. However, B cells can also limit host defence against pathogens, and protect from autoimmune pathologies. B cells can therefore act both as drivers and as regulators of immunity. Understanding how these opposite functions are mediated shall stimulate the elaboration of novel approaches for manipulating the immune system. B cells might acquire distinct functional properties depending on their mode of activation. Antigen-specific B cell responses require triggering of B cell receptor (BCR) by antigen, and provision of helper signals by T cells. B cells also express various innate immune receptors, and can directly respond to microbial products. Here, we discuss how intrinsic signalling via Toll-like receptors contributes to the suppressive functions of B cells during autoimmune and infectious diseases.

2. INTRODUCTION

B lymphocytes have multiple functions in immunity, producing antibodies, presenting antigen to T cells, and contributing to development of secondary lymphoid organs. In addition, B cells can secrete cytokines such as interleukin(IL)-4, IL-6, IL-10, IL-12 and interferon(IFN)-γ, which can influence polarization of T cell responses or progression of immune reactions (1-4). For instance, B cells controlled resolution of experimental autoimmune encephalomyelitis (EAE) through provision of IL-10 (5). IL-10-producing B cells also had regulatory activities in models of arthritis and ulcerative colitis (6-7). These findings led to the notion that B cells, similarly to particular T cell subsets, can regulate autoimmune responses. IL-10 from B cells also inhibited immune defence against the bacterial pathogen *Salmonella typhimurium* in mice, indicating that IL-10-producing B cells provide a general mechanism of immune regulation (4). The signals controlling the production of IL-10 and the regulatory functions of B cells are starting to be identified. Remarkably, Toll-like receptors (TLR) agonists were

essential for IL-10 secretion by naïve B cells *in vitro*, and TLR controlled the regulatory activities of B cells *in vivo* (8-9). Thus, the suppressive functions of B cells are part of a counter-regulatory circuit promoted directly by the signals that stimulate immunity (10). Human B cells might also regulate immunity through secretion of IL-10 because IL-10 production by B cells was impaired in patients with autoimmune diseases (11-12). Here, we discuss the importance of intrinsic TLR signalling for the IL-10-mediated suppressive functions of B cells during autoimmune and infectious diseases, and highlight the possible implications of these findings for the design of novel therapeutic strategies.

3. SUPPRESSIVE ROLE OF INTRINSIC TLR SIGNALLING IN B CELLS DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Some of the initial observations demonstrating a suppressive role for B cells during autoimmune diseases were made in EAE, an animal model for relapsing-remitting multiple sclerosis (RR-MS) (13). EAE can be provoked in rodents or non-human primates by immunization with central nervous (CNS) homogenate or purified myelin antigens. Disease is associated with development of inflammatory demyelinating lesions in CNS and is clinically manifested by paralysis (14). T lymphocytes play a critical role in EAE because activated myelin-reactive $CD4^+$ T cells can provoke disease in naïve recipient mice upon adoptive transfer, and conversely, depletion of $CD4^+$ T cells prevents EAE induction in animals immunized with myelin antigens (15-16). T cells might participate in pathogenesis of RR-MS because polymorphisms in genes controlling T cell activation (human leukocyte antigen class II, IL-2 receptor, or IL-7 receptor) are associated with disease susceptibility (17).

B cells are implicated in RR-MS because B cell-depletion with rituximab dampened disease progression in treated patients, leading to reduced occurrence of new relapses and diminished formation of new lesions in CNS, compared to controls (18). B cell depletion also attenuated EAE course when applied to mice already showing signs of paralysis (19). Paradoxically, depletion of B cells before mice were immunized with myelin antigen resulted in an aggravated course of EAE, implying that B cell depletion could have different outcomes, being beneficial or deleterious, depending on time of treatment (19). B cell-deficient mice also developed a more severe EAE course than wild-type mice upon immunization with various myelin antigens (5, 20). The beneficial effect of B cells in EAE involved their production of IL-10 because mice in which only B cells could not produce IL-10 developed a severe chronic form of EAE, while mice with wild-type B cells recovered after a short episode of paralysis (5). IL-10-producing B cells also provided protection in models of inflammatory bowel disease, and collagen-induced arthritis (6-7). These pathologies are mediated by distinct immune mechanisms i.e. T_H1 and T_H17 cells for EAE, T_H2 cells for ulcerative colitis, and B cells for arthritis, implying that IL-10-producing B cells have a general role in immune regulation (21-24).

B cells do not secrete IL-10 constitutively. Naïve B cells produced large amounts of IL-10 upon activation with agonists for Toll-like receptor (TLR)-2, -4, or -9 *in vitro*, but not upon stimulation via CD40 or B cell-receptor for antigen (BCR) either alone or in combination (2). In contrast to B cells, dendritic cells and macrophages did not secrete detectable amount of IL-10 when stimulated with TLR agonists in the same conditions *in vitro* (2). The notion that intrinsic TLR signalling controls the suppressive functions of B cells was confirmed *in vivo*: mice in which only B cells lacked either both TLR-2 and TLR-4 or the major TLR-signalling adaptor myeloid differentiation factor 88 (MyD88) developed a chronic form of EAE, alike mice with IL-10-deficient B cells, while mice with wild-type B cells recovered after a short episode of paralysis (2). These mice also developed exacerbated autoreactive T cell responses of T_H1 and T_H17 types, indicating that TLR-activated B cells might promote resolution of EAE by counter-regulating the expansion of encephalitogenic T cells (2). The suppressive function resulting from MyD88 signalling in B cells was singular because mice lacking MyD88 in all cells were, in contrast, completely resistant to EAE (2). MyD88-deficient mice also developed reduced autoreactive T cell response of T_H1 type, and T_H17 cells were barely detectable in these mice (2). Thus, the signalling adaptor MyD88 had a dual role during EAE, controlling both initiation and termination of disease flare. These opposite functions were segregated in distinct cell types: MyD88 signalling in cells other than B cells (most likely dendritic cells or macrophages) drove the development of EAE, while MyD88 signalling in B cells controlled disease resolution. Signalling via MyD88 in these different cell types also exerted opposite influences on inflammatory T cell responses. Thus, it might be possible to therapeutically interfere with T cell-mediated autoimmune diseases by modifying the balance between TLR signalling in distinct cell types, for example by blocking this signalling selectively in cells mediating inflammatory functions.

It is currently debated whether a particular B cell subset is specialized in the inhibition of pathogenic autoimmune responses. To identify protective B cells, Yanaba *et al.* stimulated mouse cells from spleen, peritoneal cavity, peripheral lymph nodes, mesenteric lymph nodes, Peyer's patches, or blood for 5 hours with lipopolysaccharides (LPS), ionomycin and phorbol myristate acetate (PMA), and then investigated the phenotype of the B cells induced to express intracellular IL-10 by this treatment (25). IL-10-expressing B cells were detectable in cultures from spleen and peritoneal cavity, but not from peripheral lymph nodes, Peyer's patches, mesenteric lymph nodes, or blood (25). In spleen, IL-10-producing B cells were $CD1d^{high}CD5^+$, and about 18% of $CD1d^{high}CD5^+$ B cells expressed intracellular IL-10 (25). These cells had high levels of IgM and CD24, and lacked CD23 and CD93 ($CD19^+CD1d^{high}CD5^+IgM^{high}CD24^{high}CD23^-CD93^-$), suggesting that they were marginal zone B cells (25-26). Splenic $CD1d^{low}$ B cells did not express IL-10 in this setting, indicating that B cell subsets differ in their capacities to produce IL-10 upon short-term TLR

stimulation (25). However, all B cell subsets could make IL-10 when activated for a longer time *in vitro* (2, 26). This difference between "rapid" and "slow" IL-10 production suggests that distinct B cell subsets might provide IL-10 at different stages, possibly in distinct microenvironments, and with specific impacts during immune responses. Supporting this notion, $CD1d^{high}CD5^{+}$ B cells (but not $CD1d^{high}CD5^{+}$ B cells deficient in IL-10, and non-$CD1d^{high}CD5^{+}$ B cells) protected recipient mice from EAE upon adoptive transfer (19). B1 cells and transitional T2-like B cells also limited inflammation through IL-10 in different models of immunopathology (27-28). Remarkably, $CD1d^{hi}CD5^{+}$ B cells, T2-like B cells, and B1 cells share as a common feature their rapid responsiveness to TLR engagement (29). The capacity of B cell subsets to regulate immunity *in vivo* therefore seems to correlate with their capacity to secrete IL-10 rapidly upon TLR stimulation *in vitro*. This might be due to the fact that IL-10 has stronger suppressive effect on the priming of immunity rather than on already established immune reactions. Protective B cells might be generally defined from a functional viewpoint as cells that rapidly secrete IL-10 upon TLR triggering. It will be important to address whether "rapid" IL-10-producing B cell subsets can mediate pathogenic activities, prior to using them in adoptive cellular therapy. On this point, it is intriguing that mice depleted of B cells and then reconstituted with $CD1d^{high}CD5^{+}$ B cells displayed a EAE course similar to wild-type mice, in which B cells acted both as drivers and regulators of the disease, suggesting that $CD1d^{high}CD5^{+}$ B cells indeed have pathogenic functions (19).

Human B cells can produce IL-10, and might have protective functions during autoimmune diseases. B cell depletion with rituximab resulted in exacerbation of intestinal pathology in a patient with ulcerative colitis, and had unexpected effects in some MS patients (30-31). It also led to novel types of immunopathology in some cases: a patient with Grave's disease developed ulcerative colitis subsequently to rituximab treatment (32). Little information is available on the immunological changes accompanying these disease exacerbations, except for a patient with ulcerative colitis who showed a loss of IL-10 expression in intestinal mucosa at the time of disease aggravation after B cell depletion (30). The notion that IL-10-producing human B cells can protect from autoimmune diseases is also supported by the fact that B cells from RR-MS or diabetes mellitus patients produced less IL-10 than B cells from healthy individuals upon TLR activation (33-34). In contrast, B cells from diabetes mellitus patients produced more IL-8 than B cells from healthy individuals (34). B cells might therefore promote autoimmune diseases by secreting unbalanced amounts of pro- versus anti-inflammatory cytokines upon stimulation. Human B cells express a broad TLR repertoire including TLR-1, TLR-6, TLR-7, TLR-9, and TLR-10, and possibly TLR-4 during chronic inflammation (35-38). Dying cells, endogenous retroviruses, or exogenous microbes could provide TLR agonists. Are there particular modes of TLR stimulation that increase the protective functions of B cells? B cells from RR-MS patients chronically infected by helminth parasites produced increased amounts of IL-10 upon stimulation *in vitro* compared to cells from non-infected RR-MS patients, and this was associated with a milder progression of disease course, suggesting that infection by helminth parasites might increase the protective function of B cells in these patients (39-40). The notion that some infections might foster the IL-10-mediated suppressive functions of B cells is relevant for the hygiene hypothesis, as discussed elsewhere (8). Taken together, these data argue for the possibility that human B cells can protect from autoimmune diseases, even though it remains challenging to obtain a definitive proof of this concept.

4. SUPPRESSIVE FUNCTIONS OF TLR-ACTIVATED B CELLS DURING INFECTIOUS DISEASES

The notion that TLR signalling in B cells can lead to suppression of immunity appears paradoxical considering the importance of these receptors in protection from infections (41-43). To test whether B cell-mediated suppression was operational in other contexts than in autoimmune diseases, the role of intrinsic TLR signalling in B cells was investigated in a model of infection by the Gram-negative bacteria *Salmonella typhimurium* (4, 44). *Salmonella* usually cause diseases that are asymptomatic or resolve spontaneously after a mild gastroenteritis, although particular strains such as *Salmonella enterica* serovars Typhi and Paratyphi can cause life-threatening diseases (45-46).

Mice with B cell-deficiency in both TLR-2 and TLR-4, or MyD88 displayed reduced bacterial load, milder liver tissue pathology, and longer survival than mice with wild-type B cells after primary infection with virulent *Salmonella* (4). TLR-signalling in B cells also led to suppression of host defence during secondary challenge because vaccinated control mice showed only a partial protection from the disease, while mice lacking MyD88 in B cells became completely resistant to virulent *Salmonella* after vaccination (4). Furthermore, depletion of B cells in vaccinated wild-type mice a week prior to challenge with the virulent bacteria resulted in an improved survival compared to control mice (4). Identification of the mechanism responsible for the inhibitory function of TLR-activated B cells during primary as well as secondary *Salmonella* infection might provide novel targets for improving host defence against infectious diseases. IL-10 played an important role in the suppressive function of TLR-activated B cells in this model. IL-10-producing B cells appeared in spleens of mice within less than 24 hours after infection, and mice lacking IL-10 only in B cells survived longer than mice with wild-type B cells after infection with virulent *Salmonella* (4). IL-10-producing B cells all expressed the plasmablast/plasma cell-specific surface receptor CD138 in the first days after infection. $CD19^{+}CD138^{+}$ cells were the first cells to produce IL-10 because dendritic cells, macrophages, and T cells did not show detectable expression of IL-10 at 24 hours after infection (4). $CD19^{+}CD138^{+}$ plasma blasts might therefore be an important source of IL-10 during immune reactions. IL-10 secretion might be a general feature of antibody-secreting cells because the master transcription factor for plasma cell differentiation prdm-1 can also

promote IL-10 transcription (47-48). The concept of "regulatory B cells" should be taken with caution as IL-10-producing B cells might contribute positively to immunity through antibody secretion.

During *Salmonella* infection, IL-10 production by B cells resulted in an inhibition of the early innate response mediated by neutrophils and NK cells, which both mediate protection against this pathogen (4, 49). The regulatory activity of TLR-activated B cells on neutrophils might be of general relevance because B cell-deficient mice showed higher neutrophil responses than wild-type mice in several infection models. Following infection with *Leishmania donovani* B cell-deficient mice displayed an enhanced neutrophil response, which correlated with better control of the parasite but more severe liver tissue damage than wild-type mice (50). After infection with *Mycobacterium tuberculosis* B cell-deficient mice also showed a higher neutrophil response and more severe lung pathology than wild-type mice, even though the two types of animals had comparable bacterial loads (51). Patients with severe tuberculosis could be distinguished from latently infected individuals by a specific neutrophil gene signature (52). Future studies shall evaluate whether B cells regulate the activity of neutrophils during *Mycobacterium tuberculosis* infection in humans. Human B cells might interfere with neutrophil homeostasis because some patients treated with rituximab showed a late-onset neutropenia during the recovery of peripheral B cells, at 2-4 months post-treatment (53-56). This might be due to competition between the two cell types in bone marrow because this neutropenia was associated with a block in bone marrow granulopoiesis in some cases (57). Furthermore, neutrophils co-localized with B cells in mouse bone marrow, and more neutrophils were present in bone marrow of lymphocyte-deficient mice than wild-type mice (58).

5. UTILIZATION OF TLR-ACTIVATED B CELLS FOR THE SUPPRESSION OF UNWANTED IMMUNE RESPONSES

TLR-activated B cells also displayed suppressive functions in adoptive transfer experiments. Fuchs and Matzinger found that LPS-activated male B cells were three times more efficient than resting B cells at inhibiting T cell immunity towards male antigens in female mice upon transfer (59), and Scott and colleagues demonstrated that antigen-expressing LPS-activated B cells could restrain antigen-specific immune responses in recipient animals (60). These findings have now been confirmed by several studies that explored the potential of TLR-activated B cells for suppression of unwanted immunity in adoptive cell therapy.

LPS-activated B cells expressing myelin antigens could inhibit development of EAE in an antigen-specific manner upon adoptive transfer in naïve recipient mice (61-62). Such B cells also protected recipient mice from severe EAE induced by combined administration of encephalitogenic T cells and immunization of recipient mice, which was fatal for control animals, while treated mice all survived and most remained healthy (62). LPS-activated B cells expressing a relevant myelin antigen could also intercept disease progression upon transfer in mice already showing signs of EAE (62). In this case, the injected B cells accumulated in CNS and spleen, suggesting that they could control pathogenesis directly in the target organ (61). These findings indicate that B cells could be isolated from MS patients to be engineered in suppressive cells for autologous cell therapy. The finding that tolerogenic B cells could be obtained from donor mice suffering from EAE supports this therapeutic concept (62). Similar findings were obtained in experimental autoimmune uveitis (EAU) (63-64). In this case, a single dose of transduced B cells was sufficient to reduce incidence and severity of disease, and to impair antigen-specific T and B cells (63-64). The protection lasted for more than two months after the B cell transfer, indicating a long lasting beneficial effect (64). These studies demonstrate that TLR-activated B cells can inhibit autoimmune diseases induced deliberately by immunization or adoptive transfer of pathogenic cells.

B cells activated with LPS and manipulated to express autoantigen could also inhibit spontaneous autoimmune diseases in adoptive cell therapy (62, 65-66). Female nonobese diabetic (NOD) mice naturally develop an autoimmune response against pancreatic islet antigens such as insulin, and glutamic acid decarboxylase (GAD) 65, which ultimately leads to destruction of islet and development of type 1 diabetes (67). LPS-activated B cells expressing GAD65 could reduce incidence and severity of diabetes in recipient mice, and the treated animals still showed a lower disease activity than controls at 6 months after the B cell infusion, indicating a durable beneficial effect (62, 65). This protection correlated with an inhibition of the autoreactive immune response (reduced levels of GAD65-specific antibodies, and lower autoreactive T cell response) indicating that LPS-activated B cells could control a complex spontaneous disease involving a broad immune response (65).

This therapeutic approach could also be useful to suppress immunity against therapeutic proteins. Haemophilia is a bleeding disorder caused in 80% of cases by deficiencies in the gene coding for factor VIII (68). This syndrome can be treated by administration of recombinant factor VIII, but a significant proportion of patients then develops neutralizing antibodies against the therapeutic protein, which limits treatment efficacy (69). Adoptive transfer of LPS-activated mouse B cells expressing parts of factor VIII tolerized antigen-specific T and B cells for more than two months in recipient factor VIII-deficient animals (70-72). This therapy was also effective in primed animals, leading to reduction of existing levels of neutralizing antibodies (70).

The suppressive properties of LPS-stimulated B cells are intriguing because activated antigen-presenting cells (APC) are generally assumed to be positive regulators of immunity (73-74). Scott and colleagues compared the tolerogenic properties of resting and LPS-activated B cells using a transgenic mouse expressing a peptide-IgG1 construct in B cells (75). Naïve and activated B cells

similarly induced antigen-specific tolerance in naïve recipient mice upon transfer, but only LPS-stimulated cells could inhibit already established immune reactions (75). These findings ask for a refinement of the two signals model formulated by Bretscher and Cohn, according to which activated APC should stimulate immune response, while resting APC should induce tolerance in reactive T cells (73). This might have important implications for immunotherapy: provision of antigen alone (as done with infusion of soluble peptides or with delivery of antigen to resting APC) might not lead to a form of antigen-presentation suitable for inhibiting ongoing immunity.

The finding that LPS-activated B cells could suppress primary and secondary immune responses asks for a better understanding of this tolerogenic effect. B cells lacking MHC-II or CD86 were not suppressive, implying that presentation of antigen and provision of appropriate co-stimulatory signals were critical (76-78). CD86 was maintained at elevated level for more than 10 days on LPS-activated B cells, while it was up-regulated transiently for less than 3 days on CpG-activated B cells, which were not tolerogenic. A durable presentation of antigen might therefore underlie the regulatory activity of LPS-activated B cells, in agreement with the fact that these B cells persisted for 3-6 months in recipient mice upon adoptive transfer (60, 79). LPS-activated B cells probably targeted effector and/or regulatory T cells because CTLA-4, a receptor expressed by both subsets of T cells, was essential for suppression (76). Furthermore, infusion of LPS-activated B cells resulted in elimination of antigen-specific $CD4^+$ T cells (60, 79), and in expansion of regulatory $CD4^+CD25^+$ T cells in treated mice (65-66). Regulatory T cells from mice treated with B cell blasts could transfer protection from diabetes to untreated recipient NOD mice (65-66). LPS-activated B cells could even convert conventional $CD4^+$ T cells into regulatory T cells *in vivo* in an antigen-specific manner (79). In a different model, LPS-activated B cells promoted the development of $CD4^+CD25^-$ suppressive T cells sharing features with IL-10-producing Tr1 cells, which possibly involved IL-10 secretion by the LPS-activated B cells (80). IL-10 secretion by the LPS-activated B cells was essential for the suppressive function of the LPS blasts in this latter disease model, yet this requirement was not observed in distinct systems (80-81). The activation of multiple subsets of regulatory T cells might explain the robustness and the duration of the unresponsiveness induced by LPS-activated B cells (60).

6. CONCLUSION AND PERSPECTIVES

The implication of TLR in the suppressive function of B cells is intriguing because these innate sensors are regarded as critical activators of protective immunity during infections. The dual roles of TLR as positive and negative regulators of immunity might be important for the optimization of dynamic and robustness of immune responses, as discussed in detail elsewhere (10). Currently, TLR agonists are considered as therapeutic tools mostly for their immunostimulatory properties. Could TLR agonists be useful for suppression of ongoing immune responses? Several observations support this notion. For instance, administration of a stimulatory TLR-9 agonist combined with an allergen almost completely protected recipient mice from acute and chronic airway inflammation (82-84). Similarly, a ragweed-TLR-9 agonist conjugate vaccine offered long-lasting improvement of clinical disease in allergic patients (85). These effects could involve B cells because B cells activated via TLR-9, and/or producing IL-10 protected recipient animals from allergy upon adoptive transfer (86-87). These results emphasize the notion that activated APC can be more tolerogenic than resting APC, particularly for ongoing immune reactions. It will be essential to have a better comprehension of the type of B cells mediating suppression. Several B cell subsets exerted IL-10-dependent suppressive effects upon adoptive transfer in recipient mice including splenic B1 B cells, T2-like B cells, and $CD1d^{high}CD5^+$ B cells. It is unknown whether the cells mediating suppression in recipient animals expressed the phenotype of the administered B cells, or carried a phenotype acquired *in vivo* as a result of their activation and differentiation. $CD19^+CD138^+$ B cells were the major IL-10-producing B cell subset in mice infected with *Salmonella typhimurium*. Further studies shall characterize the phenotype of B cells providing IL-10-dependent and -independent suppressive functions in different models (including in adoptive transfer experiments), and address their relationship with antibody-producing cells. It will also be important to better identify how TLR-activated B cells exert long-lasting suppressive effects on immunity.

7. ACKOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SFB-650 and DFG FI1238/1-1), and the Hertie Stiftung. We thank Prof. Sergei Nedospasow for critical reading of the manuscript.

8. REFERENCES

1. D. P. Harris, L. Haynes, P. C. Sayles, D. K. Duso, S. M. Eaton, N. M. Lepak, L. L. Johnson, S. L. Swain and F. E. Lund: Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat Immunol*, 1(6), 475-82 (2000)

2. V. Lampropoulou, K. Hoehlig, T. Roch, P. Neves, E. Calderon Gomez, C. H. Sweenie, Y. Hao, A. A. Freitas, U. Steinhoff, S. M. Anderton and S. Fillatreau: TLR-activated B cells suppress T cell-mediated autoimmunity. *J Immunol*, 180(7), 4763-73 (2008)

3. K. Hoehlig, V. Lampropoulou, T. Roch, P. Neves, E. Calderon-Gomez, S. M. Anderton, U. Steinhoff and S. Fillatreau: Immune regulation by B cells and antibodies a view towards the clinic. *Adv Immunol*, 98, 1-38 (2008)

4. P. Neves, V. Lampropoulou, E. Calderon-Gomez, T. Roch, U. Stervbo, P. Shen, A. A. Kuhl, C. Loddenkemper, M. Haury, S. A. Nedospasov, S. H. Kaufmann, U. Steinhoff, D. P. Calado and S. Fillatreau: Signaling via the MyD88 adaptor protein in B cells suppresses protective immunity during Salmonella typhimurium infection. *Immunity*, 33(5), 777-90 (2010)

5. S. Fillatreau, C. H. Sweenie, M. J. McGeachy, D. Gray and S. M. Anderton: B cells regulate autoimmunity by provision of IL-10. *Nat Immunol*, 3(10), 944- 50 (2002) doi:10.1038/ni833

6. A. Mizoguchi, E. Mizoguchi, H. Takedatsu, R. S. Blumberg and A. K. Bhan: Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity*, 16(2), 219-30(2002)

7. C. Mauri, D. Gray, N. Mushtaq and M. Londei: Prevention of arthritis by interleukin 10-producing B cells. *J Exp Med*, 197(4), 489-501 (2003)

8. S. Fillatreau, D. Gray and S. M. Anderton: Not always the bad guys: B cells as regulators of autoimmune pathology. *Nat Rev Immunol*, 8(5), 391-7 (2008)

9. S. M. Anderton and S. Fillatreau: Activated B cells in autoimmune diseases: the case for a regulatory role. *Nat Clin Pract Rheumatol*, 4(12), 657-66 (2008)

10. V. Lampropoulou, E. Calderon-Gomez, T. Roch, P. Neves, P. Shen, U. Stervbo, P. Boudinot, S. M. Anderton and S. Fillatreau: Suppressive functions of activated B cells in autoimmune diseases reveal the dual roles of Toll-like receptors in immunity. *Immunol Rev*, 233(1), 146-61 (2010)

11. M. E. Duddy, A. Alter and A. Bar-Or: Distinct profiles of human B cell effector cytokines: a role in immune regulation? *J Immunol*, 172(6), 3422-7 (2004)

12. M. Duddy, M. Niino, F. Adatia, S. Hebert, M. Freedman, H. Atkins, H. J. Kim and A. Bar-Or: Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J Immunol*, 178(10), 6092- 9 (2007)

13. L. Steinman: Assessment of animal models for MS and demyelinating disease in the design of rational therapy. *Neuron*, 24(3), 511-4 (1999)

14. S. Fillatreau and S. M. Anderton: B-cell function in CNS inflammatory demyelinating disease: a complexity of roles and a wealth of possibilities. *Expert Rev Clin Immunol*, 3(4), 565-78 (2007)

15. M. K. Waldor, S. Sriram, R. Hardy, L. A. Herzenberg, L. Lanier, M. Lim and L. Steinman: Reversal of experimental allergic encephalomyelitis with monoclonal antibody to a T-cell subset marker. *Science*, 227(4685), 415-7 (1985)

16. A. Ben-Nun, H. Wekerle and I. R. Cohen: The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis.*Eur J Immunol*, 11(3), 195-9 (1981)

17. J. R. Oksenberg, S. E. Baranzini, S. Sawcer and S. L. Hauser: The genetics of multiple sclerosis: SNPs to pathways to pathogenesis. *Nat Rev Genet*, 9(7), 516-26 (2008)

18. S. L. Hauser, E. Waubant, D. L. Arnold, T. Vollmer, J. Antel, R. J. Fox, A. Bar-Or, M. Panzara, N. Sarkar, S. Agarwal, A. Langer-Gould and C. H. Smith: B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med*, 358(7), 676-88 (2008)

19. T. Matsushita, K. Yanaba, J. D. Bouaziz, M. Fujimoto and T. F. Tedder: Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J Clin Invest*, 118(10), 3420-30 (2008)

20. S. D. Wolf, B. N. Dittel, F. Hardardottir and C. A. Janeway, Jr.: Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. *J Exp Med*, 184(6), 2271-8 (1996)

21. H. Park, Z. Li, X. O. Yang, S. H. Chang, R. Nurieva, Y. H. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian and C. Dong: A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immuno*l, 6(11), 1133-41 (2005)

22. V. K. Kuchroo, C. A. Martin, J. M. Greer, S. T. Ju, R. A. Sobel and M. E. Dorf: Cytokines and adhesion molecules contribute to the ability of myelin proteolipid protein-specific T cell clones to mediate experimental allergic encephalomyelitis. *J Immunol*, 151(8), 4371-82 (1993)

23. A. Mizoguchi, E. Mizoguchi and A. K. Bhan: The critical role of interleukin 4 but not interferon gamma in the pathogenesis of colitis in T-cell receptor alpha mutant mice. *Gastroenterology*, 116(2), 320-6 (1999)

24. L. Svensson, J. Jirholt, R. Holmdahl and L. Jansson: B cell-deficient mice do not develop type II collagen-induced arthritis (CIA). *Clin Exp Immunol*, 111(3), 521-6 (1998)

25. K. Yanaba, J. D. Bouaziz, K. M. Haas, J. C. Poe, M. Fujimoto and T. F. Tedder: A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity*, 28(5), 639-50 (2008)

26. K. Yanaba, J. D. Bouaziz, T. Matsushita, T. Tsubata and T. F. Tedder: The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. *J Immunol*, 182(12), 7459-72 (2009)

27. C. M. Sun, E. Deriaud, C. Leclerc and R. Lo-Man: Upon TLR9 signaling, CD5+ B cells control the IL-12-dependent Th1-priming capacity of neonatal DCs. *Immunity*, 22(4), 467-77 (2005)

28. J. G. Evans, K. A. Chavez-Rueda, A. Eddaoudi, A. Meyer-Bahlburg, D. J. Rawlings, M. R. Ehrenstein and C. Mauri: Novel suppressive function of transitional 2 B

cells in experimental arthritis. *J Immunol*, 178(12), 7868-78 (2007)

29. F. Martin and J. F. Kearney: B1 cells: similarities and differences with other B cell subsets. *Curr Opin Immunol*, 13(2), 195-201 (2001)

30. M. Goetz, R. Atreya, M. Ghalibafian, P. R. Galle and M. F. Neurath: Exacerbation of ulcerative colitis after rituximab salvage therapy. *Inflamm Bowel Dis*, 13(11), 1365-8 (2007)

31. L. Benedetti, D. Franciotta, T. Vigo, M. Grandis, E. Fiorina, E. Ghiglione, L. Roccatagliata, G. L. Mancardi, A. Uccelli and A. Schenone: Relapses after treatment with rituximab in a patient with multiple sclerosis and anti myelin-associated glycoprotein polyneuropathy. *Arch Neurol*, 64(10), 1531-3 (2007)

32. D. El Fassi, C. H. Nielsen, J. Kjeldsen, O. Clemmensen and L. Hegedus: Ulcerative colitis following B lymphocyte depletion with rituximab in a patient with Graves' disease. *Gut*, 57(5), 714-5 (2008)

33. M. Hirotani, M. Niino, T. Fukazawa, S. Kikuchi, I. Yabe, S. Hamada, Y. Tajima and H. Sasaki: Decreased IL-10 production mediated by Toll-like receptor 9 in B cells in multiple sclerosis. *J Neuroimmunol*, 221(1-2), 95-100 (2010)

34. M. Jagannathan, M. McDonnell, Y. Liang, H. Hasturk, J. Hetzel, D. Rubin, A. Kantarci, T. E. Van Dyke, L. M. Ganley-Leal and B. S. Nikolajczyk: Toll-like receptors regulate B cell cytokine production in patients with diabetes. *Diabetologia*, 53(7), 1461-71 (2010)

35. V. Hornung, S. Rothenfusser, S. Britsch, A. Krug, B. Jahrsdorfer, T. Giese, S. Endres and G. Hartmann: Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J Immunol*, 168(9), 4531-7 (2002)

36. D. Rozkova, L. Novotna, R. Pytlik, I. Hochova, T. Kozak, J. Bartunkova and R. Spisek: Toll-like receptors on B-CLL cells: expression and functional consequences of their stimulation. *Int J Cancer*, 126(5), 1132-43 (2010)

37. H. Shin, Y. Zhang, M. Jagannathan, H. Hasturk, A. Kantarci, H. Liu, T. E. Van Dyke, L. M. Ganley-Leal and B. S. Nikolajczyk: B cells from periodontal disease patients express surface Toll-like receptor 4. *J Leukoc* Biol, 85(4), 648-55 (2009)

38. M. Jagannathan, H. Hasturk, Y. Liang, H. Shin, J. T. Hetzel, A. Kantarci, D. Rubin, M. E. McDonnell, T. E. Van Dyke, L. M. Ganley-Leal and B. S. Nikolajczyk: TLR cross-talk specifically regulates cytokine production by B cells from chronic inflammatory disease patients. *J Immunol*, 183(11), 7461-70 (2009)

39. J. Correale and M. Farez: Association between parasite infection and immune responses in multiple sclerosis. *Ann Neurol*, 61(2), 97-108 (2007)

40. J. Correale, M. Farez and G. Razzitte: Helminth infections associated with multiple sclerosis induce regulatory B cells. *Ann Neurol*, 64(2), 187-99 (2008)

41. A. Rydstrom and M. J. Wick: Monocyte and neutrophil recruitment during oral Salmonella infection is driven by MyD88-derived chemokines. *Eur J Immunol*, 39(11), 3019-30 (2009)

42. P. Henneke, O. Takeuchi, R. Malley, E. Lien, R. R. Ingalls, M. W. Freeman, T. Mayadas, V. Nizet, S. Akira, D. L. Kasper and D. T. Golenbock: Cellular activation, phagocytosis, and bactericidal activity against group B streptococcus involve parallel myeloid differentiation factor 88-dependent and independent signaling pathways. *J Immunol*, 169(7), 3970-7 (2002)

43. R. Medzhitov, P. Preston-Hurlburt and C. A. Janeway, Jr.: A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature*, 388(6640), 394-7 (1997)

44. S. Fillatreau: Novel regulatory functions for Toll-like receptor-activated B cells during intracellular bacterial infection. *Immunol Rev*, 240(1), 52-71 (2011)

45. H. W. Mittrucker and S. H. Kaufmann: Immune response to infection with Salmonella typhimurium in mice. *J Leukoc Biol*, 67(4), 457-63 (2000)

46. J. A. Crump, S. P. Luby and E. D. Mintz: The global burden of typhoid fever. *Bull World Health Organ*, 82(5), 346-53 (2004)

47. M. Shapiro-Shelef and K. Calame: Regulation of plasma-cell development. *Nat Rev Immunol*, 5(3), 230-42 (2005)

48. G. A. Martins, L. Cimmino, M. Shapiro-Shelef, M. Szabolcs, A. Herron, E. Magnusdottir and K. Calame: Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. *Nat Immunol*, 7(5), 457-65 (2006)

49. M. A. Tam, A. Rydstrom, M. Sundquist and M. J. Wick: Early cellular responses to Salmonella infection: dendritic cells, monocytes, and more. *Immunol Rev*, 225, 140-62 (2008)

50. S. C. Smelt, S. E. Cotterell, C. R. Engwerda and P. M. Kaye: B cell-deficient mice are highly resistant to Leishmania donovani infection, but develop neutrophil-mediated tissue pathology. *J Immunol*, 164(7), 3681-8 (2000)

51. P. J. Maglione, J. Xu and J. Chan: B cells moderate inflammatory progression and enhance bacterial containment upon pulmonary challenge with

Mycobacterium tuberculosis. *J Immunol*, 178(11), 7222-34 (2007)

52. M. P. Berry, C. M. Graham, F. W. McNab, Z. Xu, S. A. Bloch, T. Oni, K. A. Wilkinson, R. Banchereau, J. Skinner, R. J. Wilkinson, C. Quinn, D. Blankenship, R. Dhawan, J. J. Cush, A. Mejias, O. Ramilo, O. M. Kon, V. Pascual, J. Banchereau, D. Chaussabel and A. O'Garra: An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature*, 466(7309), 973-7 (2010)

53. E. Voog, F. Morschhauser and P. Solal-Celigny: Neutropenia in patients treated with rituximab. *N Engl J Med*, 348(26), 2691-4; discussion 2691-4 (2003)

54. O. Wolach, O. Bairey and M. Lahav: Late-onset neutropenia after rituximab treatment: case series and comprehensive review of the literature. *Medicine* (Baltimore),89(5),308-18(2010)

55. B. Terrier, M. Ittah, L. Tourneur, F. Louache, V. Soumelis, F. Lavie, N. Casadevall, S. Candon, A. Hummel, X. Mariette and A. Buzyn: Late-onset neutropenia following rituximab results from a hematopoietic lineage competition due to an excessive BAFF-induced B-cell recovery. *Haematologica*, 92(2), e20-3 (2007)

56. K. Dunleavy, F. Hakim, H. K. Kim, J. E. Janik, N. Grant, T. Nakayama, T. White, G. Wright, L. Kwak, R. Gress, G. Tosato and W. H. Wilson: B-cell recovery following rituximab-based therapy is associated with perturbations in stromal derived factor-1 and granulocyte homeostasis. *Blood*, 106(3), 795-802 (2005)

57. K. Chaiwatanatorn, N. Lee, A. Grigg, R. Filshie and F. Firkin: Delayed-onset neutropenia associated with rituximab therapy. *Br J Haematol*, 121(6), 913-8 (2003)

58. Y. Ueda, M. Kondo and G. Kelsoe: Inflammation and the reciprocal production of granulocytes and lymphocytes in bone marrow. *J Exp Med*, 201(11), 1771-80 (2005)

59. E. J. Fuchs and P. Matzinger: B cells turn off virgin but not memory T cells. *Science*, 258(5085), 1156-9 (1992)

60. E. T. Zambidis, A. Kurup and D. W. Scott: Genetically transferred central and peripheral immune tolerance via retroviral-mediated expression of immunogenic epitopes in hematopoietic progenitors or peripheral B lymphocytes. *Mol Med*, 3(3), 212-24 (1997)

61. B. Xu and D. W. Scott: A novel retroviral gene therapy approach to inhibit specific antibody production and suppress experimental autoimmune encephalomyelitis induced by MOG and MBP. *Clin Immunol*, 111(1), 47-52 (2004)

62. M. E. Melo, J. Qian, M. El-Amine, R. K. Agarwal, N. Soukhareva, Y. Kang and D. W. Scott: Gene transfer of Ig-fusion proteins into B cells prevents and treats autoimmune diseases. *J Immunol*, 168(9), 4788-95 (2002)

63. W. Liang, Z. Karabekian, M. Mattapallil, Q. Xu, A. M. Viley, R. Caspi and D. W. Scott: B-cell delivered gene transfer of human S-Ag-Ig fusion protein protects from experimental autoimmune uveitis. *Clin Immunol*, 118(1), 35-41 (2006)

64. R. K. Agarwal, Y. Kang, E. Zambidis, D. W. Scott, C. C. Chan and R. R. Caspi: Retroviral gene therapy with an immunoglobulin-antigen fusion construct protects from experimental autoimmune uveitis. *J Clin Invest*, 106(2), 245-52 (2000)

65. L. Song, J. Wang, R. Wang, M. Yu, Y. Sun, G. Han, Y. Li, J. Qian, D. W. Scott, Y. Kang, N. Soukhareva and B. Shen: Retroviral delivery of GAD-IgG fusion construct induces tolerance and modulates diabetes: a role for CD4+ regulatory T cells and TGF-beta? *Gene Ther*, 11(20), 1487-96 (2004)

66. N. Soukhareva, Y. Jiang and D. W. Scott: Treatment of diabetes in NOD mice by gene transfer of Ig-fusion proteins into B cells: role of T regulatory cells. *Cell Immunol*, 240(1), 41-6 (2006)

67. M. S. Anderson and J. A. Bluestone: The NOD mouse: a model of immune dysregulation. *Annu Rev Immunol*, 23, 447-85 (2005)

68. T. Wong and M. Recht: Current options and new developments in the treatment of haemophilia. Drugs, 71(3), 305-20 (2011)

69. L. W. Hoyer: Hemophilia A. N Engl J Med, 330(1), 38-47 (1994)

70. T. C. Lei and D. W. Scott: Induction of tolerance to factor VIII inhibitors by gene therapy with immunodominant A2 and C2 domains presented by B cells as Ig fusion proteins. Blood, 105(12), 4865-70 (2005)

71. M. G. Jacquemin and J. M. Saint-Remy: Factor VIII alloantibodies in hemophilia. *Curr Opin Hematol*, 11(3), 146-50 (2004)

72. K. P. Pratt, J. Qian, E. Ellaban, D. K. Okita, B. M. Diethelm-Okita, B. Conti-Fine and D. W. Scott: Immunodominant T-cell epitopes in the factor VIII C2 domain are located within an inhibitory antibody binding site. *Thromb Haemost*, 92(3), 522-8 (2004)

73. P. Bretscher and M. Cohn: A theory of self-nonself discrimination. *Science*, 169(950), 1042-9 (1970)

74. C. A. Janeway, Jr.: Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol*, 54 Pt 1, 1-13 (1989)

75. E. T. Zambidis, R. K. Barth and D. W. Scott: Both resting and activated B lymphocytes expressing engineered peptide-Ig molecules serve as highly efficient tolerogenic

vehicles in immunocompetent adult recipients. *J Immunol*, 158(5), 2174-82 (1997)

76. M. El-Amine, M. Melo, Y. Kang, H. Nguyen, J. Qian and D. W. Scott: Mechanisms of tolerance induction by a gene-transferred peptide-IgG fusion protein expressed in B lineage cells. *J Immunol*, 165(10), 5631-6 (2000)

77. M. T. Litzinger, Y. Su, T. C. Lei, N. Soukhareva and D. W. Scott: Mechanisms of gene therapy for tolerance: B7 signaling is required for peptide-IgG gene-transferred tolerance induction. *J Immunol*, 175(2), 780-7 (2005)

78. T. C. Lei, Y. Su and D. W. Scott: Tolerance induction via a B-cell delivered gene therapy-based protocol: optimization and role of the Ig scaffold. *Cell Immunol*, 235(1), 12-20 (2005)

79. J. Skupsky, A. H. Zhang, Y. Su and D. W. Scott: B-cell-delivered gene therapy induces functional T regulatory cells and leads to a loss of antigen-specific effector cells. *Mol Ther*, 18(8), 1527-35 (2010)

80. R. R. Ahangarani, W. Janssens, L. VanderElst, V. Carlier, T. VandenDriessche, M. Chuah, B. Weynand, J. A. Vanoirbeek, M. Jacquemin and J. M. Saint-Remy: In vivo induction of type 1-like regulatory T cells using genetically modified B cells confers long-term IL-10-dependent antigen-specific unresponsiveness. *J Immunol*, 183(12), 8232-43 (2009)

81. Y. Su, A. H. Zhang, N. Noben-Trauth and D. W. Scott: B-Cell Gene Therapy for Tolerance Induction: Host but Not Donor B-Cell Derived IL-10 is Necessary for Tolerance. *Front Microbiol*, 2, 154 (2011)

82. J. N. Kline, T. J. Waldschmidt, T. R. Businga, J. E. Lemish, J. V. Weinstock, P. S. Thorne and A. M. Krieg: Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. J *Immunol*, 160(6), 2555-9 (1998)

83. V. V. Jain, K. Kitagaki, T. Businga, I. Hussain, C. George, P. O'Shaughnessy and J. N. Kline: CpG-oligodeoxynucleotides inhibit airway remodeling in a murine model of chronic asthma. *J Allergy Clin Immunol*, 110(6), 867-72 (2002)

84. K. Kitagaki, V. V. Jain, T. R. Businga, I. Hussain and J. N. Kline: Immunomodulatory effects of CpG oligodeoxynucleotides on established th2 responses. Clin Diagn Lab Immunol, *9(6), 1260-9 (2002)*

85. P. S. Creticos, J. T. Schroeder, R. G. Hamilton, S. L. Balcer-Whaley, A. P. Khattignavong, R. Lindblad, H. Li, R. Coffman, V. Seyfert, J. J. Eiden and D. Broide: Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med*, 355(14), 1445-55 (2006)

86. D. Miyazaki, C. H. Kuo, T. Tominaga, Y. Inoue and S. J. Ono: Regulatory function of CpG-activated B cells in late-phase experimental allergic conjunctivitis. *Invest Ophthalmol Vis Sci*, 50(4), 1626-35 (2009)

87. N. E. Mangan, R. E. Fallon, P. Smith, N. van Rooijen, A. N. McKenzie and P. G. Fallon: Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. *J Immunol*, 173(10), 6346-56 (2004)

Abbreviations: BCR: B cell receptor; TLR: Toll-like receptor; IL: interleukin; IFN: interferon; EAE: experimental autoimmune encephalomyelitis; RR-MS: relapsing-remitting multiple sclerosis; CNS: central nervous system; MyD88: myeloid differentiation factor 88; LPS: lipopolysaccharide; PMA: phorbol myristate acetate; EAU: experimental autoimmune uveitis; NOD: nonobese diabetic; GAD: glutamic acid decarboxylase; APC: antigen-presenting cell

Key Words: B cell, Toll-like receptor, Tolerance, Autoimmunity, Interleukin-10, Infection, Cell therapy, Review

Send correspondence to: Simon Fillatreau, Immune regulation group, Deutsche Rheuma-Forschungszentrum, a Leibniz institute, Chariteplatz 1, 10117 Berlin, Germany, Tel: 49-0-30-284-60-752, Fax: 49-0-30-284-60-603, E-mail: simonfillatreau@googlemail.com