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Integration of B cell responses through Toll-like receptors and antigen receptors

David J Rawlings[1,2,3], **Marc A Schwartz**[2,3], **Shaun W Jackson**[1,3], and **Almut Meyer-Bahlburg**[4]

David J Rawlings: drawling@uw.edu; Marc A Schwartz: mas1084@gmail.com; Shaun W Jackson: shaun.jackson@seattlechildrens.org; Almut Meyer-Bahlburg: Meyer-Bahlburg.Almut@mh-hannover.de

[1]Department of Pediatrics, University of Washington School of Medicine, Seattle, WA 98195

[2]Department of Immunology, University of Washington School of Medicine, Seattle, WA 98195

[3]Seattle Children's Research Institute, Seattle, WA 98101

[4]Department of Pediatric Pneumology and Neonatology, Hannover Medical School, Germany

Preface

Unlike other immune cells, B cells express both an antigen-specific B cell receptor (BCR) and Toll-like receptors (TLRs). Dual BCR and TLR engagement can fine-tune functional B cell responses, directly linking cell-intrinsic innate and adaptive immune programs. While most data regarding B cell-specific functions of the TLR signaling pathway has been obtained in mice, the discovery of patients with a deficiency in this pathway has recently provided insight into human B cell responses. Here, we highlight the importance of the integration of signals downstream of BCR and TLR activation in modulating B cell function, focusing when possible on cell intrinsic roles.

Introduction

Over the last decade, the unexpected success of B cell depletion therapies in human autoimmunity, combined with a growing recognition of the importance of neutralizing antibody responses in host defense, has led to an increased focus on understanding the role(s) for B cells in human immune function. B cells do not merely produce immunoglobulin, but can also secrete cytokines and serve as antigen-presenting cells and therefore B cells have a multi-faceted involvement in distinct immune responses.

A striking characteristic of B cells is expression of a clonally-rearranged, antigen-specific B cell receptor (BCR) in conjunction with expression of one or more members of a family of germline-encoded receptors termed Toll-like receptors (TLRs), capable of recognizing discrete microbiological ligands. This dual expression program permits B cells to uniquely integrate both antigen-specific and 'danger' signals via these key receptor systems. Although both B cell development and survival appear phenotypically unperturbed in the absence of TLR signals[1], patients with IRAK-4 or MyD88 deficiency possess an altered BCR repertoire with an increased proportion of autoreactive cells, presumably due to alterations in B cell selection processes[2]. Different B cell subsets exhibit variations in TLR expression patterns, and signaling via TLRs can modify B cell responses such as antibody production, antigen presentation and cytokine secretion. Therefore, individual TLR

Correspondence: David J. Rawlings, M.D., Center for Immunity and Immunotherapies, Seattle Children's Research Institute, 1900 Ninth Avenue, Seattle, WA 98101, Tel. (206) 987-7450, Fax (206) 987-7310, drawling@u.washington.edu.

expression profiles permit various effector B cell populations to manifest specific response profiles following TLR engagement[3,4].

Notably, based upon their functional responses as well as their BCR repertoire, naïve mature B cell populations have been defined as either innate-like or adaptive cells (Box 1). Innate, B-1 or marginal zone, B cells generate rapid antibody responses independent of T cell help. In contrast adaptive, follicular B cells primarily participate in T-dependent responses leading to generation of high-affinity antibodies and long-term memory. Importantly, expression of a distinct profile of TLRs and a specific BCR profile likely helps to specify the differentiation and function of these key innate vs. adaptive B cell populations. During T-independent immune responses, dual BCR and TLR signaling rapidly induce marginal zone cells and B-1 B cell migration and antibody production. Additionally, upon triggering of T-dependent immune responses, TLR responsiveness is directly modulated in activated follicular B cells thereby impacting germinal centre responses. TLR engagement, in conjunction with BCR ligation, also provides a bridge between the innate and the adaptive immune system that may impact on antigen presentation, primary antibody responses, class-switch recombination and subsequent memory responses.

Box 1

Innate-like and adaptive B cell subsets

Based on phenotypic, functional and topographical characteristics, B cells can be divided into innate-like and adaptive immune cells[109]. Follicular B cells are the main players during T-dependent immune responses and belong to the adaptive arm of the immune system. They generate a clonally rearranged antigen-specific B cell receptor (BCR) and form memory responses that are dependent on T cell help. In contrast, B-1 and marginal zone B cells are usually considered innate-like immune cells and generate rapid but lower affinity antibody responses that are independent of T cell help.

The term ‘B-1’ refers to the idea that this populations develops earlier during ontogeny than conventional B-2 cells[110]. B-1 cells are enriched in the peritoneal and pleural cavity but can also be found in the spleen. CD5 expression further subdivides mouse B-1 cells into $CD5^+$ B-1a and $CD5^-$ B-1b cells. Recently, a B-1 cell progenitor was identified in the bone marrow of adult mice[111]. The term ‘B-2’ has traditionally been used to describe the main population of mature B cells that develop from common bone marrow precursors and are located in the bone marrow, spleen and lymph nodes; B-2 cells therefore include both follicular and marginal zone subsets, which presently are referred to as separate populations because of their distinct phenotypic and functional characteristics.

Recent work has defined a B cell subset in human peripheral blood with functional responses similar to mouse B-1 cells[112]. Consistent with their innate-like immune cell phenotype, marginal zone and B-1 B cells mainly express germ-line encoded antigen receptors that have limited diversity and are enriched for specificities that recognize microbial and self-antigens. In addition to BCR ligation, activation of pattern-recognition receptors including Toll-like receptors (TLRs) on these cells is important for their immune responses. Moreover, B-1 and marginal zone B cells are the primary producers of natural IgM antibody[113]. Through these characteristics, both subsets are crucial in the early phase of T-independent immune responses[10] linking innate and adaptive immune mechanisms. Moreover, due to their polyspecific BCR repertoire, B-1 and marginal zone B cells have been implicated in driving autoimmune processes[109].

All TLRs, with the exception of TLR3, require the signaling adaptor myeloid differentiation primary-response protein 88 (MyD88) to mediate activation signals, although TLR4 can uniquely signal through both MyD88-dependent and –independent pathways[5]. Therefore, analyses of animal models and humans with deficient function of this adaptor have begun to provide important new insight into how signals via TLRs impact B cell function and immune responses. Further, recent work indicates that MyD88 also orchestrates TACI (transmembrane activator and calcium-modulator and cyclophilin ligand interactor)-mediated, BAFF-driven signals.

BAFF and the related cytokine APRIL play a major role in peripheral B cell homeostasis and survival[6]. These cytokines bind to a family of receptors expressed primarily on B cells including the BAFF receptor (BAFFR), B cell maturation antigen (BCMA), and TACI. BAFF levels are modulated by a complex interplay of factors that includes the number of B cells competing for ligand, the relative level of BAFF-binding receptors on specific B cell subsets, and inflammatory (including TLR-driven) signals that modulate BAFF production by myeloid and/or stromal cell populations. BAFF- or BAFFR-deficient mice have severely decreased numbers of B cells, whereas overexpression of BAFF increases B cell numbers and promotes systemic lupus erythematosus (SLE)-like disease. Both BCR and TLR-mediated stimulation of mouse marginal zone B cells[7,8] or B-1 cells[9] up-regulates BAFFR and TACI expression, increasing the sensitivity of these cells to BAFF and APRIL. As a result, B cell activation during immune responses directly modulates downstream outcomes of BAFF signaling by regulating BAFF-family receptor expression. Finally, an additional level of control is provided via direct participation of MyD88 in BAFF-triggered TACI signals. This dual role for MyD88 in TLR and TACI signaling thereby permits precise regulation of peripheral B cell responses to infectious challenge and other microenvironmental cues.

This review focuses on the role dual BCR and TLR (as well as other MyD88-dependent signals) in normal and dysregulated B cell immune function, with a particular emphasis on B cell-intrinsic events. We first summarize the specific roles for MyD88 signals in T-independent versus T-dependent B cell immune responses. We will highlight work that demonstrates a non-redundant role for B cell-intrinsic MyD88 signals in antiviral immune responses; describe how MyD88 controls a B cell-intrinsic, TLR-independent, pathway for immunoglobulin diversification; and review new information regarding the role for TLR signals in human immune function gained via analysis of patients with MyD88 or IL-1R-associated kinase 4 (IRAK4) deficiencies. Next, we describe how dual BCR and TLR signals may potentiate the risk for autoimmunity and discuss recent findings regarding how regulatory B cell function also requires TLR/MyD88 signals. Finally, we will discuss emerging data indicating that disregulated TLR and BCR signals collaborate during the development of B cell malignancies and, possibly, in other pathogenic B cell states.

T-independent B cell immune responses

As innate-like cells, marginal zone and B-1 B cells are the main players during T-independent immune responses. Innate B cells rapidly develop into IgM-producing plasmablasts during the early primary immune response[10] and the importance of these subsets in T-independent responses to encapsulated bacteria has been demonstrated in infectious models most notably including *Streptococcus pneumoniae*[11]. Both mice and humans with deficiencies in B-1 and marginal zone B cells, including patients with hypo- or agammaglobulinemia[12] and Wiskott-Aldrich Syndrome (WAS)[13], also exhibit increased susceptibility to infections with encapsulated bacteria; and both young children, in whom formation of the marginal zone B cell compartment is physiologically delayed until about two years of life, and splenectomized individuals are at very high risk for such infections[14].

Figure 1 provides an overview how BCR and MyD88 signal function in concert to modulate T-independent responses based on the *in vitro* and *in vivo* studies described below.

In vitro analyses of TLR-modulated T-independent responses

Stimulation of B cells via TLR ligands has been used extensively as an *in vitro* model of T-independent immune responses. *In vitro*, both B-1 and marginal zone B cells rapidly proliferate and secrete antibody in response to TLR engagement, including TLR1–TLR2, TLR4, TLR6, TLR7 and TLR9[3,15–18]. Interestingly, B-1 cells (predominantly B-1b cells) stimulated in this manner secrete large amounts of IgA, whereas marginal zone B cells primarily produce IgM[4,15]; a feature that correlates with their *in vivo* functional roles in mucosal vs. blood-borne infections. Notably, both marginal zone and B-1 B cells exhibit stronger functional responses to TLR ligands than follicular B cells, as measured by up-regulation of activation markers[15,18] and production of IL10[19,20]. Marginal zone B cells have also been shown to exhibit greater potential to act as antigen-presenting cells than follicular B cells in response to TLR stimulation[18], allowing this unique subset to efficiently bridge T-independent and T-dependent responses via shuttling antigen from the marginal zone and presenting it within the T cell zones.

Our group investigated the mechanism(s) accounting for the differential responsiveness of adaptive (follicular) vs. innate (marginal zone) B cells to the classically defined, T-independent type 1 stimulus and TLR4 ligand, lipopolysaccharide (LPS)[3]. We confirmed a delay in cell cycle entry in follicular compared with marginal zone B cells, but could not identify significant differences in proximal TLR-driven biochemical signaling events including activation of nuclear factor-κB (NF-κB) and mammalian target of rapamycin (mTOR) pathways. Notably, follicular B cells exhibited reduced basal and inducible levels of the cell cycle and growth regulator, c-MYC. Consistent with a key role for c-MYC in modulating LPS-responsiveness, enforced expression of c-MYC in follicular B cells eliminated the delay in cell cycle entry and promoted increased immune responses that mimicked functional responses of MZ B cells

Thus, consistent with their characterization as innate immune cells, B-1 and marginal zone B cells generally exhibit stronger *in vitro* responses to TLR signaling compared with follicular mature B cells and regulation of c-MYC expression levels contribute, in part, to this differential response profile.

In vivo analyses of TLR-regulation of T-independent responses

Signaling via both MyD88 and Bruton's tyrosine kinase (BTK) is required for T-independent pathogen-specific IgM production in a mouse model of *Borrelia hermsii* infection. Mice deficient in both BTK (a kinase essential for BCR signalling) and MyD88 failed to generate pathogen-specific IgM[21], whereas mice deficient in BTK alone generated specific antibodies and resolved bacteremia. In contrast, mice deficient in TLR1, TLR2 or MyD88 generated pathogen-specific antibodies with delayed kinetics and suffered more severe episodes of bacteremia, suggesting that MyD88 specifically synergizes with BCR signals and that in $Btk^{-/-}$ B cells, TLR-mediated stimulation is sufficient to rescue the defective BCR signal.

Two groups have analyzed in vivo B cell responses to *Salmonella typhimurium* in the setting of MyD88 deficiency restricted to the B cell compartment[22,23]. *Salmonella typhimurium* induces both T-independent and T-dependent immune responses. Following *S. typhimurium* infection, *S. typhimurium*-specific IgM and IgG responses were initially lower in mice with $Myd88^{-/-}$ B cells, but no difference was observed at later time points (>4 weeks) after infection. Moreover, MyD88 signaling in B cells significantly inhibited both

neutrophil and natural killer (NK) cell responses to *S. typhimurium*[23]. Based on these limited *in vivo* data, MyD88 signaling in B cells, in conjunction with BCR engagement, appears to play an important role during early T-independent immune responses to bacteria leading to both rapid production of protective IgM and IgG, and modulation of other innate effector cell populations (presumably via cytokine and/or chemokine production).

TLR signals in innate B cell positioning

TLR signals play an important role in the regulation of localization and migration of B-1 and marginal zone B cells. While enriched in the splenic marginal zone, marginal zone B cells continuously circulate between the marginal zone and splenic follicles, a property that allows this population to capture and shuttle blood borne antigens to follicular dendritic cells. *In vivo* treatment with LPS promotes near complete relocalization of marginal zone B cells into the splenic white pulp[24–26]. Sphingosine-1-phosphate receptor 1 (S1P1) represents a important regulator marginal zone B cell retention and blocking S1P1 with the inhibitor FTY720 *in vivo* leads to migration of marginal zone B cells into follicles[27]. Interestingly, LPS administration results in downregulation of S1P1 expression by marginal zone B cells and reduced chemotactic responsiveness to S1P possibly explaining this observation. Of note, TLR2, TLR3 and TLR7 ligands also promote marginal zone B cell migration[28]. Interestingly, whereas *in vivo* treatment with TLR2, TLR4 and TLR7 ligands resulted in downregulation of *S1p1* mRNA levels, no change was seen after stimulation via TLR3, implying that at least two distinct TLR dependent signalling pathways can promote migration of marginal zone B cells.

Similarly, B-1 cells express very high levels of integrins and stimulation via TLRs induces a massive egress of B-1 cells from the peritoneal cavity associated with coordinated down-regulation of integrins and CD9 expression[29]. Thus, B cell intrinsic TLR signaling *in vivo* can directly alter B-1 B cell responsiveness, thereby directing their migration to sites where rapid local antibody responses help to limit pathogen growth. This likely also allows innate B cells to modulate the local effector functions other immune cell types.

MyD88 signals in human innate-like B cell responses

While it is difficult to judge how findings generated using mouse models can be translated into the human system, some insight into the role of B cell-intrinsic TLR signals can be obtained from the analysis of patients with inborn errors in the downstream TLR signaling effectors, MyD88 or IRAK4 (Box 2)[30–32]. In these patients, *S. pneumoniae* is the most common invasive pathogen, followed by *S. aureus* and *P. aeruginosa* — all of which trigger T-independent responses[31]. In contrast, antibody responses to protein antigens are normal in these patients and no severe viral, parasitic or fungal diseases have been observed. The predominance of infections with encapsulated bacteria suggests that TLR signaling in human B cells is likely crucial for triggering T-independent immune responses that require B-1 and/or marginal zone B cells. A detailed analysis of the *in vivo* responses to polysaccharide immunization in such individuals would be a helpful means to begin to directly test this interpretation.

Box 2

Requirement for TLR responses in humans revealed by primary immune deficiency disorders

Over the last decade several monogenic primary immunodeficiencies affecting the Toll-like receptor (TLR) signaling pathway have been identified. Inborn errors of the TLR and interleukin-1 receptor (IL-1R) pathway include IL-1R-associated kinase 4 (IRAK4) and myeloid differentiation primary-response protein 88 (MyD88) deficiency identified in

patients in 2003[32] and 2008[30], respectively. The serine/threonine kinase IRAK4 and MyD88 are key adaptor molecules that function downstream of TLRs and IL-1R. Both inborn errors are inherited in an autosomal recessive manner and result in indistinguishable clinical disease[31]. Patients with these inborn errors are typically predisposed during infancy and early childhood to recurrent life-threatening bacterial infections, most commonly involving invasive pneumococcal disease with only limited signs of systemic inflammation. Interestingly, no deaths or invasive infectious episodes have been reported in patients beyond age 14 years. Moreover, no severe viral, parasitic and fungal disease have been reported and the range of bacterial pathogens that theses patients are susceptible to seems to be restricted to *Streptococcus pneumococcus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Immune responses to protein antigen were also mainly normal. Treatment of these patients with prophylactic antibiotics, anti-pneumococcal vaccination and/or intravenous immunoglobulin seemed to have a beneficial impact on the disease. The absence of severe infections in older patients may result from maturation of other signaling cascades and/or adaptive immune function that gradually compensates for the lack of TLR signaling.

Another group of inborn errors affects the TLR3–interferon β (IFNβ) and –IFNλ pathway, independent of MyD88 signaling and results from autosomal recessive UNC93B deficiency (an ER-resident transmembrane protein, deficiency in which impacts on TLR3, TLR7, TLR8 and TLR9 signaling)[114] and autosomal dominant TLR3 deficiency[115]. Both deficiencies are associated with the occurrence of Herpes simplex virus-mediated encephalitis. Overall, these data suggest that deficits in TLR signaling increase susceptibility for specific pathogens without obviously impacting responses to T-dependent immunization or immune cell development. Further studies, however, are required to fully understand the precise contributions of TLRs and IL-1R to human host defense.

Unique role for MyD88 in TACI signaling

Recent work has identified an unexpected role for MyD88 in signal transduction via the BAFF- and APRIL-binding cell surface receptor TACI (also known as TNFRSF13B) (Figure 1)[33]. Following BAFF-binding, TACI directly interacts with MyD88[33] resulting in activation of NF-κB via a signaling cascade dependent on IRAK1, IRAK4, TNFR-associated factor 6 (TRAF6), TGFβ-activated kinase 1 (TAK1) and IκB kinase (IKK). Strikingly, TACI engagement, in conjunction with cytokines or TLR ligands, markedly facilitates class-switch recombination (CSR) [33]; suggesting that TACI signaling via MyD88 may help promote extrafollicular B cell responses that lead to rapid and sustained IgG and IgA responses to T-independent antigens, including encapsulated bacteria polysaccharides. TACI–MyD88 interactions may also enhance antibody responses by delivering survival signals to activated extrafollicular B cells, including class-switched plasmablasts. This mechanism may help to explain the clinical observations in patients with MyD88 or IRAK4 deficiency, as well as those with common variable immune deficiency (CVID) syndrome and mutations in TACI, who present with hypogammaglobulinemia and impaired IgG responses to T-independent antigens[34,35].

In summary, MyD88 signaling in B cells is crucial for T-independent responses by activating and positioning B cells, thereby resulting in rapid and appropriately localized, pathogen-specific, antibody production. In addition, B intrinsic, TACI-triggered, MyD88 signals appear to promote extrafollicular class-switch recombination facilitating improved primary, protective, T-independent, antibody responses.

Role of MyD88 signals in T-dependent B cell immune responses

B cells are recruited into T-dependent immune responses primarily from the FM subset, after which they enter a germinal center and subsequently differentiate into either antibody-secreting plasma cells or memory B cells. Although innate-like B cells generally display stronger responses to TLR engagement alone, antigen-mediated B cell responses can be modulated by simultaneous BCR and TLR engagement (Figure 2). *In vitro*, follicular B cells proliferate, produce cytokines and secrete antibody following stimulation with various TLR ligands[3,4,15,36]. Purification of mouse germinal center B cells revealed enhanced TLR ligand-responsiveness and increased expression of *Myd88* mRNA compared with follicular B cells, suggesting that follicular B cells may become more sensitive to TLR engagement during the course of a T-dependent immune response[16]. Additionally, TLR4 signaling in B cells has been proposed to increase migration to lymph nodes and accumulation in germinal center dark zones, perhaps helping to sustain ongoing germinal center reactions[37].

A role for TLRs in promoting plasma cell differentiation has been proposed based on immunoglobulin secretion and expression of the differentiation factors B lymphocyte-induced maturation protein 1 (BLIMP1) and X-box-binding protein 1 spliced isoform (XBP1s) in response to TLR ligands in follicular B cells, although in the absence of co-stimulation these responses were primarily elicited in B-1 and marginal zone B cells[15]. In humans, naïve B cells are minimally responsive to TLR ligands, but memory B cells proliferate and differentiate into antibody-secreting plasma cells in response to the TLR9 ligand CpG DNA[38]. These data led to the suggestion that stimulation of human memory B cells through TLRs may contribute to long-term maintenance of serological memory[38]. A later study showed that maximal activation of naïve human B cells required a combination of BCR engagement, T cell help via CD40 signaling and TLR stimulation[39].

B cell-intrinsic MyD88 signals in response to T-dependent antigens

Together, the above data suggested that, in both mice and humans, B cell intrinsic TLR signals can promote events associated with T-dependent immune responses. This has led to significant interest in determining the *in vivo* role of MyD88 in B cells in the response to protein antigens. This question was first addressed by transferring either wild-type or MyD88-deficient B cells into μMT mice and analyzing the response to intraperitoneal immunization with human serum albumin (HSA) and LPS co-adsorbed on the adjuvant alum[40]. In this setting, production of HSA-specific IgM and IgG1 antibodies was severely impaired in mice with MyD88-deficient B cells. Similar results were obtained after immunization with flagellin, and the authors provided evidence that MyD88 signaling promotes enhanced antigen presentation by B cells, as well as differentiation of germinal center B cells and plasma cells. This study concluded that activation of TLRs in B cells is necessary for antibody responses to T-dependent antigens.

A subsequent study further tested this idea by immunizing wild-type and MyD88- and TIR-domain-containing adaptor protein inducing IFNβ (TRIF)-double deficient mice with trinitrophenol-hemocyanin (TNP–Hy) or TNP–keyhole limpet hemocyanin (TNP–KLH) as antigens in combination with various adjuvants[1]. TNP-specific antibody responses were largely intact in double deficient mice, although IgG2b and IgG2c titers were slightly lower in response to TNP–KLH in Ribi adjuvant. In a third study, wild-type or MyD88-deficient B cells were transferred to μMT mice, which were then immunized with the antigen 4-hydroxy-3-nitrophenylacetyl (NP)–chicken γ-globulin (CGG) in alum[16]. During both the primary immune response and following re-challenge with NP–CGG 4 months after initial immunization, NP-specific IgM and IgG levels were unaffected in recipients of MyD88-deficient B cells. However, addition of LPS during the primary immunization greatly increased the antibody response to NP–CGG, and increased NP-specific IgM and IgG2a

levels required MyD88 expression in B cells, whereas increased NP-specific IgG levels did not. The ability of TLR signals to activate memory B cells was also tested by injecting μMT mice that had received wild-type or MyD88-deficient B cells with LPS several months after a series of NP–CGG immunizations. LPS induced a transient increase in both total and NP-specific IgM and IgG levels. Together, these latter two studies support a model in which B cell MyD88 signaling is not required to generate T-dependent antigen-specific antibody responses, but such signals can augment early antibody production, influence CSR and promote differentiation of memory B cells into plasma cells.

Several factors may help explain the discrepancy in the results from these studies. TLR engagement on B cells might be required in settings where BCR engagement or perhaps T cell co-stimulation is limited. Thus, it is possible that decreased levels of BCR crosslinking by HSA compared with TNP–KLH (or NP–CGG) may affect the requirement for MyD88 signals in B cells[41]. Additionally, a comparison of responses to HSA with or without the hapten dinitrophenol (DNP) indicated that antibody response to HSA with LPS required MyD88 signaling, whereas DNP–HSA in incomplete Freund's adjuvant elicited equivalent responses in wild-type versus MyD88- and TRIF-double deficient mice, leading to the suggestion that haptenated antigens are inherently immunogenic, thereby limiting the requirement for MyD88 signals[42].

In addition to variability in the antigens used in these studies, recent reports suggest an important role for the physical form of the TLR ligands during adaptive immune responses. Using either myeloid cell- or B cell-specific deletion of MyD88, DeFranco and colleagues investigated how various forms of a TLR ligand influence T-dependent immune responses[43]. Ovalbumin (OVA)-specific antibody responses to OVA with soluble CpG DNA, OVA with an aggregated form of CpG DNA, and OVA covalently linked to CpG DNA depended on MyD88 expression in DCs but not B cells. In contrast, antigen-specific IgG responses to immunization with CpG DNA incorporated in proteinacious virus-like particles (VLPs) that were derived from the Qβ bacteriophage depended largely on MyD88 expression in B cells but not DCs. B cell-specific MyD88-deficent mice were deficient in IgG2b and IgG2c Qβ-specific antibodies and this correlated with a dramatic defect in differentiation of germinal center B cells. Consistent with a critical role for B cell intrinsic MyD88 signals in the humoral response to viruses, the IgG influenza virus-specific response generated following immunization with inactivated H1N1 virus was significantly reduced in B cell-specific MyD88-deficient mice[43].

These data are consistent with the previous demonstration that human papillomavirus type 16 (HPV16) major caspid protein L1-containing VLPs can directly induce CSR in B cells, dependent on intrinsic MyD88 expression[44]. In another study, μMT mice reconstituted with TLR9-deficient B cells and challenged with Qβ VLPs containing CpG DNA showed a dramatic reduction in VLP-specific IgG2a titers, further supporting a role for B cell intrinsic MyD88 signaling in driving CSR[45]. Of note, major T-dependent immune deficits have not been identified in MyD88 or IRAK4 deficient patients: however, published data are largely limited to total IgG responses to robust T-dependent antigens (for example, tetanus toxoid- and diptheria toxin-derived antigens)[31].

MyD88-dependent T-dependent B cell responses in infection

Infection of mice with live pathogens has supported a role for MyD88 in modulating humoral immunity, although this has not always been shown to be B cell intrinsic. Both TLR7 and MyD88 were shown to be important for CSR to IgG2a and IgG2c in response to influenza virus infection[46]. Alternatively, B cell-specific MyD88 expression was shown to be dispensable for the early T-dependent antibody response to mouse polyoma virus infection but was required for the maintenance of long-term antibody production[47]. A recent

study examining the failed response to respiratory syncytial virus (RSV) vaccines in infants concluded that insufficient TLR signaling in B cells resulted in low affinity, non-protective antibody production[48]. Further, infection with live RSV failed to elicit virus-specific IgG in B cell-specific MyD88-deficient μMT mice[48].

These findings are not limited to viral infections; one report described a B cell intrinsic role for MyD88 signaling to generate an appropriate primary Th1 cell response to *Salmonella enterica*[36]. In that study, mixed bone marrow chimeras were used in which the B cell compartment was deficient in MyD88. At one week post-infection, T cells isolated from these chimeras exhibited deficient IFNγ and IL-10 secretion following challenge with *S. enterica* antigen. Using similar mixed bone marrow chimeras in which B cells lacked expression of specific cytokines, a supporting role for B cell secretion of IL-6 and IFNγ for the development of Th17 and Th1 responses, respectively, was identified[36].

Collectively, these data demonstrate that MyD88 signaling in B cells can make important contributions to T-dependent antibody responses, and the dependence on B cell MyD88 varies depending on the nature of both the protein antigen and the TLR ligand. In particular, B cell MyD88 signaling appears to contribute to: driving CSR to IgG2a (or its equivalent, e.g. IgG2c, in C57BL6 mice) during primary T-dependent responses; promoting the differentiation of germinal center and memory B cells into antibody-secreting cells; and supporting effector T cell differentiation through cytokine secretion (Figure 2).

MyD88 and B cells in autoimmunity

Recent work demonstrates that MyD88 signals and most notably, dual TLR and BCR engagement, influence mechanisms of B cell tolerance (Figure 3). Further, new data suggest that genetic changes that alter BCR and/or TLR signaling thresholds likely promotes these events; and autoreactive B cells triggered in this way may directly break T cell tolerance facilitating germinal centre reactions that lead to pathogenic autoantibody production.

Dual BCR and TLR activation in promoting B cell autoimmunity

Despite a diverse repertoire of potential autoantigens, many autoimmune diseases are characterized by a restricted autoantibody repertoire. In addition to pathogens, TLRs can also recognize self-ligands, in particular nuclear antigens released from apoptotic cells. Further, dual TLR and BCR engagement in B cells has been implicated in the initial activation of autoreactive B cells, helping to explain the propensity of these cells to develop antinuclear antibodies (ANAs)[49–51].

The importance of dual BCR and TLR activation was initially demonstrated *in vitro* using B cells expressing the AM14 BCR, which is a low-affinity BCR for autologous IgG2a[52]. Stimulation of AM14 B cells with DNA–IgG2a or DNA-associated protein–IgG2a immune complexes resulted in B cell proliferation, while protein-specific immune complexes did not. MyD88 signaling downstream of TLR9 was critical for activation of AM14 B cells suggesting involvement of dual BCR and TLR signals[52]. Similarly, RNA- or RNA-associated protein-containing immune complexes activated AM14 B cells via TLR7[53]. Furthermore, a critical role for MyD88 signaling in the generation of RNA- and DNA-specific autoantibodies *in vivo* was demonstrated in MyD88-deficient MRL-*lpr* and MRL-*gld* mice. In contrast to littermate controls, these mice do not have detectable levels of ANAs, with significantly reduced RNA- and DNA-specific autoantibodies and decreased glomerulonephritis[53,54].

Importantly, two alternative mechanisms could explain the importance of MyD88-dependent signaling for autoantibody development *in vivo* in these autoimmune models: e.g. direct B

cell intrinsic signals mediated via dual BCR and TLR activation vs. an indirect effect of TLR- and immune complex-mediated activation of plasmacytoid dendritic cells leading to increased type I interferon production[55]. To date, the requirement for B cell-intrinsic MyD88 signals has only been investigated in a limited number of autoimmune models. Using a mixed bone marrow chimera strategy, Groom *et al.* demonstrated that deficiency of MyD88 specifically in B cells decreases autoantibody production and glomerular immunoglobulin and complement deposition in BAFF-transgenic mice, stressing the importance of B cell-intrinsic MyD88 signaling in BAFF-triggered autoimmunity[56]. MyD88 deletion also prevented CSR to pathogenic IgG2a and IgG2b in FcγRIIb-deficient B cells expressing an anti-DNA specific BCR heavy chain, suggesting that the role of MyD88 is B cell-intrinsic in this model[57].

Our group recently characterized a model of autoimmunity in which B cells, but not other hematopoietic lineages, lack Wiskott–Aldrich syndrome protein (WASP)[49]. In the absence of WASP, peripheral B cells are mildly hyperresponsive to BCR and TLR ligands, and mice with WASP-deficient B cells develop SLE-like autoimmunity characterized by spontaneous germinal center formation, generation of pathogenic IgG2b and IgG2c autoantibodies, glomerulonephritis and early mortality. Chimeric mice with B cells deficient in both WASP and MyD88, however, fail to develop any autoantibodies or signs of autoimmune disease, demonstrating a critical role for B cell-intrinsic MyD88 signaling in this model. Disease development also required wild-type T cells, suggesting that enhanced BCR and TLR signals are sufficient to drive a B cell-intrinsic, MyD88-dependent loss of T cell tolerance.

The relative contribution of specific TLR signals to MyD88-dependent autoimmune disease has been addressed by several groups[50,51]. Notably, TLR7 and TLR9 exhibit divergent roles in mouse autoimmune disease. Briefly, TLR7-deficient MRL–*lpr* mice[54] have decreased RNA-specific autoantibody titers but preserved autoimmune responses to double-stranded DNA (dsDNA). In contrast, TLR9-deficient MRL–*lpr* mice have markedly decreased levels of autoantibodies specific for dsDNA and chromatin, but elevated titers of autoantibody specific for RNA and RNA-associated protein[58,59]. *Tlr7*$^{-/-}$ autoimmune-prone mice are protected from immune complex-mediated glomerulonephritis, whereas glomerulonephritis and mortality is exacerbated in the absence of TLR9[58,60]. While the events responsible for this TLR9-mediated protective effect remain unclear, it is interesting that accelerated autoimmunity in *Tlr9*$^{-/-}$ mice depends on intact TLR7 signaling[54,60]. Importantly, whether TLR7 and TLR9 signaling influences autoimmunity in a B cell intrinsic manner has not been addressed.

In addition, MyD88 signaling may impact autoimmunity independently of TLR7 and TLR9 via TACI signaling[33] or, alternatively, via its role in IL1 or IL18 signaling. TACI-deficient mice develop spontaneous lymphoproliferative and autoimmune disease and CVID patients with TACI mutations exhibit a higher risk for autoimmunity[61], suggesting a protective role for TACI. Overall, both B cell-intrinsic and B cell–extrinsic MYD88-dependent TLR signals likely promote autoimmune disease, with TLR7 and TLR9 playing opposing roles. However, the cell-intrinsic requirements for individual TLR signals remain to be defined.

Role for MyD88 signals in human autoimmunity

Direct evidence for B cell-intrinsic MyD88 signaling in the pathogenesis of human autoimmune disease is lacking. However, genome-wide association studies have implicated genetic polymorphisms that affect TLR signaling pathways in susceptibility to SLE. Notable among these are mutations in IFN-regulatory factor 5 (*IRF5*)[62], a transcription factor involved in production of IFNα following TLR ligation, and in *IRAK1*[63]. Two genes involved in ubiquitin-mediated downregulation of NF-κB signaling, TNF-induced-protein 3 (TNFAIP3; also known as A20) and its ligation partner TNFAIP3-interacting protein 1

(TNIP1; also known as ABIN1), have also been associated with human SLE, possibly implicating TLR-mediated NF-κB activation in disease pathogenesis[64,65]. In murine studies, B cells from B cell-intrinsic *A20*−/− mutant[66] and polyubiquitin-binding defective *Abin1*[D485N] knockin mice[67] demonstrate enhanced TLR-mediated NF-κB activation and both B cell-restricted *A20*−/− and *Abin1*[D485N] mice develop lupus-like systemic autoimmunity. Disease development in *Abin1*[D485N] mice is MyD88-dependent, thus implicating TLR-mediated NF-κB activation in the pathogenesis of human SLE. Although not validated via genome-wide association studies, candidate-gene approaches have suggested associations between TLR7 or TLR9 polymorphisms and autoimmune disease[68–70].

Interestingly, despite the role for TLR–MyD88 signaling in activation of autoreactive B cells, an unanticipated increase in autoreactive naive B cells was observed in patients with MyD88 or IRAK4 deficiency[2]. A high number of immature B cells from these patients exhibit BCRs that bind nuclear antigens and deletion of these autoreactive B cells prior to entry into the mature peripheral compartment is defective. Together, these data suggest that the normal mechanisms of central and peripheral B cell tolerance may depend on intact MyD88 signaling. However, despite the increase in autoreactive B cells, patients with MyD88 or IRAK4 mutations did not display increased serum ANA titers, perhaps consistent with a requirement for MyD88 also in the activation of such autoreactive B cells.

MyD88 signals in regulatory B cell function

Functional and phenotypical definitions of regulatory B cells

Although B cell-intrinsic TLR signals can potentially break tolerance and trigger autoimmune disease, these same signals also play a role in regulatory B cell function, helping to suppress immune responses and maintaining self-tolerance (Figure 4). It has been long-recognized that both murine and human B cells produce IL-10[71,72]. Recently, this capacity to generate high levels of IL-10 has defined populations of B cells with regulatory function. Two distinct phenotypic definitions for regulatory B cells have been described in mice- $CD19^{+}CD21^{hi}CD23^{hi}CD1d^{hi}$ marginal zone-precursor B cells[73] and $CD1d^{hi}CD5^{+}$ B cells[74]. Based on their function, these populations have been termed regulatory B cells (Breg cells)[75,76] and B10 B cells[74], respectively. As IL-10 production can be triggered via a variety of stimuli, it remains unlikely that these cells comprise a distinct B cell developmental subset. Also, although not discussed here, B cells may also utilize transforming growth factor-β (TGFβ) secretion to exert their regulatory activity[77,78].

The importance of IL-10-producing B cells has been demonstrated in a range of mouse models of autoimmune disease including inflammatory bowel disease[75], collagen-induced arthritis[73], experimental autoimmune encephalomyelitis (EAE)[79], and SLE[20]. Additional data indicate a regulatory function for B cells in infectious models with *Leishmania major*[80], *Schistosoma mansoni*[81], *Brugia pahangi*[82], *Salmonella typhimurium*[23] and *Helicobacter pylori*[83].

Role for TLR signals in modulating regulatory B cell activity

Intrinsic TLR stimulation appears to be crucial for modulating the activity of IL-10-producing B cells, predominantly via promoting their differentiation and expansion. *In vitro*, stimulation of mouse splenic B cells via TLR9 results in high levels of IL-10 production and, interestingly, the amount of IL-10 is significantly higher in B cells from lupus-prone than wild-type mice[84]. In splenic marginal zone and marginal zone-precursor B cells, IL-10 production can be triggered via TLR4 or TLR9 engagement[20]. TLR4 ligation, in addition to polyclonal B cell stimulation, induced cytoplasmic IL-10 expression and rapid clonal expansion of splenic $CD1d^{hi}CD5^{+}$ B cells[74]. While the development of IL-10-producing

CD1d^{hi}CD5^{+} B cells was normal in *Myd88*$^{-/-}$ mice, direct LPS-induced IL-10 production and secretion was significantly reduced. In a mouse model of EAE, B cell-intrinsic TLR signals resulted in the suppression of both Th1 and Th17 cell-mediated inflammatory T cell responses and facilitated recovery from disease[85].

MyD88-dependent, IL-10-producing B cells can also modulate the response to infectious challenge. B cells exert regulatory function in *S. typhimurium* infection that requires both B cell intrinsic MyD88 signalling and IL-10 secretion[23]. MyD88-deficient mice infected intravenously with virulent *S. typhimurium* exhibit increased mortality, whereas mice with B cell-specific MyD88 deficiency exhibit prolonged survival. Notably, B cell-intrinsic MyD88 signals appear to drive two distinct programs in this setting. First, B cell-specific *Myd88*$^{-/-}$ mice exhibit lower basal levels of total and *S. typhimurium*-specific natural IgM and delayed *S. typhimurium*-specific IgM and IgG responses after infection, indicating that MyD88 signals accelerate humoral immunity. In contrast, bone marrow chimeras with IL-10 deficiency restricted to B cells exhibit increased survival[23], suggesting that B cell-derived IL-10 limits immunity to *S. typhimurium.*

In a model of *H. pylori*-induced gastric immunopathology, infection activates B cells in a MyD88- and TLR2-dependent manner[83]. Activated B cells subsequently promote IL-10 production by CD4^{+}CD25^{+} T regulatory type 1 cells *in vitro* and *in vivo*, suppressing excessive gastric *H. pylori*-associated pathology. In contrast to the *S. typhimurium* infection model, however, these events do not require B cell intrinsic IL-10 expression.

IL-10-producing B cells with a regulatory function have also been identified in humans by two independent groups[86,87]. IL-10-expression was identified in B cells derived from peripheral blood, spleen and tonsils following stimulation with either LPS or CpG DNA plus CD40 ligation[86,87]. Notably, suppressive function and IL-10 production was impaired in peripheral B cells derived from patients with SLE[87].

In summary, B cells with regulatory function differentiate and exert their suppressor function mainly by IL-10 production upon TLR signaling. Most data to date have been obtained from murine studies and the importance of MyD88 signals in initiating suppressive B cell function has been demonstrated in at least two infectious models. Additional studies are required to determine whether MyD88 signaling is required for the generation or function of IL-10-producing B cells in humans.

MyD88 in additional pathogenic B cell responses

Role for BCR and MyD88 signals in B lymphoid malignancies

Approximately 90% of human lymphomas arise from B cells, and diffuse large B cell lymphoma (DLBCL) comprises the most common type of non-Hodgkin's lymphoma. Several distinct DLBCL subtypes have been defined based on genetic signatures reflecting their putative origins, including (treatment-resistant or poor-prognosis) activated B cell-like (ABC) DLBCL[88]. ABC DLBCL probably originate from early stages of a germinal center reaction and this tumor type is characterized by high-level constitutive NF-κB activation[89] and coordinate high level expression of PKCβ, the BCR-dependent upstream activator of this signaling cascade[90,91].

RNA interference screens of ABC DLBCLs identified the importance of the CARD11–BCL-10–MALT1 complex downstream of the BCR/PKCβ in driving NF-κB activation[92]. Consistent with this, somatic mutations in the scaffold protein caspase recruitment domain-containing protein 11 (CARD11; also known as CARMA1)[93] and in BCR-associated signaling effector molecules CD79A and CD79B[94] are present in ~10% and 20% of ABC

DLBCLs, respectively. Recently, MyD88 mutations have also been identified in 39% of ABC DLBCLs, thereby also implicating TLR-mediated activation of NF-κB in lymphomagenesis[95]. Strikingly, a single substitution (Leu256Pro) within the Toll/IL-1-receptor (TIR) domain of MyD88 accounted for the majority of these mutations. Leu256Pro MyD88 was noted to be a gain-of-function mutant resulting in spontaneous assembly of a signaling complex with IRAK1 and IRAK4 and enhanced NF-κB activation. Interestingly, Leu256Pro MyD88 also activated JAK–STAT3 (Janus kinase–signal transducer and activator of transcription 3) signaling resulting in enhanced production of IL-6 and IL-10, likely promoting autocrine survival signals [96,97].

Most remarkably, MyD88 mutations were noted in ABC DLBCLs that also contained CARD11 or CD79A and CD79B mutations that impact on BCR signaling[95]. Thus, in a manner analogous to both viral-driven B cell activation and B intrinsic autoimmunity, dual BCR and TLR signals appear to promote the survival of a subset of refractory B cell lymphomas. Finally, MyD88 mutations are not limited to ABC DLBCLs. The same Leu256Pro mutation was also observed in 9% of gastric mucosa-associated lymphoid tissue lymphomas and 3% of chronic lymphocytic leukaemia[98] highlighting the importance of dysregulated TLR signaling in B cell-derived malignancies.

B cell MyD88 signals in atherosclerosis

Atherosclerosis is a chronic inflammatory disease that is impacted by TLR responses. TLRs, in particular TLR4, can be activated by endogenous oxidized lipid epitopes and apolipoprotein E (*Apoe*)$^{-/-}$ mice that lack express of MyD88 or TLR4 have decreased levels of atherosclerosis compared with *Apoe*$^{-/-}$ control mice[99,100]. Furthermore, a human Asp299Gly TLR4 polymorphism that decreases TLR4 signaling is associated with decreased risk of atherosclerosis[101].

Although the atheroprotective effects of MyD88 and TLR4 deficiency have been hypothesized to involve an absence of TLR signals in tissue macrophages or foam cells within atherosclerotic plaques, there is now growing interest in the atheroprotective and atherogenic roles for B cells in disease. While B cell-deficient[102] and serum IgM-deficient[103] low-density lipoprotein receptor (LDLR)-deficient mice develop increased aortic atherosclerotic lesions, an effect attributed to the loss of protective natural IgM antibodies, B cells can also actively promote atherosclerosis, particularly in autoimmune settings[104]. Further, B cell depletion with CD20-specific antibody decreases the development of atherosclerosis in both *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mouse models of atherosclerosis[105,106] and protection correlates with a decline in oxidized low density lipid (oxLDL)-specific IgG and coordinate preservation of oxLDL-specific IgM titers[106]. Patients with SLE have a markedly increased risk of atherosclerotic cardiovascular disease[107], and those with a history of cardiovascular events have elevated oxLDL-specific and malondialdehyde-modified LDL-specific IgG antibody titers compared with other SLE patients or population controls[108], suggesting that these autoantibodies may promote atherogenesis. While not yet directly addressed, given the role of B cell-intrinsic MyD88 signals in the pathogenesis of autoimmunity, it will be interesting to determine whether B cell MyD88-TLR signals promote accelerated atherosclerosis in animal models and patients with autoimmune disease.

Conclusions and future perspectives

B cell-intrinsic MyD88 signals and, most notably, integration of dual TLR and BCR signals play a crucial role in B cell responses to pathogen challenge. This unique functional program is crucial for protective immune responses to specific viruses and, most likely, also to encapsulated bacteria. Strikingly, new findings suggest that this hard-wired program also

predisposes to pathogenic B cell responses including humoral autoimmunity and B cell malignancies. Key open questions include how developmental events as well as local microenvonmental and inflammatory cues (or other cellular co-stimuli) function to specifically modulate these events; and most importantly, whether is will be possible to manipulate such signals to develop novel immunization strategies. Finally, while most of our knowledge to date is derived from mouse studies, it will be important to progressively extend investigation to human B cells to optimally leverage or curtail these responses for translational applications.

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Glossary

T-dependent and T-independent immune responses	
Marginal-zone B cell	A mature B cell that is enriched mainly in the marginal zone of the spleen, which is located at the border of the white pulp
B-1 cells	$IgM^{hi}IgD^{low}MAC1^{+}B220^{low}CD23^{-}$ cells that are dominant in the peritoneal and pleural cavities. The size of the B-1-cell population is kept constant owing to the self-renewing capacity of these cells. B-1 cells recognize self components, as well as common bacterial antigens, and they secrete antibodies that tend to have low affinity and broad specificity
Follicular B cell	A re-circulating, mature B-cell subset that populates the follicles of the spleen and lymph nodes
Germinal center	A lymphoid structure that arises within follicles after immunization with, or exposure to, a T cell-dependent antigen. It is specialized for facilitating the development of high-affinity, long-lived plasma cells and memory B cells. The germinal center can be divided into the morphologically distinct dark zone and light zone. Activated B cells are classically thought to proliferate in the dark zone and then move into the light zone where selection is mediated by competition for antigen on the surface of follicular dendritic cells
Wiskott–Aldrich syndrome	A life-threatening X-linked immunodeficiency caused by mutation in the WAS protein. It is characterized by thrombocytopaenia with small platelets, eczema, recurrent infections caused by immunodeficiency, and an increased incidence of autoimmune manifestations and malignancies
Systemic lupus erythematosus (SLE)	An autoimmune disease in which autoantibodies that are specific for DNA, RNA or proteins associated with nucleic acids form immune complexes that damage small blood vessels, especially in the kidney. Patients with SLE generally have abnormal B and T cell function

Class-switch recombination (CSR)	The process by which a heavy-chain variable region gene segment attached to one heavy-chain constant region gene segment in the expressed heavy-chain gene is recombined with a downstream constant region gene segment to express a new antibody class
Common variable immunodeficiency syndrome (CVID)	The most common symptomatic primary antibody deficiency, characterized by decreased levels of serum immunoglobulin, and a low or normal number of B cells. Most patients suffer from recurrent infections, predominantly of the respiratory and gastrointestinal tracts. The incidence of malignancies, such as gastric carcinoma or lymphoma, is increased in patients with CVID
μMT mice	These mice carry a stop codon in the first membrane exon of the -chain constant region. They lack IgM^+ B cells and B cell development is arrested before the differentiation stage at which IgD can be expressed
Ribi adjuvant	An emulsion containing a metabolizable oil, detergent, and bacterial products including the TLR4 ligand monophosphoryl lipid A
hapten	A molecule that can bind antibody but cannot by itself elicit an immune response. Antibodies that are specific for a hapten can be generated when the hapten is chemically linked to a protein carrier that can elicit a T cell response
Virus-like particles (VLPs)	Virion-like structures that are formed from the self assembly of viral envelope or capsid proteins in vitro. VLPs are not infectious because they do not contain a viral genome
Antinuclear antibodies (ANAs)	Heterogeneous autoantibodies against one or more antigens present in the nucleus, including chromatin, nucleosomes and ribonuclear proteins. ANAs are found in association with many different autoimmune diseases
Immune complexes	Complexes of antigen bound to antibody and, sometimes, components of the complement system. The levels of immune complexes are increased in many autoimmune disorders, in which they become deposited in tissues and cause tissue damage
MRL–*lpr* mouse	A mouse strain that spontaneously develops glomerulonephritis and other symptoms of systemic lupus erythematosus (SLE). The lpr mutation causes a defect in CD95 (also known as FAS), preventing apoptosis of activated lymphocytes. The MRL strain contributes disease-associated mutations that have yet to be identified
MRL–*gld* mouse	A mouse strain that has a naturally occurring mutation in CD95 ligand that causes a generalized lymphoproliferative disease, similar to that of MRL–*lpr* mice
T regulatory type 1 (T_R1) cells	A subset of $CD4^+$ regulatory T cells that secrete high levels of IL-10 and that downregulate T_H1 and T_H2 cell responses *in vitro* and *in vivo* by a contact-independent mechanism(s) mediated by the secretion of soluble IL-10 and TGF 1

Apolipoprotein E (*Apoe*)$^{-/-}$ mice	A widely used mouse model that is prone to develop atherosclerosis because the mice have high levels of types of atherogenic lipoprotein called remnant lipoproteins. This lipoprotein abnormality is cause by the genetic absence of apolipoprotein E (APOE), which normally clears remnant lipoproteins from the bloodstream by interacting with hepatocytes

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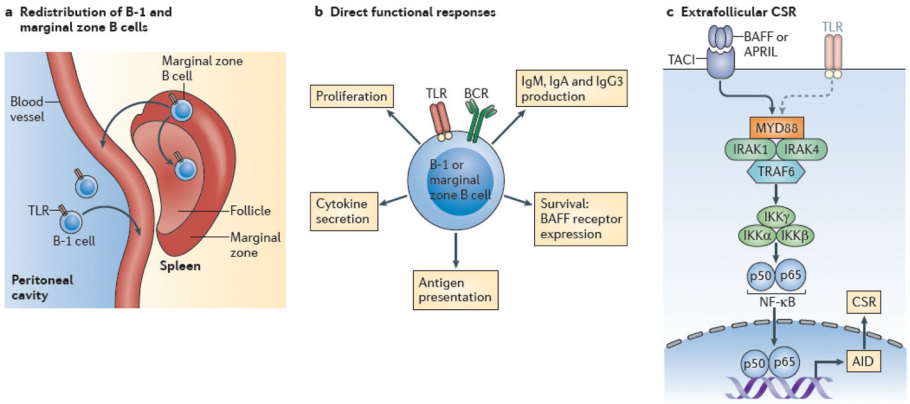

Figure 1. Role of BCR and MyD88 signaling in B cells during T-independent responses

(**A**) Innate-like B cells, that is B-1 and marginal zone B cells, are most crucial for T-independent immune responses. Upon TLR ligation these cells downregulate integrin receptor expression, resulting in redistribution of the cells from the peritoneal cavity to the blood, lymph node and spleen. (**B**) Subsequently, B-1 and marginal zone B cells start to proliferate and secrete immunoglobulins and cytokines. Up-regulation of B cell-activating factor (BAFF) receptor expression leads to prolonged survival of these cells. Increased expression of co-stimulatory molecules also enables activated innate-like B cells to act as antigen-presenting cells, thereby linking T-independent and T-dependent immune responses. Most of these functional responses have been demonstrated to occur in response to direct TLR engagement *in vitro*; however, *in vivo*, these events probably require synergistic B cell receptor (BCR) and TLR engagement. (**C**) Recent data demonstrate the direct involvement of MyD88 in transmembrane activator and CAML interactor (TACI)-dependent nuclear factor-κB (NF-κB) signals. This can result in activation-induced cytidine deaminase (AID; an enzyme that is required for somatic hypermutation and class-switch recombination (CSR) in the germinal centre) expression and extrafollicular CSR, independently of T cell help. This pathway might also be directly activated by TLR signals, as *in vitro* stimulation of B cells with TLR ligands induces AID expression. Moreover, because TLR stimulation leads to upregulation of BAFF receptors, signaling via this pathway and induction of extrafollicular CSR might be enhanced by dual TACI and TLR engagement.

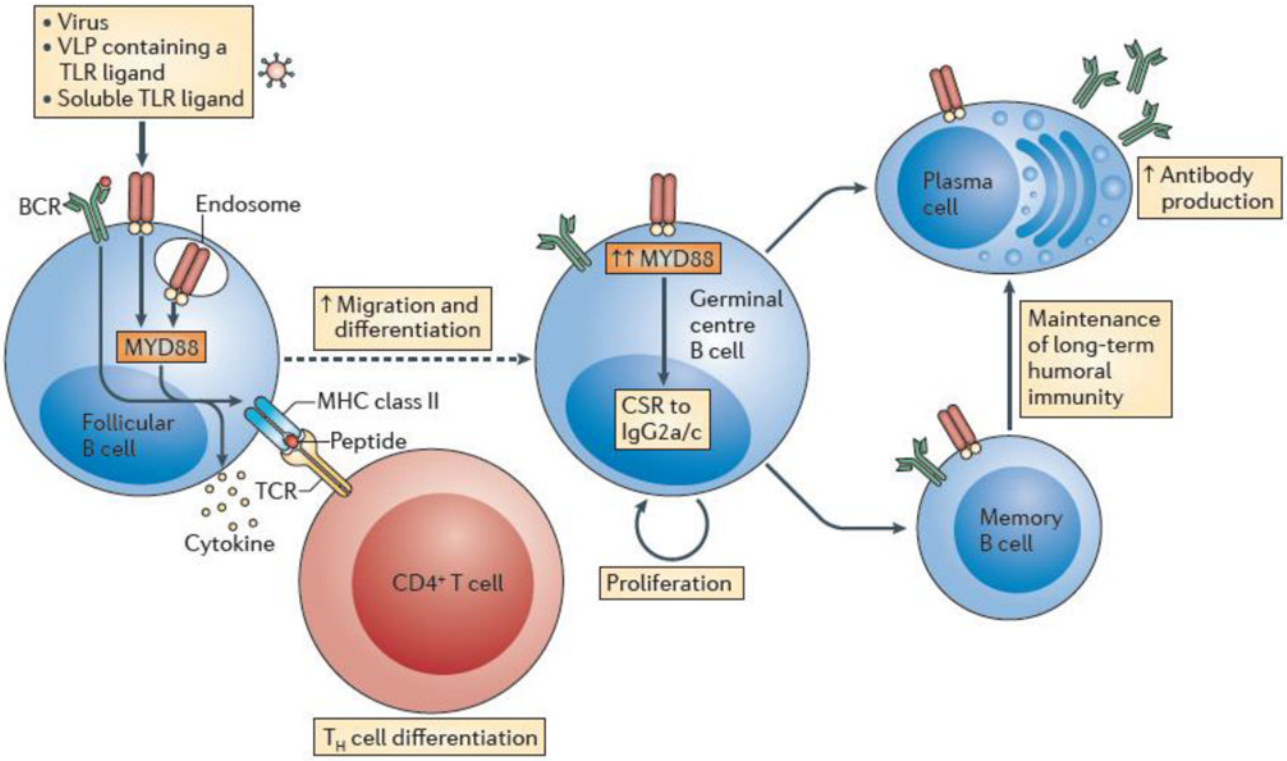

Figure 2. Role of BCR and MyD88 signaling in B cells during a TD immune response

Follicular B cells are activated following interaction with cognate $CD4^+$ T cells and B cell receptor (BCR) engagement. Signaling through myeloid differentiation primary-response protein 88 (MyD88) during the primary response may enhance antigen presentation by B cells to T cells, as well as the secretion of cytokines such as interleukin-6 (IL-6) and interferon-γ (IFNγ) that drive T cell differentiation. Recent data suggests that TLR ligands present in viruses or viral-like particles (VLP) can directly stimulate B cells. After entering a germinal center, B cells upregulate MyD88 expression and become more responsive to TLR ligands. Signaling through MyD88 at this stage can drive increased germinal center B cells proliferation and promote class-switch recombination, especially to IgG2a and IgG2c subclasses. Soluble TLR ligands may increase B cell migration and entrance into ongoing germinal center reactions. Finally, MyD88-dependent signals can drive the differentiation of B cells into antibody-secreting plasma cells, helping to generate primary and sustain memory humoral immune responses.

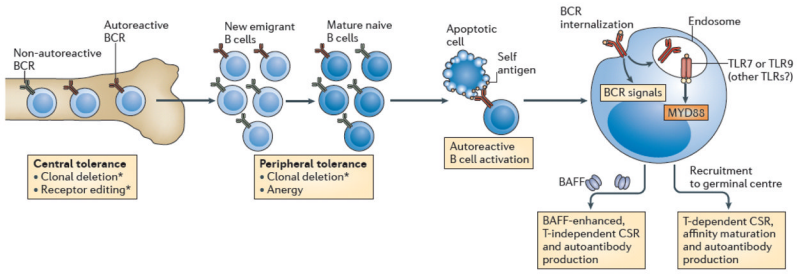

Figure 3. Role of B cell intrinsic Myd88 signaling in B cell tolerance and autoimmunity

B cell development frequently results in the generation of autoreactive B cells that are removed at distinct checkpoints in the bone marrow (central tolerance) and periphery (peripheral tolerance), via a combination of mechanisms including B cell clonal deletion, receptor editing and functional anergy[116]. Although the mechanisms remain to be determined, MyD88 signaling may impact on tolerance mechanisms, as greater autoreactivity is noted in both new emigrant and mature naïve B cell populations in patients with inborn errors in *MYD88*, *IRAK4* and *UNC93B*[2]. Autoreactive B cells also enter the mature compartment in healthy individuals, despite intact tolerance mechanisms. Murine models have demonstrated the critical importance of dual BCR- and TLR-mediated activation of autoreactive B cells. Following engagement of antigen receptors on DNA- or RNA-reactive B cells, BCR internalization shuttles DNA- or RNA-associated antigens to TLR7- and TLR9-containing intracellular compartments resulting in MyD88-dependant activation. The potential requirement for additional TLRs in activating autoreactive B cells with different specificities has not yet been addressed. Activated autoreactive B cells either undergo T-independent class-switch recombination and autoantibody production that is enhanced by BAFF; or, recruit autoreactive T cells to germinal centers resulting in T-dependent class-switch recombination, affinity maturation and pathogenic autoantibody production.

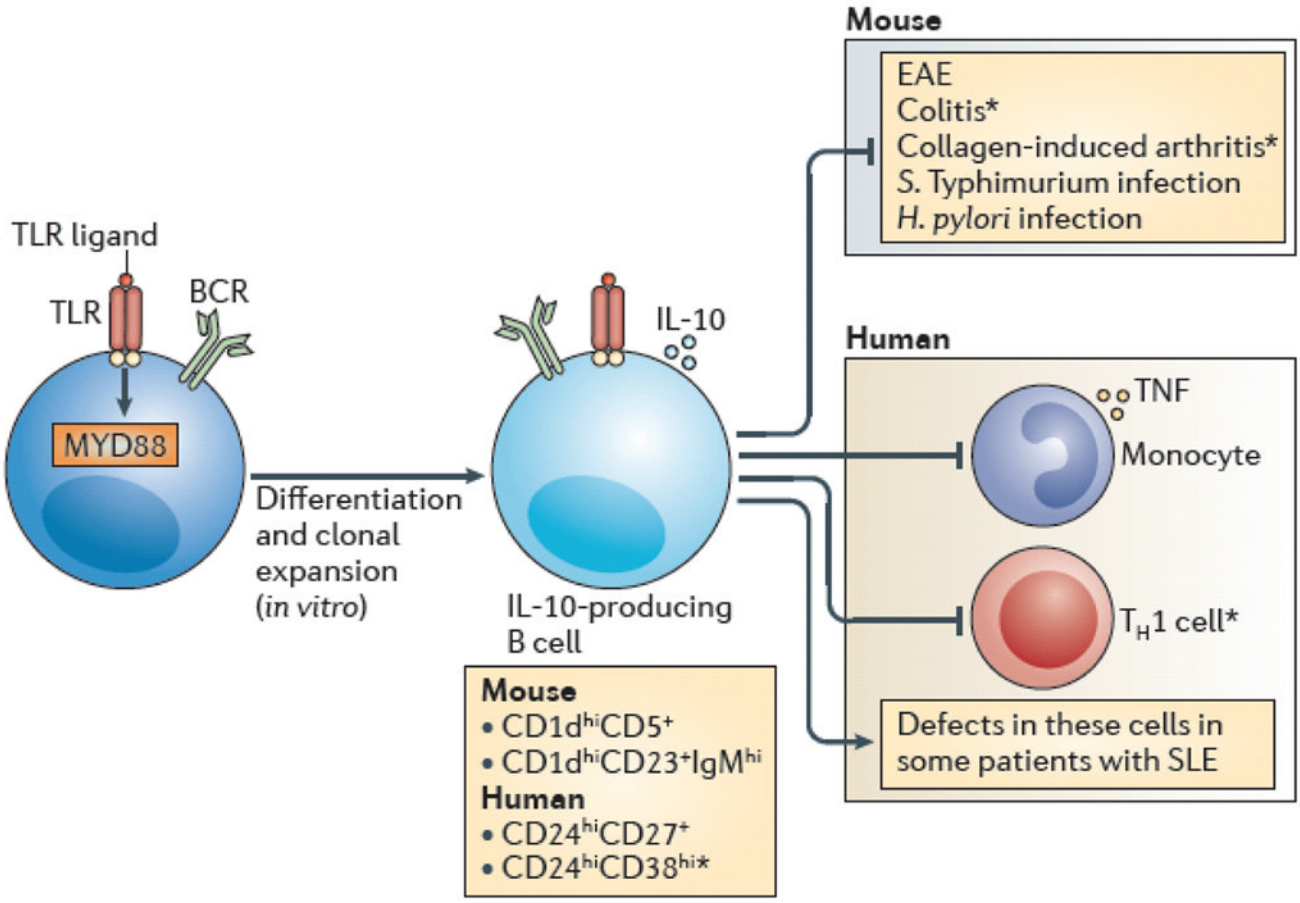

Figure 4. Role of MyD88 signaling in B cells with a regulatory function

B cells with a regulatory function can be identified in both humans and mice based on various phenotypiccharacteristics as indicated. Toll-like receptor (TLR) signals are not required for their development, but stimulation via MyD88 leads to maturation and expansion of interleukin-10 (IL-10)-expressing B cells *in vitro*. In mice, the suppressor function of B cells is evident in several autoimmune and infectious models. Human B cells with a regulatory function can suppress tumour necrosis factor (TNF) production by monocytes and functional deficits in human regulatory B cell function were found in patients with systemic lupus erythematosus. In contrast, increased numbers of IL-10 producing B cells have also been observed in some patients with autoimmune diseases.