



Marginal zone B cells: From housekeeping function to autoimmunity?



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ABSTRACT

Marginal zone (MZ) B cells comprise a subset of innate-like B cells found predominantly in the spleen, but also in lymph nodes and blood. Their principal functions are participation in quick responses to blood-borne pathogens and secretion of natural antibodies. The latter is important for housekeeping functions such as clearance of apoptotic cell debris. MZ B cells have B cell receptors with low poly-/self-reactivity, but they are not pathogenic at steady state. However, if simultaneously stimulated with self-antigen and pathogen- and/or damage-associated molecular patterns (PAMPs/DAMPs), MZ B cells may participate in the initial steps towards breakage of immunological tolerance. This review summarizes what is known about the role of MZ B cells in autoimmunity, both in mouse models and human disease. We cover factors important for shaping the MZ B cell compartment, how the functional properties of MZ B cells may contribute to breaking tolerance, and how MZ B cells are being regulated.

1. Introduction

B lymphocytes play an important role in the immunopathogenesis of several autoimmune diseases. B cell targeted therapy has been successful in treating rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and multiple sclerosis (MS). However, despite this undisputed involvement of B cells, little is known about B cell subpopulations with distinct immune functions that may play a role in the spectrum of autoimmunity. One distinct subset that is implicated in the autoreactive B cell response are the innate-like marginal zone (MZ) B cells. Their principal function is in defense against blood-borne pathogens and in so-called “housekeeping” functions to clear e.g. apoptotic cell debris. However, an expanded MZ B cell compartment, with abilities to present self-antigen and secrete autoantibodies, has been demonstrated in several autoimmune conditions.

This review summarizes recent advances on the ontogeny, activation, and effector function of MZ B cells, all in the context of autoimmunity. We describe mechanisms regulating MZ B cell responses and what happens if these fail. Finally, we review data suggesting that MZ B cells participate in the development of autoimmunity in humans and in mouse models of autoimmune disease.

2. Introducing MZ B cells

The B cell compartment in mice and humans is mainly made up of MZ, follicular (FO), and B-1 B cells (Fig. 1). The MZ B cells derive their name from their microanatomical location in the marginal zone of the spleen, a specialized area demarcating the border between the white and the red pulp. Here, arterial blood filters through and is scavenged for pathogens by specialized marginal zone macrophages, marginal metallophilic macrophages, and MZ B cells. Cells in the MZ are continually exposed to sphingosine-1-phosphate (S1P) in the bloodstream and localization of MZ B cells to this particular area is maintained by their high expression of sphingosine 1-phosphate receptor 1 (S1P₁) and the integrins lymphocyte function associated (LFA)-1 and $\alpha 4\beta 1$ [1,2].

In contrast to FO B cells, which primarily express monoreactive B cell receptors (BCRs) and give rise to highly specific, high-affinity antibodies, the innate-like MZ B cells express poly-reactive BCRs and rapidly produce low affinity antibodies with self-reactivity to clear pathogens and apoptotic cell debris. In addition, MZ B cells are highly sensitive to stimulation through Toll-like receptors (TLRs), which contributes to their accelerated primary antibody response, particularly to blood-borne pathogens and T-independent antigens [3,4]. These unique qualities make the MZ B cells poised for action in response to pathogens, but also put them in danger of crossing the threshold to autoimmunity.

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The MZ B cell compartment, predominantly studied in mice and humans, consists of CD19^{+/hi} B cells that express high levels of surface IgM and low levels of surface IgD (Figs. 1 and 2). They have high expression of the complement receptors CD21 and CD35, the non-classical class I MHC molecule CD1 (CD1c in humans, CD1d in mice), MHCII, CD80/CD86, and CD9 (mice only), as well as the negatively regulatory proteins Fc gamma receptor IIIB (FcγRIIB) and CD22 (Fig. 2) [5–11]. Human MZ B cells also express the memory marker CD27 [10, 11] (Fig. 2), and MZ B cells were therefore previously believed to be *bona fide* IgM⁺ memory B cells [11]. However, recent findings suggest that human CD27⁺IgM⁺IgD⁺ MZ B cells do not share a differentiation path with memory B cells and thus constitutes a distinct lineage [9].

Human MZ B cells comprise around 15–20% of splenic B cells and around 15% of B cells in peripheral blood [9–12]. In mice, this number varies between 5 and 15% of splenic B cells depending on mouse strain, with the commonly used BALB/c and C57BL/6 mice at the lower end of the spectrum compared to DBA/1, NOD, and CBA [4,8,13–17]. The reasons for this difference are not known, but transcriptional differences in MZ B cells from different mouse strains further stresses the importance of genetics in shaping this compartment and thus potentially also in its function [18]. Interestingly, activation and/or expansion of the MZ B cell compartment is observed in several murine models for autoimmunity, including RA [4,15,19], SLE [20–23], type I diabetes [13,24], and Sjögren's syndrome (SS) [25–27]. This MZ B cell expansion is associated with mouse strain, age and sex [15,24], and is generally seen before production of pathogenic autoantibodies and clinical signs of disease [15,20]. Similarly, MZ B cells have been described to be activated also in human autoimmunity [28–30].

3. Shaping the MZ B cell compartment

Commitment of an immature B cell to the MZ B cell fate is directed by a number of different receptor-ligand interactions. In view of the presence of self-reactive antibodies in many autoimmune diseases, the involvement of the BCR for the MZ B cell fate decision is of particular interest in the context of autoimmunity. MZ B cells share developmental origin with FO B cells and derive mainly from B cell progenitors in the bone marrow that upon BCR assembly are allowed to enter the circulation as immature B cells, if not strongly binding to self-antigen. The

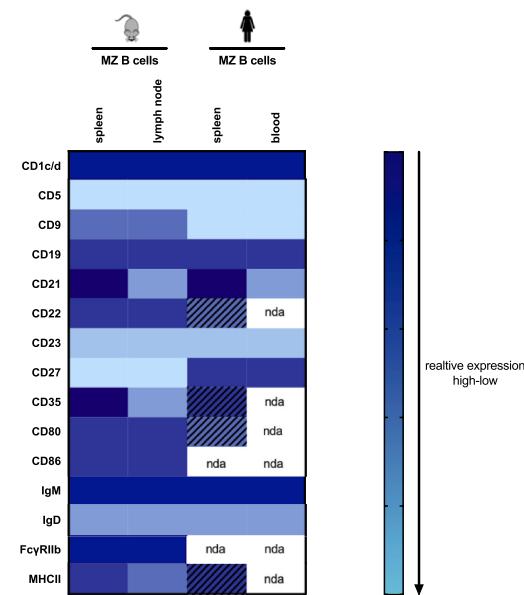


Fig. 2. Relative expression levels of surface proteins on MZ B cells in mice and humans. Expression levels of surface proteins on mouse (spleen/lymph nodes) and human (spleen/blood) MZ B cells relative to the expression on follicular B cells from the respective tissue; ranging from high (dark blue) to low/negative (light blue). Open boxes indicate protein expression, striped boxes indicate mRNA expression. MZ, marginal zone; nda, no data available.

decision to differentiate into MZ or FO B cell fate is then subsequently made in the spleen, at the transitional B cell stages T1 and T2 [31–33].

One key interrogation point is at the T1 transitional stage where functional signaling through the BCR causes relocation of ADAM10 to the cell surface. ADAM10 is a sheddase needed for cleavage of Notch2 [34], which in turn controls the transcriptional program for the MZ B cell fate [35–43]. However, if an antigen interacts too strongly with the BCR it will activate Bruton's tyrosine kinase (BTK), which may block the signals from Notch2 [44]. Consequently, weak BCR signaling in transitional B cells will favor commitment to the MZ B cell lineage, while a strong signal will favor differentiation to FO B cells. Furthermore,

	FO B cell	MZ B cell	B-1 B cell
Primary localization	Secondary lymphoid organs; mainly in B cell follicles	Secondary lymphoid organs; mainly in the splenic marginal zone	Peritoneal and pleural cavities
Distinguishing surface markers	B220 ⁺ , CD19 ⁺ , CD1d ^{lo} , CD21 ⁺ , CD23 ^{hi} , CD43 ⁻ , IgM ^{lo} , IgD ^{hi}	B220 ^{lo/+} , CD19 ^{+/hi} , CD1d ^{hi} , CD21 ^{hi} , CD23 ^{lo} , CD43 ⁻ , IgM ^{hi} , IgD ^{lo}	B220 ^{-/lo} , CD19 ^{hi} , CD1d ⁺ , CD23 ⁻ , CD43 ⁺ , IgM ^{hi} , IgD ^{lo}
Principal function	Participation in T-dependent immune responses	Housekeeping function (natural IgM), first responders to blood-borne pathogens	Housekeeping function (natural IgM), first responders to mucosal pathogens

Fig. 1. Overview of the three main B cell subsets. FO, follicular; MZ, marginal zone.

deficiency in negative regulators of BCR signaling such as CD22 or the transcription factor Aiolos, resulting in overall enhanced BCR signaling, will cause a reduction in the MZ B cell population [45,46]. In fact, MZ B cell precursors express the highest levels of CD22 of any B cell subset [47], reflecting the effect on BCR signaling on B cell fate decisions.

To some extent there is also direct repertoire-based selection into the MZ B cell pool, further indicating that certain B cells are positively selected into this subset based on how and what their BCR binds [33,48,49]. Given that immature B cells are only allowed to leave the bone marrow for the periphery if they have a low enough affinity for self-antigen, the interaction of a poly-/self-reactive transitional B cell with its cognate antigen in the spleen will result in optimally weak BCR signaling, consequently favoring entry into the MZ B cell compartment [37,44,45,49,50].

Unlike their murine counterpart, human MZ B cells carry mutated BCRs [10]. It was recently suggested that they complete their maturation not in the spleen, but rather in gut-associated lymphoid tissue [9]. Here they can interact with gut bacteria, mutate, and then be selected for (self-/poly-reactive binding abilities before circulating back to the spleen. Thus, the microbiota may play a crucial role in shaping the MZ B cell compartment in humans. This may be one of the mechanisms whereby the microbiome, influenced both by genetics and diet, can play a significant role in the pathogenesis of several autoimmune conditions, e.g. SLE, SS, and RA [51]. It has also been indicated that the gut microbiota has an impact on mouse MZ B cell selection [52]. This may be owing to their anatomical location as the first cell population to encounter antigens acquired through the gut [53,54]. In addition, NOD mice fed a specialized diet high in the short-chain fatty acid acetate experience a change in the gut microbiota, accompanied by a dramatic decrease in transitional and MZ B cells [55]. Interestingly, this finding was accompanied by a high protection from diabetes. Although still a field in need of further study, the available evidence thus suggests a link between changes in microbiome, a dysregulated MZ B cell compartment, and autoimmunity.

In summary this points towards a profound mechanism for purposely retaining low poly-/self-reactivity within the MZ B cell pool [50,56–59], probably as a means to provide fast B cell responses against pathogens.

4. Extrasplenic localization of MZ B cells is associated with autoimmunity

While most literature support full restriction of MZ B cells to the spleen in the mouse, human MZ B cells are also found in extrasplenic locations such as the subcapsular sinus of lymph nodes, in tonsils, and in Peyer's patches, and there is evidence suggesting they are recirculating [10,11]. However, an increasing body of work now suggests that this may be true also for murine MZ B cells. By using flow cytometry and/or immunohistochemistry imaging we, and others, have demonstrated the presence of MZ B cells in the subcapsular sinus of lymph nodes and/or in target organs both at steady state and under autoimmune conditions [13,15,25,60,61]. Worth noting is that the early studies in mice concluding restriction to the spleen use high expression of CD21 as the defining marker for MZ B cells. CD21 is downregulated on recirculating human MZ B cells compared to the same subset when resident in the spleen (Fig. 2) [11], and a lower CD21 expression is also seen for MZ B cells in mouse lymph nodes [15]. This makes detection of MZ B cells in extrasplenic locations difficult unless the population is markedly increased (e.g. under autoimmune conditions) or if additional markers are used [13,15,60]. In our hands, CD1d^{hi}CD23^{lo}IgM^{hi}IgD^{lo} MZ B cells are found in lymph nodes from naïve DBA/1, BALB/c, and CBA/J mice [15], but not from C57BL/6 mice (unpublished data). These nodal (n) MZ B cells are mainly located in the subcapsular sinus; an area that, similar to the splenic marginal zone, is rich in macrophages and is where antigen enters. Others have described nMZ B cells in diabetic NOD mice and in mice with manifestations of SS, but again not in naïve C57BL/6 mice [13,60]. In fact, the C57BL/6 strain has a smaller MZ B cell

population compared to several other mouse strains [4,8,13–16]. Moreover, MZ B cells in C57BL/6 mice have a relatively high expression of S1P₁, possibly explaining the more rigid retention to the spleen for MZ B cells in this strain [18]. Interestingly, C57BL/6 mice are less susceptible to many autoimmune models [62–64], potentially due to the smaller and more strictly confined MZ B cell compartment.

In humans, an expanded MZ B cell compartment with MZ B cell infiltration into target tissue is typically seen in SS patients [28,29]. MZ B cells are also found in the thyroid gland in Graves' disease [30]. Further studies are needed to fully understand the function of such extrasplenic MZ B cells, but the evidence currently available suggest that they might be involved in presentation of self-antigen to T cells [13,15] or increased antigen delivery to ectopic germinal centers (GCs).

5. Functional properties of MZ B cells

The strategic positioning of MZ B cells in the splenic MZ and the lymph node subcapsular sinus places them right where the circulatory and immune systems meet and where concentration of antigens is high. Here, their principal function is to act as the first line of defense, especially against encapsulated bacteria [65–67]. The self-reactivity of MZ B cells also helps to clean up altered self-antigens, such as apoptotic and dying cells, a housekeeping function that prevent any potential triggers of autoimmunity. MZ B cells that have engaged antigen can then be differentiated, with or without T cell help, to plasmablasts in extrafollicular foci in the spleen or in the medullary cords of lymph nodes [68]. In particular, antibodies produced in response to thymus-independent type 2 (TI-2) antigens have been shown to be the result from extrafollicular expansion of MZ B cells.

The highly repetitive structure of TI-2 antigens enables MZ B cells to use their BCRs similarly to pattern-recognition receptors; recognizing structural elements on antigens. Extensive BCR crosslinking by TI-2 antigen, together with the high sensitivity to TLR-stimulation, thus results in a lower threshold for activation of MZ B cells compared to FO B cells [3,4,6,53,69,70]. The TLR-stimulation is particularly important for the MZ B cell to respond to self-antigen [17]. Upon TRL-activation, MZ B cells rapidly start to produce high amounts of low-affinity antibodies and cytokines [4,71]. In fact, MZ B cells secrete substantial amounts of cytokines such as TNF, IL-6, and IL-10 following direct TLR stimulation [3,4], but also upon signals generated *in vivo* e.g. during collagen-induced arthritis (CIA) progression by antigen and adjuvant [15]. An altered cytokine profile of the MZ B cells may influence autoimmune development considering the enhanced IL-6 secretion by splenic and nodal MZ B cells in mouse models of SS and RA [15,25]. On the other hand, regulatory IL-10 secreted by activated MZ B cells may contribute to the regulation of systemic autoimmunity in mice [72]. Thus, MZ B cells can use both their canonical B cell features as well as their more innate-like qualities, such as being hyper-responsive to TLR-stimulation, in their quick response to antigenic stimulation.

5.1. Production of natural autoantibodies

Another important feature of MZ B cells and other innate-like B cells is the production of natural antibodies. These antibodies are mainly of IgM isotype and are produced independently of foreign antigen stimulation [73]. They are often self- and/or poly-reactive, thus recognizing conserved molecules that are shared by foreign and autologous antigen, such as phospholipids, carbohydrates and nucleic acids. Due to this self-/poly-reactivity, natural antibodies are characterized by low-affinity antigen interactions. However, the ten binding sites of pentameric IgM still enable high-avidity binding. In this way, natural antibodies and MZ B cells can facilitate both neutralization of invading pathogens and promote "housekeeping" functions such as apoptotic cell clearance [74]. Importantly, natural IgM preferentially bind to particulate antigens, e.g. apoptotic cells, but not soluble antigens. This may be the key to immune-complex mediated antigen capture attributed to

expanded MZ B cells. When in complex with antigen the natural IgM are sufficient to activate the classical complement pathway. Although the natural IgM antibodies thus play a protective role, they also carry a risk to initiate injury in conjunction with complement. If natural IgM extravasates into tissues, such as during endothelial damage and leakage, the presence of IgM can cause complement activation *in situ* with ensuing tissue damage; mechanisms often seen in autoimmune diseases. However, in a non-inflammatory environment, the size of IgM prevents its easy diffusion and it remains mainly at the site of production in secondary lymphoid tissues.

While natural autoantibodies are important for house-keeping functions there is a possibility that such antibodies may initiate an autoimmune reaction. We have previously demonstrated that naïve mice harbor splenic B cells secreting natural autoantibodies to collagen type II (CII), a cartilage-specific protein [4,19]. Natural IgM reactive to CII can also be found in lymph nodes and in sera of naïve mice [4,15]. CII is used to trigger CIA, and antibodies to CII are often found in RA patients [75]. Notably, the CII molecule is a TI-2 antigen with highly repetitive epitopes. One CII molecule can thus simultaneously cross-link multiple BCRs to enhance the BCR signaling, a typical mechanism in MZ B cells. Interestingly, natural antibodies to CII can be demonstrated in DBA/1, BALB/c, and NOD mice, while barely detectable in C57BL/6 mice [19]. This is consistent with that genetic background influences natural antibody titers and reactivity [74]. The natural autoantibody response to CII in naïve DBA/1 mice is specifically associated with the MZ B cells (both splenic and nodal), and is not detected in B-1 B cells, nor in FO B cells [4,15]. Similarly, natural autoantibodies to mucin 2, a component of intestinal goblet cell granules and a self-antigen in colitis, are produced by MZ B cells in naïve mice [76]. This IgM anti-goblet cell/mucin2 autoreactivity is also detectable in serum, where levels differ between mouse strains. Titers were highest in BALB/c mice followed by C57BL/6 mice, while levels were below detection limits in C57BL/6 mice, again possibly reflecting the smaller MZ B cell population in this strain. Together, these data support MZ B cells as being responsible for the production of natural antibodies to self-antigens.

Humans have a large number of circulating natural autoantibodies directed against well-conserved cell structures (e.g. receptors, nucleosome, DNA, nuclear and mitochondrial proteins). For example, red blood cell (RBC) autoantibodies are detected in healthy individuals, likely as a homeostatic mechanism for clearing altered self-RBC [77]. The natural autoantibodies may be a contributing factor for developing autoimmune disease in genetically susceptible individuals. Anti-goblet cell autoantibody can be found among clinically healthy relatives of ulcerous colitis patients. This can be interpreted as although production of specific natural autoantibody does not necessarily result in clinical disease, it may indicate a genetic risk for development of overt autoimmune disease if adequately stimulated. Indeed, infection with hepatitis C virus, a well-described polyclonal immune activator, can, in rare cases, trigger autoantibody production targeting RBCs causing autoimmune hemolytic anemia [78]. Comparably, when naïve DBA/1 mice are immunized with CII in a potent adjuvant, such as mycobacteria-containing Freund's complete adjuvant, the natural MZ B cell response to CII is amplified, serum IgM anti-CII levels increase, and pathogenic IgG anti-CII antibodies and arthritis will develop [4,19]. Similarly, stimulation with bacterial CpG DNA motif (TLR9-agonist) in lupus-prone NZB/NZW mice leads to lupus pathology by inducing MZ B cell activation, costimulatory molecule expression, and polyclonal immunoglobulin secretion [79]. Thus, although MZ B cells do not secrete pathogenic autoantibodies *per se*, the MZ B cell compartment is enriched in autoreactive clones that may promote autoimmune disease if unfavorably stimulated and/or if regulation fails.

5.2. Antigen transport and follicular inclusion

It is well recognized that MZ B cells continuously shuttle antigen between the marginal zone and the follicle. The high surface expression

of CD21/35 (Fig. 2) allow the MZ B cells to readily capture complement-opsonized particulate antigen directly from the blood [80]. The interaction of complement proteins with CD21/35 causes downregulation of S1P₁ in the MZ B cells, allowing the MZ B cells to leave the marginal zone. In combination with the resulting net-predominance of CXCR5, MZ B cells translocate along a CXCL13 gradient into the follicle where the immune complexes are deposited onto follicular dendritic cells, thus facilitating the GC reaction [1,81–85]. GCs are not only present in response to foreign antigen, but are also an important feature of autoimmune responses [86,87]. Antigen transport by MZ B cells is likely to take place also in autoimmunity; particularly if the self-antigen is opsonized by complement, e.g. as a strategy for marking it for clearance. Importantly, such shuttling of (self-)antigen will not activate the MZ B cell unless it carries a BCR that binds the antigen. If the BCR does bind the antigen, it will crosslink with CD21, providing activation signals to the MZ B cell, which may then endocytose and present the antigen to T cells instead of depositing it onto follicular dendritic cells. Indeed, we have observed increased movement of MZ B cells into the follicles following CII-immunization for CIA [19]. Moreover, expression of *Sle* susceptibility loci results in follicular inclusion of MZ B cells, especially when the MZ B cells express a self-reactive BCR [7,88]. Importantly, autoimmune pathology is significantly reduced when follicular exclusion of MZ B cells is restored in this model [7], suggesting that relocation of MZ B cells to the follicle is important for breakage of tolerance. Downregulation of S1P₁ may also cause MZ B cells to leave the spleen and infiltrate into target tissue in autoimmune conditions, e.g. the salivary glands in mice in a SS model [25,60,61], the pancreas in NOD mice developing T1D [13], or the thyroid gland in Grave's disease [30]. Tertiary lymphoid structures, including ectopic GCs, are often formed in target tissues in autoimmune disease [89–95], and consequently such migration of self-reactive MZ B cells could help drive these ectopic GC reactions resulting in the formation of high-affinity pathogenic autoantibodies.

Thus, allowing MZ B cells into follicles and/or ectopic lymphoid structures may promote the autoimmune processes, e.g. by increasing self-antigen delivery and/or presentation of self-antigen to CD4⁺ T cells. Notably, expansion of MZ B cells without them entering follicles does not result in production of pathogenic autoantibodies [96].

5.3. Presentation of self-antigen

MZ B cells express high levels of MHCII and CD80/CD86 (Fig. 2), making them highly efficient antigen-presenting cells for CD4⁺ T cells [54,71,97,98]. Importantly, one of the main risk loci for developing autoimmune disease is the MHCII locus [99], which suggests that presentation of self-antigen to T cells is key to breaking tolerance. Indeed, we have demonstrated that MZ B cells present self-antigen to T cells in CIA, triggering activation and proliferation of the antigen-specific T cells [4,15]. Other groups have also demonstrated similar findings in models for type I diabetes and SLE [13,88,100]. Given that any autoreactive T cells that are allowed to leave the thymus should be anergic, the antigen-presenting cell must display high concentration of antigen-derived peptide on its MHCII, together with a high expression of costimulatory molecules, for them to be able to overcome tolerance mechanisms. Thus, the high expression of MHCII and co-stimulatory molecules on MZ B cells together with their recognized poly-/self-reactive BCR consequently make this particular subset likely to break tolerance in T cells.

6. Regulation of MZ B cells

6.1. Complement receptors

MZ B cells are exquisitely sensitive to TLR- and CD40 ligation, as well as to S1P-dependent chemotaxis. The high expression of the complement receptors CD21 and CD35 (both encoded by the *Cr2* gene in mice)

together with CD19 mounts additional activating signals when their cognate antigen is opsonized with complement [101]. Accordingly, MZ B cells are easily activated and therefore require tight regulation. Any defective regulatory processes may increase their activity, as well as may dispose for development of autoimmunity. Notably, the signaling of CD21 and CD35 is also important in the maintenance of peripheral tolerance [102,103], as alterations in the CD21/CD35 pathway can predispose to autoimmunity. Support for this is provided by the significantly reduced CD21 and/or CD35 expression on B cells from patients with RA, lupus, SS, and systemic sclerosis [104–106], as well as the CD21^{lo} B cells associated with joint damage in RA patients [107]. Moreover, reduction in CD21 and CD35 expression is also found in animal models of autoimmunity. MRL/lpr mice developing lupus have decreased CD21 and CD35 [108], and *Cr2*-deficient mice, which have an increased MZ B cell compartment [45,109–111], demonstrate enhanced susceptibility to CIA [112]. In particular, *Cr2*-deficiency in MZ B cells contributes to increased proliferation, both spontaneous and upon TLR9 stimulation, and TLR-induced TNF, IL-10, and IL-6 is significantly enhanced [4]. Furthermore, human MZ B cells low in CD21 are enriched in clones expressing autoreactive BCR and are linked to autoimmunity [113]. The CD21^{lo} MZ B cells are unresponsive to BCR stimulation, suggesting that they are controlled by anergy instead of being eliminated. However, the CD21^{lo} MZ B cells are responsive to TLR9 stimulation which indicates that exogenous agents can overcome the anergy and trigger autoreactivity in the CD21^{lo} MZ B cells.

6.2. Negative regulators of the BCR

MZ B cells are further restricted by Fc γ RIIB and CD22, two inhibitory receptors containing intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that regulate BCR-mediated signals. When the BCR is co-ligated with the inhibitory Fc γ RIIB, it provides tyrosine kinase activity that phosphorylates the ITIM and the BCR signaling will be dampened. A decrease of Fc γ RIIB represents a significant risk factor for the development of autoimmunity in both mouse and man [114–116]. We have previously demonstrated that the expression level of Fc γ RIIB on naive murine MZ B cells in spleen and lymph nodes is significantly higher compared to naive murine FO B cells, possibly indicating the need for a higher level of regulation of MZ B cells compared to FO B cells [15]. We have also shown that the level of Fc γ RIIB on MZ B cells increase even further after CII immunization for induction of CIA [15,19]. That Fc γ RIIB control MZ B cells is evident by the increased secretion of TNF and IL-10 in TLR-activated Fc γ RIIB-deficient MZ B cells compared to wild type MZ B cells [4]. Fc γ RIIB-deficient MZ B cells are also more efficient at presenting self-antigen to naïve T cells, further demonstrating the important control function for this receptor [4].

The role of CD22 as an inhibitor of B cell activation lies in its ability to regulate calcium signaling, especially downstream of the BCR and associated molecules. Phosphorylation of proteins involved in the calcium cascade such as CD19 is increased in B cells from CD22-deficient mice after anti-IgM stimulation [117]. Although CD22 is mostly important in BCR signaling, it can also regulate TLR signaling [118], similarly to Fc γ RIIB [4]. Accordingly, CD22-deficient B cells proliferate *in vitro* more strongly than WT B cells to TLR7 and TLR9 agonists, and antibody responses to LPS are elevated [119–122]. In contrast, antibody responses to TI-2 antigens are impaired in CD22-deficient mice, probably as a result of the decreased number of MZ B cells in these mice [46]. Despite a diminished MZ B cell population, CD22-deficiency accelerates the development of autoimmunity in autoimmune-susceptible mice [121]. However, CD22-deficient mice on a C57BL/6 background do not develop an autoimmune phenotype spontaneously. This highlights the complexity of autoimmune pathogenesis, and the need to further elucidate the role of CD22 in regulating MZ B cells and a possible link between MZ B cell decrease and increased autoimmunity in mice. Importantly, polymorphisms in the human CD22 gene have been linked

to RA [123].

Altogether these data highlight CD21, CD35, Fc γ RIIB and CD22 as important regulators of MZ B cells. A decreased regulation of MZ B cells seems to increase the incidence of autoimmunity, but not necessarily increase disease severity. Thus, a tight regulation of MZ B cells appears essential for preventing the initial activation and the ensuing complete breakage in tolerance.

6.3. Estrogen promotes MZ B cells

It is widely known that estrogens are immune system modulators, which may influence the induction of autoimmunity [124]. Indeed, a strong female bias is seen in many autoimmune diseases. While estrogens are present in both males and females they are usually found at significantly higher levels in females of reproductive age. In murine disease models of autoimmunity, it has been established that estrogen potently exacerbate B cell autoimmunity by promoting the expansion and activation of autoreactive MZ B cells, which are consequently induced to secrete antibodies and undergo class-switch recombination [21]. Accordingly, the increased frequency of MZ B cells in aged female mice may be an effect of long term estrogen stimulation [15]. Furthermore, in a prospective case-control study in women it was demonstrated that a short-term *in vivo* increase in circulating estrogen levels lead to an expansion of mature MZ B cells [125]. Together, this suggests that MZ B cell expansion and activation due to prolonged estrogen exposure may partly explain the female bias in autoimmune diseases.

7. Concluding remarks

The pathogenic role of B cells in autoimmune diseases have been extensively studied and their role further confirmed by the efficacy of B cell depletion in autoimmune diseases. Although these therapies are very promising, long-term B cell depletion may lead to immunopathology and increased susceptibility to infection. A more tailored depletion of particular pathogenic B cell subset in autoimmunity would therefore be advantageous. Selective elimination of MZ B cells, with minimal reduction of the conventional B cell compartment, could be an interesting therapeutic approach in autoimmune disorders.

It is probable that B cells have a previously neglected or unsuspected role as antigen-presenting cells in the initiation of autoimmunity. Evidence presented here do not exclusively prove that MZ B cells contribute to autoimmunity by their antigen-presentation capability, but they certainly open up this possibility. A dysregulated MZ B cell compartment that involves MZ B cell expansion accompanied by follicular inclusion and/or extrasplicenic localization (Table 1) may support antigen activation of self-reactive T-helper cells, which in turn can promote expansion of self-reactive GC FO B cells and production of high-affinity autoantibodies (Fig. 3). In strong support of this model are our observations from CIA, in which the early IgM response to the immunogen bovine collagen type II comes from MZ B cells, while the later IgG response is derived from FO B cells and is more directed towards the self-antigen murine collagen type II [4,19]. This IgG response is preceded by an increased movement of MZ B cells into the follicles as well as potent presentation of self-antigen to and activation of CD4⁺ T cells by MZ B cells.

Manipulation or depletion of MZ B cells will possibly answer if they play a key role in the pre-clinical phase of autoimmunity. However, it should also be emphasized that autoimmunity can develop even when MZ B cells are reduced or absent, as seen in some models for SLE [126, 127]. This suggests that while MZ B cells certainly seem to contribute to breakage of immunological tolerance, other B cell subsets may also play a role.

In conclusion, further characterization of the functional features of MZ B cells are needed to provide a more complete understanding of the roles MZ B cell play in autoimmune pathogenesis. An advantage in this respect is the finding that mice, similarly to humans, display MZ B cells also in the lymph node subcapsular sinus, which will aid in the

Table 1

Description of MZ B cells in autoimmunity.

MZ B cell characteristics	Disease [ref.]				
	Rheumatoid arthritis	Systemic lupus erythematosus	Sjögren's syndrome	Type 1 diabetes	Graves' disease
Enlarged compartment	Yes [4,15,16,19]	Yes [20–23,72]	Yes [25,28,29,60,61]	Yes [13,17,24]	Yes [30]
Infiltration into target tissue			Yes [25,28,29,60,61]	Yes [13]	Yes [30]
Follicular inclusion	Yes [19]	Yes [7,88]	Yes [25,28]		
Contribution of natural autoantibodies	Yes [4,15]				
Hyper-responsive to TLR-ligation	Yes [4,19]	Yes [79,88]	Yes [25]	Yes [13]	
Secretion of cytokines (TNF, IL-6, IL-10)	Yes [15]	Yes [72,79]	Yes [25]		
Presentation of self-antigen	Yes [15]			Yes [13]	
Induction of CD4 ⁺ T cell response	Yes [15]	Yes [88]		Yes [13]	
Reduced expression of CD21		Virus-infection related autoimmunity [113]			

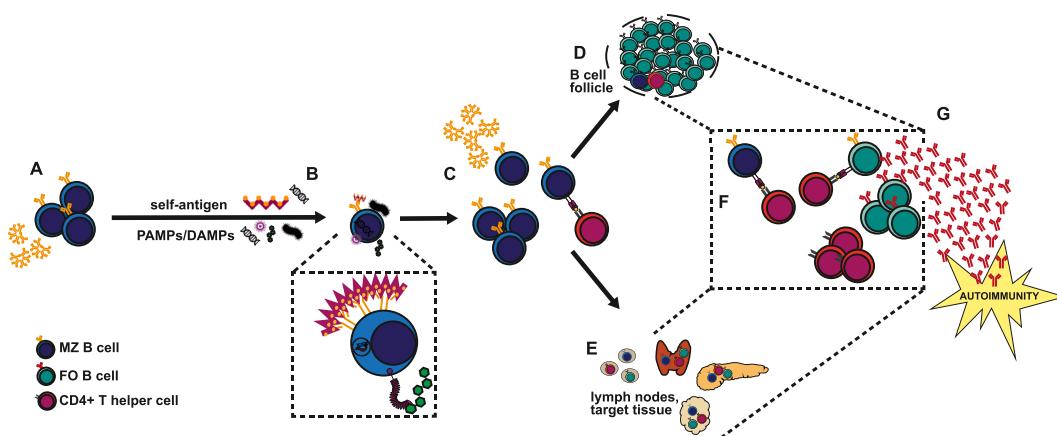


Fig. 3. A model for the role of MZ B cells in autoimmunity. A At steady state, MZ B cells with low affinity for self-antigen secrete natural IgM for housekeeping functions. B If these MZ B cells are exposed to self-antigen, particularly with repetitive epitopes, they can be activated when simultaneously stimulated with PAMPs and/or DAMPs. C The activated MZ B cells will expand extrafollicularly, increase secretion of low-affinity IgM autoantibodies, and present self-antigen to cognate T helper cells, thus initiating breakage of tolerance. D-E MZ B cells activated in this manner will gain access to B cell follicles (D) and lymph nodes (E), and/or infiltrate target tissue such as salivary glands, the pancreas, or the thyroid gland (E). F Here, further presentation of antigen to T cells takes place, resulting in activation and expansion of self-reactive FO B cells and T cells followed by initiation of GC reactions. G The output of these GCs are pathogenic autoantibodies mainly of IgG isotype produced by the FO B cells. Ultimately, the threshold is passed and autoimmune disease ensues. DAMPs, damage-associated molecular patterns; FO, follicular, GC, germinal center; MZ, marginal zone; PAMPs, pathogen-associated molecular patterns.

understanding of human MZ B cell biology better. The knowledge gained may lead to new insights into the role of MZ B cells in the context of human autoimmune diseases.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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