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Natural IgM and the Development of B Cell-Mediated Autoimmune Diseases

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Abstract

Most serum immunoglobulin M (IgM) is "natural IgM," which is produced apparently spontaneously by a distinct subset of B cells requiring no exogenous antigenic or microbial stimuli. Natural IgM is an evolutionarily conserved molecule and reacts with a variety of epitopes expressed on both self- and non-self antigens. It has long been understood that secreted (s) IgM contributes to the removal of altered self-antigens, such as apoptotic and dying cells. As we outline in this review, it is thought that this sIgM housekeeping function removes potential triggers of autoresponse induction. However, we recently demonstrated an unexpected and distinct role for sIgM in the control of autoreactive B cells: the regulation of bone marrow B cell development. The absence of sIgM blocked pro- to pre- B-cell transition and greatly altered the BCR repertoire of the developing B cells and the peripheral B-cell pools in genetically engineered mice. This finding strongly suggests that IgM is critical for B-cell central tolerance induction. Given that treatment of sIgM-deficient mice with polyclonal IgM corrected these developmental defects, therapeutic application of IgM could be of clinical relevance in the treatment of some B-cell–mediated autoimmune diseases.

Keywords

IgM-deficiency; B-1 cells; B-cell development; tolerance induction

I. INTRODUCTION

Secreted IgM (sIgM) is the most evolutionary conserved antibody isotype, present in all vertebrate species, and it is also the earliest isotype to be expressed during immune development. Despite its short half-life, sIgM presents at high levels in the serum of both mice and humans (mean concentrations in human sera: 147 ± 84 μg/μl mean ± SD with t ½ of 5 days, and in sera from C57BL/6 mice: 220 ± 90 μg/μl with t ½ of 2 days).[1–5] Most circulating IgM is "natural" IgM; thus, IgM is produced spontaneously without known

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exogenous antigenic or microbial stimuli. Even mice, raised under germ-free conditions and fed a diet that is free from foreign antigens, develop normal serum levels of IgM and an apparently normal natural antibody repertoire.[6–8]

Studies by numerous groups have shown that in mice, at least 80% of circulating IgM is derived from IgM-secreting B-1 cells residing predominantly in the spleen and bone marrow.[9–13] A recent study by Kelsoe et al., however, suggested that non B-1 cell-derived bone marrow plasma cells are a main source of serum IgM in naïve mice.[14] Ongoing studies in our laboratory have confirmed that these plasma cells contribute to natural IgM production. We find, however, that these cells are also B-1 cell-derived (Savage H.P. Yenson, V. and Baumgarth N, 2016, under review). Murine B-1 cells, phenotypically identified as IgM+ $IgD^{lo/-}$ CD23− $CD19^{hi}$ CD43+ CD5+/−, are distinct in development, tissue distribution and function from the majority of conventional B cells.[15,16] Their development appears to be dependent on a positive selection step requiring intact BCR signaling and binding to self-antigens.[16,17] This explains the skewed repertoire of B-1 cells, which are enriched in particular for VH11 and VH12 heavy chains.[16,18–23] Given the independence of natural IgM production on exposure to foreign antigen, and the requirement for self-antigen selection of B-1 cells, the data suggest that natural IgM production is triggered by interaction with self-antigens. However, the mechanisms controlling natural IgM production remain unknown.

While it is clear that natural IgM is present in humans, as it is in all other jawed vertebrates,[24] the cellular origins of natural IgM in humans are still controversial. An orthologous human B-1 cell population has not been clearly identified, and the presence and phenotype of human B-1 cells is still debated.[25–28]

Natural IgM is encoded by unmutated germline variable gene segments associated with polyreactive binding specificities.[23] We define here polyreactivity as the ability to bind shared structures and epitopes that may be present on a variety of unrelated self-and non–self-antigens. For example, natural IgM can recognize epitopes on phosphorylcholine (PC), which is present not only on cell membranes of apoptotic cells but also on cell-wall polysaccharides of many microbes and parasites, including *Streptococcus pneumonia, Neissaeria meningitides, Haemophilus influenzae, Aspergillus fumigatus,* and *Heligmosomoides polygyrus.*[29–32] Other known specificities of natural IgM include nucleic acids, phospholipids, and carbohydrates, such as PC, phosphatidyl choline (PtC), lipopolysaccharide (LPS), low-density lipoprotein (LDL), single-stranded DNA (ssDNA), and double-stranded DNA (dsDNA).[15,33,34] Frequencies of polyreactive IgM are higher in neonates than in adults in both mice and humans.[30,35–37]

There are three main known forms of secreted IgM: monomeric, pentameric, and hexameric. The monomeric form is not regularly seen in healthy individuals, but it is frequently found in patients with autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA).[38,39] The mechanistic association linking monomeric IgM with autoimmune disease has not been elucidated. In healthy people, circulating IgM exists predominantly in the pentameric form. Pentameric IgM is formed in the endoplasmic reticulum, containing 5 monomers of IgM linked together by a J- (joining) chain.[40,41] Thus,

the pentameric structure has 10 potential Ag-binding sites enabling IgM high avidity interactions with antigens despite potential low affinity for each antigen-antibody binding site interaction. The least frequent form of IgM in healthy people is the hexameric form, which lacks a J-chain.[42] Its functional significance is largely unknown.

sIgM plays various roles as part of the immune system. During infections, sIgM directly neutralizes harmful pathogens, induces complement activation, enhances antigen presentation, and thereby enhances subsequent adaptive immune responses.[43–48] In the absence of overt infections, IgM seems crucial in enforcing immune homeostasis by preventing the development of autoimmune antibody production and dampening inflammatory responses. In this review, we focus on the latter. We summarize existing evidence demonstrating the importance of IgM in immune homeostasis and then discuss the potential mechanisms that allow natural IgM to play such a critical role in ensuring system health.

II. AUTOREACTIVE IGM AND IGG IN AUTOIMMUNE DISEASES

Studies of mice genetically engineered to lack sIgM (μs−/− mice) have demonstrated a need for sIgM in protection against antibody-mediated autoimmunity diseases. μs−/− mice showed greatly enhanced spontaneous IgG autoantibody development, which included development of antibodies to nuclear autoantibody components (ANA), dsDNA, and ssDNA as well as increased susceptibility for development of arthritis and lupus-like diseases.[49–51] Breeding μs−/− mice onto a lupus-prone (lpr) genetic background resulted in offspring that developed more severe lupus nephritis than the control lpr mice. Disease prevalence further increased with age.[49] Lpr/μs−/− mice suffer from more severe glomerulonephritis and reduced survival compared to lpr mice. After 1.5 years, the survival rates were only 48% for Lpr/μs−/− mice compared to 78% for controls.[49] Increased morbility and mortality correlated with increased deposits of autoreactive IgG immune complexes in glomeruli.

Selective IgM deficiency is a relatively rare primary immunodeficiency of humans, which reportedly occurs at a rate of 0.03% in both children and adults without gender bias.[52] A study showed that 13% of patients with selective IgM deficiency had pathogenic antinuclear antibodies (ANA) and 14% developed autoimmune diseases such as arthritis and SLE.[53] Thus, in humans as well as in mice, deficiency in sIgM increases an individual’s susceptibility for autoimmune diseases.

Autoreactive IgM levels are greatly elevated in various autoimmune diseases, such as SLE, rheumatoid arthritis, and autoimmune liver diseases.[34,54,55] This association likely does not reflect a causative relationship between autoreactive IgM and disease occurrence. Rather, it could reflect a compensatory mechanism by which the increased IgM might dampen systemic chronic inflammation, accumulation of autoantigens, and/or the potential for increased exposure of antigen-reactive B and T cells to self-antigens. Consistent with this hypothesis, the presence of autoantibodies of the IgM isotype seem to be protective, while antibodies with similar specificity, but of the IgG isotype, are often pathogenic. Higher titers of IgM anti-dsDNA as well as and a higher ratio of IgM to IgG anti-dsDNA are negatively correlated with the occurrence of glomerulonephritis and renal diseases in SLE patients.[54,56]

Furthermore, SLE patients that have higher levels of sIgM and a more diverse repertoire are associated with reduced disease development and lower incidence of atherosclerotic cardiovascular diseases.[57] Thus, even though IgG and IgM autoantibodies in patients with autoimmune disease have similar specificities and can bind to the same antigens, mostly the self-reactive IgG antibody is pathogenic. This finding suggests that the function of the constant region of immunoglobulins, the Fc-region rather than their specificity, directs the biological functions of antibodies, potentially through binding to specific Fc receptors. Overall, these studies suggest that autoreactive IgM serves as a regulatory molecule that helps to maintain tissue homeostasis and to prevent the development of inflammatory- and autoimmune diseases.

III. NATURAL IGM BINDS TO SELF-ANTIGENS ON APOPTOTIC CELLS AND ENHANCES THEIR PHAGOCYTIC CLEARANCE

One of the fundamental functions of the immune system is the recognition and removal of cells having undergone apoptotic cell death. It is well understood that apoptotic cell death avoids the release of damage-associated molecular patterns (DAMPs) and other triggers of inflammation. Apoptotic cells are frequently cleared by phagocytes (macrophages and dendritic cells (DCs)) in a process called efferocytosis. [58–60] Multiple studies have shown that polyclonal IgM and some monoclonal IgM antibodies can enhance the clearance of apoptotic components released from dying cells.[61–66] Polyclonal IgM binds to apoptotic cells and has been shown to enhance the clearance of these cells by alveolar macrophages in the lung.[67] Monoclonal T15 IgM, which recognizes PC determinants, enhances apoptotic clearance by DCs and macrophages.[64,65] Mice lacking sIgM showed delayed clearance of apoptotic cells after their injection into the peritoneal cavity compared to controls.[62]

Natural IgM might help not only to remove auto-antigens but also to actively dampen inflammatory responses. Recently, it was shown that the presence of sIgM led to enhanced production of the anti-inflammatory cytokine IL-10 by T and B cells following apoptotic cell transfer.[51] Furthermore, monoclonal T15 IgM inhibited the secretion of pro-inflammatory IL-6 and TNF-α by LPS-stimulated macrophages *in vitro,*[64] and the transfer of autoreactive IgM reduced frequencies of pro-inflammatory Th17 cells in FcγRIIB/TLR9 double-knockout mice.[68] Together, these studies highlight the contribution of natural IgM in enhancing apoptotic cell clearance and in preventing the development of inflammation.

The binding of natural IgM to apoptotic cells recruits the early complement factors C1q, which activates the classical pathway of complement, and promotes opsonization and phagocytosis of apoptotic cells by phagocytes (macrophages and dendritic cells) (Fig. 1A).[62,64,65] Mice, deficient in C1q or sIgM, showed reduced apoptotic cell clearance and cellular C3 deposition.[62] Moreover, C1q deficiency in humans is associated with severe lupus-like autoimmune disease,[69–71] potentially linking the IgM with complement activation for removal of self-antigens.

However, the roles of the complement receptors (CR1 or CR2) in autoimmune disease development are incompletely resolved. In one study, deficiency in CR2 or CR1 on an lpr/lpr background was shown to cause more severe autoimmune disease. Lpr/CR2−/− mice had

significantly higher autoantibody titers (ANA, anti-dsDNA and Rh factor) and autoimmune disease manifested at an earlier age than in the lpr/WT controls.[72] In contrast, another study that generated CR1/CR2-deficient MRL/lpr mice showed that disease development was for the most part unaffected. [73] Indeed, in that study significantly lower levels of pathogenic IgG3 autoantibodies were measured compared to controls.[73] The discrepancies on the impact of the CR2 are potentially due to distinct genetic backgrounds of the knockout mouse strains used.[72–74] On mixed C57BL/6 x 129/lpr backgrounds, lack of CR2 led to severe disease development with early onset disease induction and glomerulonephritis development.[72] When CR2−/− mice were fully backcrossed onto C57BL/6 background, the effects of CR2 deficiency in lpr diseases appeared to be milder and to occur later in life.[74] CR2-deficiency on a MLR/Lpr background caused higher autoantibody titers (ANA, anti-dsDNA), without affecting the rate of autoimmune disease manifestations, such as lymphadenophathy and splenomegaly, compared to control lpr mice.[75] While a link between complement activation and immune response induction has been made previously,[76] studies with mice harboring a point mutation in the third constant domain of μ-heavy chain, which renders IgM unable to activate complement, still showed normal humoral immune responses.[77] Whether the effect of natural IgM in enhancing apoptosis clearance is mediated mainly or at least in part via the activation of complement might depend on the specific tissue location or presence of particular cell types. Further studies are required to fully elucidate the interaction of IgM with complement and CR and their effects on autoimmune response induction.

Thus, convincing evidence exists showing that natural IgM is polyreactive, can bind to complement, and can contribute to auto-antigen clearance. However, despite previous assertions, there is actually very little direct evidence that enhanced clearance of apoptotic cells and other cellular components by sIgM complement interactions leading to the control of autoimmune disease development. Various BCR transgenic and knock-in mice, which express a highly restricted oligoclonal or monoclonal B-cell compartment and often lack B-1 cells and/or B-1 cell–derived IgM, do not appear to suffer from autoimmune diseases, despite lacking polyreactive IgM.[78–81] As we outline below, these data could suggest that sIgM has other/additional functions that contribute to appropriate control of autoimmune disease development.

IV. NATURAL IGM CONTROLS B-CELL DEVELOPMENT AND SELECTION

Immunological tolerance is tightly controlled at multiple checkpoints during lymphocyte development to eliminate pathogenic self-reactive B- and T-cell clones. These checkpoints serve to prevent the development of autoimmune disease. Our recent study has shown that sIgM regulates B-cell selection steps that usually prevent autoreactive B cells from escaping mechanisms of “central tolerance” induction in the bone marrow (Fig. 1B).[82] μs−/− mice, i.e., mice that lack sIgM, showed reduced B-cell output from the bone marrow compared to wild-type mice, as measured by B-cell reconstitution in spleen and bone marrow 12 days after sublethal irradiation.[82] FACS analysis and PCR analysis of V-gene usage showed that non-manipulated μs−/− mice have altered frequencies of bone marrow pre-B cells and immature B cells and altered BCR repertoires at multiple checkpoints during B-cell development. Surprisingly, the absence of sIgM even seemed to block the very early pro-to

pre-B-cell transition stage, as frequencies of pro-B cells were increased and those of pre-B cells decreased. An effect was also notable at the transitional B-cell stage (T1) in the spleen and all peripheral B-cell subsets. The noted changes were consistent with a failure of central tolerance induction in the bone marrow, as the periphery contained high frequencies of anergic CD5+ B cells in the spleen and other tissues that seemed to have escaped appropriate tolerance induction.

Given these significant alterations in B-cell development, it was not surprising that μs−/− mice had high titers of pathogenic autoreactive IgG in sera, even despite lower numbers of mature B cells when compared to wild-type mice. These data strongly suggest that the developmental defects in B-cell development altered selection in the bone marrow and that the resulting repertoire changes caused the accumulation of pathological autoreactive B cells.

Lack of sIgM also affected the B-1 cell compartment. Various earlier studies reported increases in CD5+ B-1a cell subsets in the peritoneal cavity of μs−/− mice.[43,83,84] While our studies confirmed increased frequencies and numbers of CD5+ B cells in spleen and peritoneal cavity, the CD5+ cells were $CD43^{neg}$ $CD45R^{hi}$ and $CD19^{int}$, thus distinct in phenotype from that of B-1a cells. In addition, these CD5+ B cells were short-lived and unresponsive to BCR-mediated stimulation, suggesting that they are anergic B cells that escape central tolerance induction in the bone marrow. A comprehensive analysis of the B-1a and B-1b cell subsets ($CD19^{hi}$ $CD45R^{lo}$ IgM^{hi} IgD^{lo} CD43+ CD5+/−) in μs−/− mice showed that both B-1a (CD5+) and B-1b (CD5−) cells were strongly reduced in the peritoneal cavity. This finding was consistent with a 10-fold drop in the number of B-1 cells binding to phosphatidyl choline (PtC) and a near complete lack of B-1 cells expressing IgHV11, two hallmarks of peritoneal cavity B-1 cells.[85,86] Splenic B-1 cell numbers, however, were unaffected.[82] Thus, sIgM modulates bone-marrow B-cell development, and it supports the development of peritoneal cavity, but not splenic, B-1 cells.

In contrast to findings in μs−/− mice, frequencies of total B cells as well as follicular (FO) and marginal zone (MZ) B cells in PBMC of adults with primary selective IgM deficiency (SIGMD) were comparable to gender and age-matched healthy controls.[87,88] This may be explained by the different B-cell compartment analyzed (blood versus spleen) and/or genetic heterogeneity of SIGMD patients. Interestingly, frequencies of a subset of purported human B-1 cells (CD20+ CD70− CD27+ CD43+) were unaffected in SIGMD patients, consistent with splenic B-1 cells in μs−/− mice.[88] In addition, SIGMD patients show increased frequencies of $CR2^{low}$ (complement receptor 2/CD21)-expressing B cells compared with healthy controls.[88] $CD21^{low/-}$ B cells are expanded in patients having increased risks of autoimmune disease development,[89] such as patients suffering from rheumatoid arthritis (RA) and common variable immunodeficiency. $CD21^{low/-}$ B cells express autoreactive B-cell receptors (antinuclear and anticytoplasmic) and are unresponsive to B-cell receptor and/or CD40 stimulation.[89] The expansion of autoreactive and anergic $CD21^{low}$ B-cell population in patients with SIGMD is thus similar to findings in μs−/− mice, and our studies with μs−/− mice provide a mechanism for the expansion of $CD21^{low}$ autoreactive anergic B cells in SIGMD.

Various mouse models showed that strong BCR signaling during B-cell development promotes B-1 and FO B cells, while weaker BCR signals favors MZ B-cell development.[90–92] The observed increases in MZ, decreases in FO B cells and body cavity B-1 cells, and the presence of large populations of anergic B cells in the μs−/− mice are consistent with sIgM altering BCR-mediated signaling in the developing B cells. In support of these findings, others have demonstrated that B cells from μs−/− mice show reduced phosphorylation of downstream targets of BCR signaling after BCR cross-linking: Erk, Syk, and Lck.[93] We find that splenic μs−/− B cells also have reduced basal expression of catalytic (p110) subunit of PI3K enzyme, phosphorylation of Akt (pAkt) and Btk (pBtk) (Fig. 2). Together, these data demonstrate that a lack of sIgM leads to reductions in BCR signaling. Importantly, polyclonal serum IgM can rescue normal B-cell development and reduce autoantibody production by μs−/− B cells.[82,83]

We conclude that sIgM crucially affects B-cell development and selection, ensuring appropriate enforcement of central tolerance induction. We suggest that this regulatory function of sIgM controls autoantibody production, either alone or in conjunction with the other known housekeeping functions of natural IgM. Patients with SIGMD also showed reduced frequencies of GC, and switched memory B cells, suggesting that sIgM might regulate B-cell differentiation.[87, 88] A careful analysis of B-cell development and differentiation in humans with selective IgM deficiency seems indicated.

V. IGM RECEPTORS IN AUTOIMMUNE DISEASEs

Various receptors are reported to bind to sIgM on the surface of B cells and other cell populations. Important for the above findings, sIgM was shown to directly bind to B-cell precursors in the bone marrow, as well as to mature B cells both *in vivo* and *in vitro.* These data open up the possibility that direct B-cell–IgM interaction might regulate B-cell development.[82] However, the above data demonstrate that IgM does not have to be secreted by the developing B cells themselves to regulate normal development. Instead, sIgM could bind to cells other than B cells and thus function indirectly on B-cell development. We provide a summary below on the receptors known to bind IgM and discuss whether they could be involved in the regulation of B-cell development and selection by sIgM.

A. Complement Receptors

Complement receptors are expressed broadly by a number of cell types. B cells express complement receptors type 1 (CR1/CD35) and complement receptor type 2 (CR2/CD21) on the cell surface. These receptors can bind to IgM-antigen complexes via activated complement molecules, including C3b and C4b binding to CR1, and iC3b, C3d, g, C3d, and C4d binding to CR2.[94] CR1/CR2 are first expressed at the transition stage of B cell development thus after B cells leave the bone marrow. Thus, it is not surprising that CR1/CR2−/− mice show normal B-cell development and immunoglobulin levels.[95] Based on their late expression during B-cell development these receptors are therefore unlikely responsible for the observed effects of sIgM on B-cell development.

B. Fcα/μ Receptors (R)

The Fcα/μR is a type I transmembrane protein that binds both IgA and IgM isotypes. The receptor is broadly expressed in humans and mice, and it was reported that B cells and macrophages express this molecule.[96,97] However, Fcα/μR-deficient mice have shown normal B-cell development and normal levels of serum immunoglobulins. Autoimmune disease activity has not been reported in these mice.[98] Furthermore, our own studies failed to find Fcα/μ receptor expression on B cells (Nguyen and Baumgarth, unpublished). Thus, we conclude that Fcα/μR cannot be responsible for the role of sIgM in preventing autoimmune disease or affecting B-cell development.

C. Polymeric Immunoglobulin Receptor (pIgR)

The pIgR is another receptor with dual specificity for IgA and IgM. This receptor binds polymeric IgA and IgM via the J-chain and mediates the transport of polymeric J-chain–containing immunoglobulins at mucosal sites.[99] The pIgR is expressed only on epithelial cells, but not on B cells. pIgR-deficient mice showed accumulation of serum IgA, but strong reduction of IgA in secretions, supporting transepithelial transport of IgA as a major function for this receptor.[100] In addition, serum IgM levels appear to be unaffected in pIgR-deficient mice, and the mice have not been shown to develop autoimmune-related diseases.

D. Fcμ Receptor

The FcμR is the only identified FcR that binds selectively to IgM. Originally identified as “Fas apoptosis inhibitory molecule 3” (FAIM3), this receptor was recently rediscovered as an IgM-specific Fc receptor. The receptor is a type I transmembrane sialoglycoprotein that binds to the CH3 and/or CH4 region of IgM.[101,102] The protein contains an intracellular domain with several tyrosine residues, but it lacks classical immunoreceptor tyrosine-based activation (ITAM) and inhibition (ITIM) motifs.[102] The signaling pathways downstream of the FcμR are still not well understood.

Gene and protein expression analysis showed that the FcμR is present in a variety of cell types, such as macrophages, dendritic cells, T cells; expression is highest in B cells.[103–105] HeLa cells transfected with the FcμR, but not non-transfected cells, bound to and, internalized sIgM, from where it was transported into lysosomal compartments for degradation. In humans, dysregulated FcμR expression has been correlated with the development of various lymphomas.[106] Three independent lines of FcμR deficient mice have been generated to study the functions of the FcμR.[103,107–110] FcμR was reported to be expressed relatively early in development, the pre-B cell stage. Together with the increased serum IgG autoantibody titers in FcμR −/− mice, the data suggest that natural IgM - FcμR interactions may regulate B-cell homeostasis and development.[108,109] However, in contrast to the μs−/− mice, FcμR−/− mice have no overt B-cell developmental defects.[82,103,108,109] Thus, the mechanism by which the FcμR controls autoantibody production seems to be distinct from that noted for sIgM and requires further study.

E. Sialic Acid-Binding Immunoglobulin-Like Lectin (Siglec)

Siglec-G and CD22 both belong to the sialic acid-binding immunoglobulin-like (Siglec) family of lectins. They were identified as negative regulators of BCR signaling.[111,112] Both

receptors are expressed on B-cell membranes and can bind sialic acid residues on sIgM. CD22 is expressed in pre-B cells (CD45R+ IgM−) at lower levels than on mature B cells (CD45^{hi} IgM+).[113] CD22 and Siglec-G–deficient mice showed increased BCR signaling, leading to increased numbers of peritoneal cavity B-1 cells and serum IgM levels.[114] This finding contrasts with the phenotype of μs−/− mice, suggesting that Siglecs may not be involved in regulation of B-cell development by sIgM. Interestingly, the absence of either Siglec-G or CD22 alone did not result in autoimmune disease development, but the absence of both resulted in spontaneous lupus-like disease in mice.[112] In humans, Siglecs are also associated with several autoimmune diseases. In the absence of Siglec-G and CD22, hyper-reactivity of B cells due to increased BCR signaling may lead to autoimmune disease development.[115,116]

We conclude that none of the receptors currently known to interact with sIgM are responsible, or solely responsible, for the observed defects in B-cell development and selection noted in the μs−/− mice. Redundancies in the expression of multiple known sIgM receptors, or the presence of an as-of-yet unknown IgM receptor may explain these findings. It is also possible that sIgM has additional effects on the control of autoantibody production by mechanisms that are independent of B-cell development. While frequencies of total B cells as well as CD4 and CD8 T cells are unaffected in patients with SIGMD,[87,88] they show increases in Bregs and CD8 Tregs.[88] Whether these are compensatory changes that aim to control autoimmune disease development or that contribute to the disease phenotype require further study. At a minimum, these data demonstrate that sIgM directly or indirectly also regulates cells other than B cells.

Given the impact of sIgM on B-cell development and autoimmune response induction, identification of the underlying mechanisms that cause these devastating diseases will be of great importance.

VI. THERAPEUTIC SIGM IN AUTOIMMUNE DISEASES

Some polyclonal and monoclonal IgM treatments have shown promising results in reducing autoimmunity and inflammatory diseases in mice and humans. In mice, transfer of polyclonal IgM was shown to rescue B-cell development and reduce harmful IgG autoantibody titers.[82] Treatment with monoclonal IgM T15 clone, which binds to oxidized LDL and PC, was shown to suppress inflammatory arthritis in both the anti-collagen induced and the anti-collagen antibody passive transfer models.[64,65] Furthermore, In murine models of lupus-prone diseases, treatment with anti-dsDNA monoclonal IgM at a dosage of 100 μg/week given for at least 8 weeks reduced glomerulonephritis and renal pathology, causing a delay in the onset of lupus nephritis and it improved the survival of (NZBxNZW) F1 and MRL/lpr mice.[117,118] However, in contrast to this finding, passive transfer of a monoclonal IgM specific for α-1,3-glucose, thus not a specificity of natural IgM, was unable to improve B-cell development in μs−/− mice.[82] This finding suggests that the specificity and ability to bind to self-antigen components is a crucial requirement for the protective effects of sIgM.

In human studies, passive transfer of pooled IgM from 2,500 healthy donors containing 90% pure IgM (IVIgM) suppressed the activities of IgG-autoantibodies from patients with a

variety of autoimmune diseases *in vitro.*[119] Furthermore, IVIgM has been shown to have promising therapeutic effects in ameliorating inflammatory diseases, myasthenia gravis in experimental models, and multiple sclerosis.[119–123]

VII. CONCLUSION

In summary, sIgM protects against autoimmune diseases by regulating B-cell development and preventing autoreactive B cells to escape central tolerance selection. Despite its short half-life and its polymeric structure, treatment regimens based on transfer of natural IgM show significant promise in the treatment of certain autoimmune diseases.

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ABBREVIATIONS

ANA	anti-nuclear autoantibody
CD	cluster of differentiation
CR1/CR2	complement receptor type 1/2
DC	dendritic cell
dsDNA	double-stranded DNA
Fcα/μR	Fc α/μ receptor
FcμR	Fc μ receptor
FO	follicular
MZ	marginal zone
PC	phosphorylcholine
pIgR	polymeric immunoglobulin receptor
RA	rheumatoid arthritis
sIgM	secreted immunoglobulin M
SIGMD	selective IgM deficiency
SLE	systemic lupus erythematosus
μs−/− mice	secreted immunoglobulin M–deficient mice

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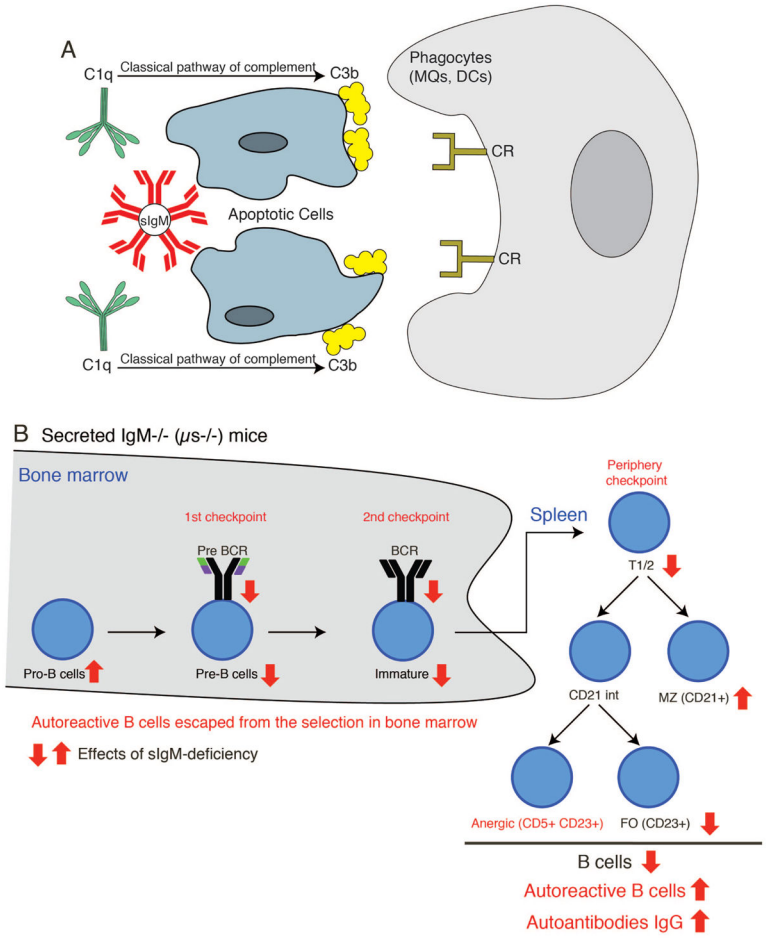

FIG. 1.

The functions of natural IgM in homeostasis maintenance and autoimmune diseases prevention; (A) sIgM enhances apoptotic cell clearance. sIgM binds to apoptotic cells thereby forming antigen:antibody complexes. C1q binds to the Fc portion of sIgM. This leads to the activation of the classical complement pathway Complement Receptors (CR) on phagocytes (macrophages (MQs), dendritic cells (DCs)) can bind to C3b on the immune complexes which triggers their uptake. (B) sIgM prevents the escape of harmful autoreactive B cells during development and selection in the bone marrow and periphery, apparently by enhancing BCR signaling. The absence of sIgM affects all stages of B-cell development and leads to the development of a peripheral B-cell compartment that contains increased self-reactive and anergic CD5+ B cells as well as shifts in the composition of the mature B-cell compartment.

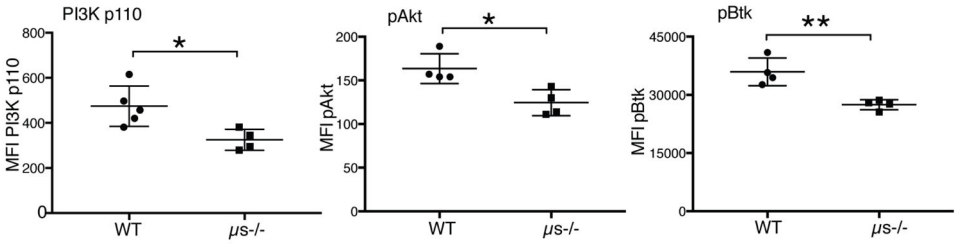

FIG. 2.

Reduced BCR signaling in μs−/− B cells. Graph summarizes mean fluorescence intensity (MFI) ± SD of PI3K-p110, phosphorylated Akt (pAkt), and phosphorylated Btk (pBtk) levels in total spleen B cells from sIgM-deficient (μs−/−) and wild-type (WT) mice (n=4–5 mice/group). Fixed and permeabilized spleen cells were stained with anti-PI3K p110 Alexa Fluor 488 (Abcam ab202666, clone EPR5515(2)), anti-phospho-Akt-PE (pS473; BD phosphoflow) or with anti-phospho-Btk PE (Y551/Y511; eBioscience) on ice for 30 minutes. Data are representative of two independent experiments. *p<0.05, **p<0.005 by unpaired two-tailed Student's *t*-test.