

Review

B cells in autoimmunity

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Published: 14 October 2009

This article is online at <http://arthritis-research.com/content/11/5/247>

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Arthritis Research & Therapy 2009, **11**:247 (doi:10.1186/ar2780)

Abstract

B-cell development is tightly regulated, including the induction of B-cell memory and antibody-secreting plasmablasts and plasma cells. In the last decade, we have expanded our understanding of effector functions of B cells as well as their roles in human autoimmune diseases. The current review addresses the role of certain stages of B-cell development as well as plasmablasts/plasma cells in immune regulation under normal and autoimmune conditions with particular emphasis on systemic lupus erythematosus. Based on preclinical and clinical data, B cells have emerged increasingly as both effector cells as well as cells with immunoregulatory potential.

and focus on human systemic lupus erythematosus (SLE) as a prototypic autoimmune disease.

Under normal resting conditions, B cells follow a tightly regulated life cycle (Figure 1) with a large number of check points at indicated stages (antigen-dependent and antigen-independent selection) to prevent the development of autoimmunity [1]. In the bone marrow, B cells develop from stem cells through a series of precursor stages during which they rearrange their variable immunoglobulin (Ig) genes to generate a wide range of unique antigen-binding specificities. Immature CD10⁺ transitional B cells expressing surface IgM/IgD emigrate from the bone marrow into the peripheral blood and then mature into naïve B cells. In the mouse, this occurs in the spleen, although the site of maturation in humans is not known [2]. After encountering antigen and T-cell help in follicles of secondary lymphoid organs, mature naïve B cells undergo germinal center (GC) reactions leading to their clonal expansion, somatic hypermutation of Ig gene rearrangements, and Ig heavy-chain class-switch recombination. Notably, these complex molecular processes are unique capacities of B cells and ensure specific higher avidity binding by the B-cell receptor (BCR) and also the production of antibodies with altered effector function. During the GC reaction, naïve antigen-specific B cells mature into either memory B cells or Ig-secreting plasma cells.

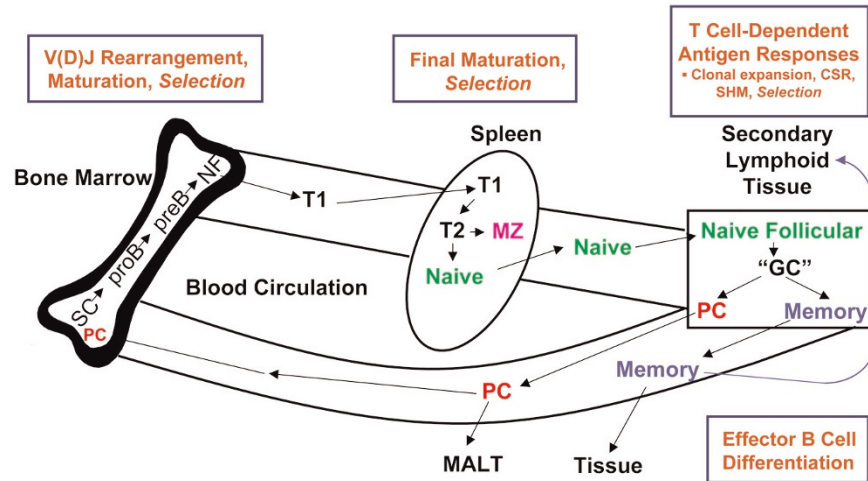
Introduction

One of the major roles of cells of the B-cell lineage is to generate antibody-secreting plasmablasts and plasma cells and also memory B cells with an enhanced capability to respond to the specific initiating antigen. These effector functions of the B lineage are well recognized and their roles in autoimmune diseases are accepted. Knowledge about the immunoregulatory role of B cells has also been substantially expanded within the last decade and their functions have been reconsidered. Historically, B cells have not been thought to play a major regulatory function in the development of autoimmunity and autoimmune diseases, although the identification of autoantibodies produced by autoreactive plasma cells and their pathogenic consequences are widely accepted. It is important to emphasize that B cells increasingly emerge as part of a tightly regulated immune activation process with numerous intimate interactions with other immunocompetent cells that have been identified. Thus, B cells are considered effector cells as well as cells with immunoregulatory potential. This review will consider B-cell involvement as both effector cells and immunoregulatory cells in the induction and maintenance of systemic autoimmunity

In mice, B1 B cells producing natural antibodies are important for the immediate defense against encapsulated bacteria. Whether they contribute to abnormalities of peripheral B cells in SLE [3] and primary Sjögren syndrome (pSS) [4] is not known. The reduced susceptibility of B1 B cells at mucosal sites after depletion by anti-CD20 therapy [5] suggests particular survival conditions of these cells in mice. The B1 B-cell equivalent subset and its role in human autoimmunity

anti-dsDNA = anti-double-stranded DNA; APRIL = a proliferation-inducing ligand; BAFF = B cell-activating factor; BCR = B-cell receptor; BILAG = British Isles Lupus Assessment Group; BLyS = B-lymphocyte stimulator; GC = germinal center; ICOS = inducible co-stimulator; ICOS-L = inducible co-stimulator ligand; IFN = interferon; Ig = immunoglobulin; IL = interleukin; ITIM = immunoreceptor tyrosine-based inhibitory motif; MZ = marginal zone; pSS = primary Sjögren syndrome; SLE = systemic lupus erythematosus; TLR = Toll-like receptor; TNF = tumor necrosis factor.

Figure 1



Schematic B-cell development of B2 B cells emigrating as immature B cells from the bone marrow and differentiating further into naïve/marginal zone (MZ) B cells in the spleen, which subsequently undergo T cell-dependent differentiation into memory B cells and plasma cells. Secondary lymphoid tissue refers to spleen, tonsil, lymph node, and Peyer's patches. CSR, class-switch recombination; GC, germinal center; MALT, mucosal-associated lymphoid tissue; NF, nuclear factor expression; PC, plasma cells; SC, stem cell; SHM, somatic hypermutation; T1, transitional 1; T2, transitional 2.

diseases, however, remain to be delineated. Although there is an increase in CD5⁺ B cells in both SLE and pSS, these cells may represent an expanded population of pre-naïve conventional B2 cells and not the human equivalent of B1 cells [6]. In addition, CD5 can be regarded as a B-cell activation marker in humans and there are no convincing data providing evidence that it can be used as a lineage marker as accepted in mice.

On the other hand, B2 B cells comprise the adaptive portion of humoral immune responses. B2 cells participate preferentially in T cell-dependent GC reactions, during which they can hypermutate their IgV gene rearrangements, switch Ig classes, and differentiate into memory cells and long-lived plasma cells. However, B2 cells can also be activated during T cell-independent responses [7]. B2 precursor cells are generated in the bone marrow and are subjected to central tolerance mechanisms. The immature survivors with functional BCRs leave the bone marrow and migrate to the periphery and are thought to be exposed to further selection (peripheral tolerance). Although it has been suggested that B2 B cells differentiate into either a mature follicular B-cell or a marginal zone (MZ) B-cell program [8], there are insufficient data that confirm this in humans. Alternatively, MZ B cells and B2 B cells can derive from precursor transitional 2 (T2) B cells that still need additional confirmation. Mouse models suggest that both B1 and MZ B-cell responses occur independently of T-cell help and B1 and MZ B cells are thought to be excluded from undergoing GC reactions. In conclusion, there appear to be substantial differences between mice and humans in terms of the specifics of the

differentiation of B-lineage cells. Moreover, the distinct contribution of B1, B2, and MZ B cell-equivalent subsets in human systemic autoimmunity as well as the role of T cell-independent (TI) and T cell-dependent (TD) B-cell activation remain to be fully delineated. A more precise understanding of these processes in human autoimmunity would allow us a more targeted approach to treat specific autoimmune diseases.

Important for the interaction with T cells and the generation of GC reactions are a series of ligand-receptor interactions, including those mediated by CD154/CD40 and inducible co-stimulator ligand/inducible co-stimulator (ICOS-L/ICOS). Defects in these interactions have been shown to lead to hyper-IgM syndrome, resulting in impaired plasma cell and memory B-cell generation, including B lymphopenia and adult-onset common variable hypogammaglobulinemia, respectively [9,10]. Moreover, the presence of certain cytokines, such as interleukin (IL)-6, lymphotoxin-β, IL-4, and IL-21, is required in order to facilitate specific stages of B-cell differentiation by providing essential co-stimulatory signals.

Because of the complexity of the abnormalities of immune regulation in systemic autoimmunity, a few key B-cell abnormalities will be highlighted since they provide insight into the nature of perturbations of B-cell function that could contribute to autoimmunity, either in a causative manner or as a pathway that amplifies disease. In this regard, it is not clear whether the identified abnormalities of B cells in SLE are intrinsic or secondary to the disturbed internal milieu characteristic of SLE.

1. Defects in proper selection against autoreactivity during B-cell development

A. Autoantibody production

Currently, the detailed nature of defects in immunologic check points during B-cell development in SLE is unclear. However, autoantibodies against double-stranded DNA (dsDNA) and nucleosomes, serologic hallmarks of lupus, and other nuclear antigens reflect the breakdown of immune tolerance. Notably, autoantibodies have been observed in some patients 6 to 10 years before the onset of the disease [11-13], indicating that the breakdown of tolerance may precede and not be secondary to disease activity in SLE. The processes of recombination and somatic hypermutation for affinity maturation in the bone marrow and subsequently in several lymphoid organs, respectively, are followed by strong selective pressures ('check points') under normal conditions to protect the body from the emergence of B cells with self-reactivity. In this regard, a number of check points in B-cell development have been proposed between immature and mature naïve B cells [14,15].

Examples of abnormalities in selection in patients with SLE have been reported. The idiotype defined by the 9G4 monoclonal antibody that is encoded by V_H4-34 heavy-chain gene rearrangements and frequently used by autoantibodies has been shown to circumvent negative selection in GCs in tonsils from SLE patients with subsequent expansion into the memory B-cell and plasma cell pool [16]. Moreover, the level of 9G4-expressing B cells as well as 9G4-containing anti-dsDNA antibodies is related to disease activity in SLE [17]. Compelling evidence of the failure of peripheral tolerance has also been found in an analysis of somatically mutated V_H gene rearrangements encoding anti-DNA antibodies [18,19], where back-mutation clearly resulted in loss of binding activity. These data are consistent with the conclusion that most but possibly not all anti-DNA antibodies can arise by somatic mutation from precursors that lack autoantibody specificity. Therefore, the induction of some autoantibodies requires activation-induced cytidine deaminase for somatic hypermutation and Ig switching [20], and their development occurs *de novo* in the periphery fostered by a defect in peripheral tolerance.

B. Plasmablasts/plasma cells

Although we have acquired a broad knowledge in the use of autoantibodies in the diagnosis of autoimmunity, less is known about how autoantibodies are generated in humans. Clinical data suggest that in patients with active SLE there are short-lived plasmablasts that are CD27^{high}/HLA-DR^{high}-producing anti-DNA antibodies and their frequency in the blood correlates with disease activity [17,21,22], whereas long-lived plasma cells (likely CD27^{high}HLA-DR^{low}) produce stable autoantibody titers, such as anti-Sm, -Ro, or -La, independently of disease activity. Experimental evidence from mice provided further evidence that plasmablasts and plasma cells reside in the spleen of lupus-prone mice [23] during

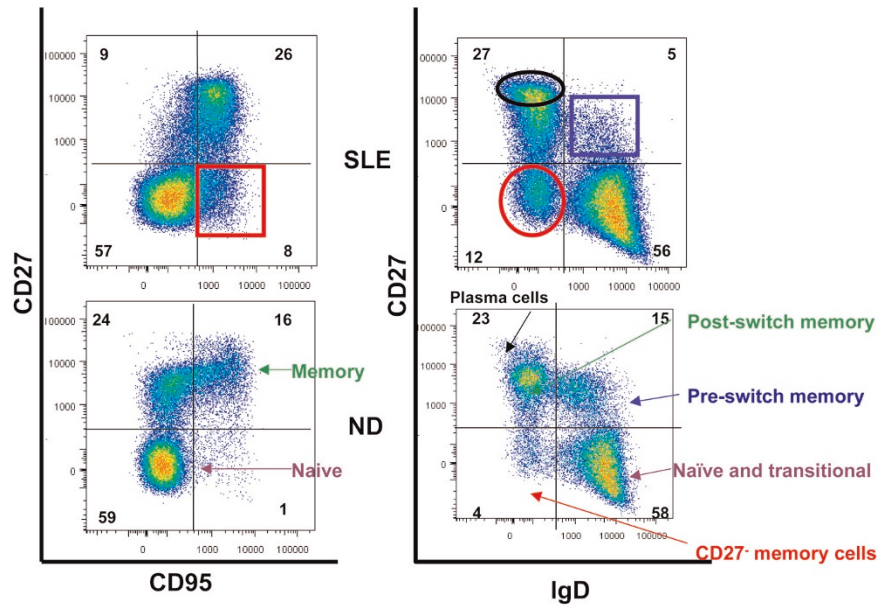
early stages of the disease and that only proliferating plasmablasts showed susceptibility to cyclophosphamide treatment. One critical question is whether these major subsets of Ig-producing cells are generated differently in autoimmunity [24].

Recently, in SLE patients, a more specific plasmablast subset that expresses HLA-DR very brightly and that clearly represents freshly generated plasmablasts was identified [22,25]. Notably, this cell fraction but not the remaining HLA-DR^{low} plasma cells correlated with lupus activity (Systemic Lupus Erythematosus Disease Activity Index score) and anti-DNA titers, which indicates that they directly reflect the activity of SLE. Thus, there is an apparent defect of negative selection or regulation of newly generated plasmablasts in SLE, or alternatively, the process is normal but not appropriately terminated. In this context, proper regulation of antigen-specific plasmablast generation can be seen after secondary tetanus vaccination, when these cells appear in the circulation between days 6 to 8, but their appearance is downregulated as the immune response wanes [26]. The data on plasmablasts in murine and human lupus provide evidence that these cells are susceptible to anti-proliferative immunosuppressive agents, whereas more differentiated non-dividing plasma cells show resistance to these drugs [23]. Their distinct contribution to the disease and susceptibility to therapeutics remain to be assessed.

C. Memory B-cell compartments

There are a number of abnormalities of peripheral B-cell subsets in human SLE, including an expanded population of transitional B cells and post-switched CD27⁺ B cells as well as the appearance of a distinct population of CD27⁻/IgD⁻ B cells with a memory phenotype that is usually not found under normal conditions (Figure 2). Although their induction and nature remain poorly understood, it appears that major perturbations occur in the memory compartment that could explain disease pathogenesis or resistance to therapy or both (Figure 3). This is consistent with molecular data that the peripheral B-cell repertoire in SLE can be shaped by exaggerated somatic hypermutation, whereas the Ig repertoire initially generated by V(D)J recombination appears similar to normal controls [27]. Although the frequency of memory B cells is increased in the peripheral blood of SLE patients undergoing immunosuppressive therapy [21], the specificity of these therapy-resistant memory B cells is unknown. It is also unclear whether this predominance of memory B cells reflects a loss of peripheral tolerance or abnormal selection processes or whether their appearance is simply the result of an enhanced activation and differentiation of naïve B cells or their insusceptibility to immunosuppressive drugs (Figure 3). Independently of these considerations, an enlarged pool of memory B cells poses a risk for autoimmunity since these cells have lower activation thresholds and have passed all check points of the immune system for negative selection. The enhanced risk for

Figure 2



Major differences of peripheral B-cell compartments between systemic lupus erythematosus (SLE) patients and normal controls as shown in a representative dot plot. Please note the increased frequency of Ig-class-switched CD27⁺ memory B cells and CD27⁻/IgD⁻ B cells. ND, normal donors.

autoimmunity is emphasized by data showing that some memory B cells acquire polyreactivity and autoreactivity induced by somatic hypermutation [18,19,28].

D. Naïve B cells

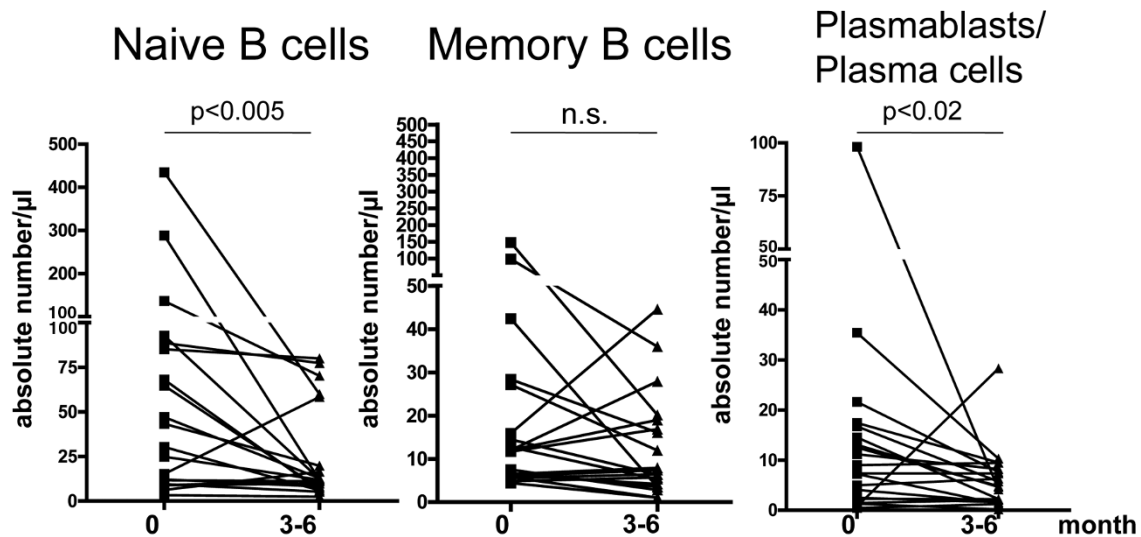
Of further interest remains the question of the extent to which autoimmunity is already contained in the naïve B-cell repertoire of SLE before an encounter with antigen in the periphery. This is a difficult question to address in humans. SLE patients clearly have defects in check points which result in the appearance of an increased frequency of B cells capable of polyreactive autoantigen reactivity. Interestingly, a comprehensive study [15] showed that even inactive SLE patients fail to remove self-reactive BCRs expressed by naïve B cells. Consistent with this, earlier studies demonstrated that self-reactivity or loss of proper selection during early B-cell development from immature (CD10⁺ CD27⁻ IgM⁺ B cells) to mature (CD10⁻ CD27⁻ IgM⁺ B cells) naïve B cells is a key feature in SLE [14]. Although these cells appeared to be influenced neither by antigen nor by T-cell help, the available data do not allow a conclusion about the detailed impact of a primary autoimmune predisposition or secondary influences such as cytokines or co-stimulatory signals. While it has been debated that polyreactive B cells can represent a source of autoantibody-secreting cells, formal evidence is lacking. On one hand, the IgM^{-/-} mouse develops 'autoimmunity' [29]. By contrast, polyreactive IgG antibodies in SLE may impact on autoimmune manifestations [19]. Although it is tempting to conclude that SLE has defects in

'check points against autoimmunity' that explain the development of pathologic IgG autoantibodies, the possibility cannot be excluded that these 'check points' are active in SLE to prevent serious autoimmunity but are simply overwhelmed by chronic polyclonal B-cell activation. Alternatively, extensive cytokine release and enhanced co-stimulation may bypass these check points. It is also important to consider that B cells with a memory phenotype appear among CD27⁻ B cells previously considered to be exclusively naïve [30,31]. A subset of CD27⁻/IgD⁻ B cells that express CD95 were characterized as activated memory B cells with mutated and Ig-class-switched BCRs. Interestingly, their appearance correlated with lupus activity, which is in striking contrast to the CD27⁺ memory B-cell population that does not vary much with disease activity. This CD27⁻ memory subset was found in patients with SLE but not in patients with infection. The role of this CD27⁻ memory B-cell subset in the pathogenesis of SLE remains to be determined.

E. Pre-naïve B cells

Recently, a unique pre-naïve peripheral B-cell population representing an intermediate stage between transitional and naïve B cells was identified at enhanced numbers in human SLE [6]. These cells are CD5⁺ and express levels of CD38, CD10, CD9, and the ABCB1 transporter which are intermediate between transitional and naïve B cells. Therefore, these cells were considered pre-naïve B cells that could be induced to differentiate into naïve B cells *in vitro*. These pre-naïve B cells showed defective responses to BCR stimulation

Figure 3



CD27⁺ B cells with a memory phenotype are less susceptible to cyclophosphamide treatment in patients with systemic lupus erythematosus (SLE) ($n = 20$). Severely active SLE patients undergoing monthly intravenous cyclophosphamide bolus therapy were followed for a period of 3 to 6 months. Whereas CD27⁻ B cells and CD27⁺⁺ plasmablasts/plasma cells showed a decrease, the absolute numbers of CD27⁺ memory B cells did not change significantly. n.s., not significant.

but intact responses to CD40 ligation, whereas spontaneous apoptosis and cell death were enhanced compared with naïve B cells. Of note, B cell-activating factor/B-lymphocyte stimulator (BAFF/BLyS) was not an essential survival factor of these pre-naïve cells compared with naïve B cells. Finally, these cells had the capacity to differentiate into plasma cells after stimulation and the ability to function as antigen-presenting cells. The contribution of this population to lupus pathogenesis remains unknown. It is clear, however, that disturbances of early stages of B-lymphocyte homeostasis are also present in SLE and indicate that not only memory compartments are affected.

2. Aspects of disturbed immunoregulation in systemic lupus erythematosus

A. Regulation of B-cell activation by Fc receptors

The immune system has evolved to defend the organism against a variety of pathogens and applies threshold mechanisms for regulation. Independently of the co-stimulatory mechanisms, the coupling of activating and inhibitory receptors is able to use thresholds for immune cell activation. IgG immune complexes have long been recognized to have potent immunoregulatory functions ranging from a strong enhancement to complete suppression of antibody responses [32] using selective engagement of specific FcγRs on discrete cell types, which result in either arrest or progression of an immune response. Four different classes of Fc receptors have been defined: FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIV [33]. Functionally, there are two different classes of Fc receptors: the activating and the

inhibitory receptors, which transmit their signals via immunoreceptor tyrosine-based activation (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Co-expression of activating and inhibitory molecules on the same cell allows a balanced immune response, and the biochemical structure of IgG has substantial influence of the effects on Fc receptors, resulting in either a pro- or anti-inflammatory response [33].

B. The inhibitory Fcγ receptor IIB

The inhibitory Fcγ receptor IIB is part of the family of immune inhibitory receptors and its loss leads to autoimmunity and autoimmune disease [34,35]. FcγRIIB is a single-chain receptor that carries an ITIM in its cytoplasmic domain, a hallmark of this inhibitory protein family, and signals via inositol phosphatase SHIP (Src homology 2-containing inositol phosphatase) [36]. With the exception of T cells and natural killer cells, FcγRIIB is expressed on all cells of the immune system, including B cells, where it regulates activating signals delivered by immune complexes. As a consequence of its role in regulating BCR signals, which ultimately will decide whether a B cell will undergo proliferation and differentiation into an antibody-secreting plasma cell, FcγRIIB has been suggested to play an important role in maintaining peripheral tolerance [34,35]. The capacity of FcγRIIB to trigger B-cell apoptosis has been proposed to be another mechanism for controlling B-cell responses and maintaining self-tolerance. This hypothesis was supported by the generation of FcγRIIB-deficient mice that spontaneously develop a lupus-like disease characterized by the production of autoantibodies and premature death because of severe glomerulonephritis

[37]. Recently, it was suggested that Fc γ RIIB co-ligation inhibits BLyS signaling and BLyS-R upregulation following BCR engagement [38].

C. Polymorphisms in the human Fc γ RIIB promoter and autoimmunity

Polymorphisms in the human Fc γ RIIB promoter linked to lupus have been identified. One polymorphism leads to decreased transcription and surface expression of Fc γ RIIB on activated B cells of human lupus patients [39]. Another polymorphism in the transmembrane domain of Fc γ RIIB is linked to human lupus in several ethnic populations [33]. It has been suggested that this allelic variant of the inhibitory receptor loses its function because of the inability to associate with lipid rafts [40,41].

Autoreactive B cells can potentially be generated at several stages during B-cell development. There is accumulating evidence that Fc γ RIIB mediates its function during late antigen-dependent stages of B-cell maturation, thus representing a distal check point against autoimmunity [33]. Of note, Fc γ RIIB deficiency did not impact on early events in the bone marrow, such as receptor editing, nor did it prevent the development of IgM⁺ autoreactive B cells. After class-switching to IgG, however, Fc γ RIIB was essential to prevent the expansion of autoreactive B cells and their maturation into plasma cells [33]. Considering the higher pathogenic potential of IgG compared with IgM antibody isotypes, this relatively late stage of Fc γ RIIB-mediated negative regulation has a major role in preventing the initiation of severe autoreactive processes.

Central check points, including receptor editing, deletion, and anergy of self-reactive BCR species, ensure that the majority of B cells with an autoreactive BCR are deleted in the bone marrow [42], which occurs independently of Fc γ RIIB. In contrast, autoreactive B cells can be generated *de novo* by somatic hypermutation, which is supported by the finding that many autoantibodies are encoded by somatically mutated V_H gene rearrangements and switched Ig. Here, Fc γ RIIB might serve as the final barrier to prevent these B cells with potentially autoreactive BCR specificities from maturing into plasma cells.

3. Abnormalities of immune activation in systemic lupus erythematosus

Co-stimulation results from a complex mix of factors involved in effective immune activation, which includes antigen presentation, the provision of soluble and insoluble co-stimulatory factors as well as the anatomic organization of secondary lymphoid organs, including the GCs. One important function of memory B cells is antigen presentation, which is facilitated by the expression of high-avidity BCR and also major histocompatibility complex class II molecules. Under sufficient co-stimulation, memory B cells can effectively present antigen primarily to memory T cells. In mice, MZ

B cells were even shown to provide co-stimulatory activating signals to naive T cells [43]. In addition, CD80/CD86 expression on B cells has been shown to be indispensable for the activation of autoreactive T cells in a murine arthritis model [44]. The function of B cells as antigen-presenting cells has also been suggested by instructive data from animal models [45], in which a lupus-like disease developed when autoimmune-prone mice were reconstituted with B cells that lacked the ability to secrete Ig but not when they were deprived of B cells completely. These studies provided data on the possible role of antigen presentation by B cells and its pathogenic relevance.

Antigen presentation by B cells, in particular by memory B cells, may be important in the amplification and maintenance of autoimmunity after it has been initiated. Memory B-cell subsets in SLE [46] provide a sensitive pool of cells that immediately react to various stimuli, such as Toll-like receptor (TLR) ligands, IL-21, BAFF, IL-10, BCR activation, or co-stimulatory signals, resulting in the production of Ig-secreting cells. Although SLE memory B cells are mainly Ig-class-switched and show an activated phenotype, it remains to be determined whether these cells are also effective antigen-presenting cells. However, while the impact of individual stimuli is still a matter of debate, the decrease of IgM memory B cells [47] could represent the result of continuous memory B-cell activation by elevated BAFF, IL-21, or IL-10 levels [48-51].

Although B cells produce pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and IL-6, and immunoregulatory cytokines, such as IL-10, the immunologic relevance of B cell-derived cytokines is less clear. Furthermore, convincing evidence for a substantial production of interferon-alpha (IFN α), an important cytokine in SLE [52], by B cells has not been provided. However, it has been shown that IFN α is produced by tissue-resident plasmacytoid dendritic cells, which are reduced in the blood of SLE patients and may ultimately contribute to B-cell hyperactivity, especially in lupus nephritis. In this context, a direct effect of IFN α on B cells is less likely, but IFN α can induce BAFF/BLyS production of myeloid cells, which can result in enhanced B-cell survival and potentially activation [53]. BAFF/BLyS or alternatively APRIL (a proliferation-inducing ligand), which belong to the TNF ligand family, increase survival of most B-cell subsets as well as plasma cells. Enhanced BAFF/BLyS levels could contribute to prolonged survival of autoreactive cells, which otherwise would be deleted. BAFF/BLyS and APRIL, therefore, may lead to a vicious cycle of continuous antibody-mediated inflammation and tissue destruction. Immune cells of mesenchymal origin produce functional BAFF/BLyS in response to pro-inflammatory cytokines (TNF α and IFN γ). Although BAFF transgenic mice [54] develop autoimmunity with an SLE/Sjögren phenotype, blockade of this cytokine in human SLE so far has not shown striking clinical effect but induced changes in Ig and B-cell levels. The regulatory control of BAFF production by IFN γ and TNF α

[53,55] in myeloid cells suggests that BAFF may act as a kind of secondary cytokine translating mainly IFN and TNF effects.

An important immunoregulatory cytokine produced by B cells is IL-10, which is able to activate dendritic cells to be more effective antigen-presenting cells and, with the help of T cells, to enhance the differentiation of memory B cells into plasma cells in the presence of IL-2 and which may likely be an important factor of the immunoregulatory function of B cells [56]. Recently, it was suggested that triggering of TLRs can induce IL-10 production, which appears to play a role in protection from chronic inflammatory diseases. Therefore, immunoregulatory effects of B cells may result from their IL-10 production in autoimmune diseases. Such a regulatory function would have important implications for B-cell depletion therapies, which so far have not been substantiated by clinical trials. Whether low IL-10 levels after B-cell depletion ultimately lead to higher IFN γ and TNF α expression, translating into high BAFF levels, remains open.

With regard to the expression of co-stimulatory ligands involved in plasma cell and memory B-cell differentiation, it has been shown that T and B cells from SLE blood spontaneously express CD154, which is an indicator that GCs in this disease abnormally release activated lymphocytes into the periphery and implies the presence of overactive GCs [57] or insufficient control mechanisms allowing an egress of premature memory B cells. Similarly, CD4⁺ and CD8⁺ T cells from lupus patients show enhanced ICOS expression, whereas memory B cells downregulate ICOS-L, likely as a result of continuous interaction with T cells [58]. Since these interactions of the CD28 family are important for memory B-cell and plasma cell generation but not for the formation of GCs, they are consistent with the conclusion that there is overactivated adaptive immunity in SLE and that this represents an important therapeutic target. All of these disturbances of B-cell subsets in adults and children with lupus with a predominance of memory B cells may also contribute to the increases of plasmablasts during active lupus [21], which could be sufficiently blocked by anti-CD154 therapy [57]. Unfortunately, this therapy [59] had severe side effects that stopped the trials.

The role of the type I cytokine, IL-21, in the pathogenesis of SLE has been suggested by a number of recent findings. First, findings in both humans and mice have indicated an essential role for this cytokine in co-stimulating B cells to differentiate into plasma cells [29]. IL-21R^{-/-} mice have a diminished capacity to produce IgG1 in response to immunization, whereas IL-21 transgenic mice develop hypergammaglobulinemia [60]. Overexpression of IL-21 is found in the BXSB_{yaa} and the sanroche murine models of SLE [61]. Finally, blocking of IL-21 activity successfully treats the manifestations of lupus in the MRL mouse, whereas crossing the BXSB_{yaa} mouse with the IL-21R^{-/-} mouse prevents all

manifestations of SLE [62]. IL-21 is produced by CXCR5⁺ follicular helper T cells, which require ICOS stimulation for their generation [61]. These findings link ICOS and IL-21 in a definable pathway required for B-cell stimulation in secondary lymphoid organs and suggest that blocking IL-21 may be effective in human SLE. Formation of ectopic GCs has been identified in the kidneys of patients with SLE, in the salivary glands of patients with Sjögren syndrome, in the thymus in patients with myasthenia gravis, and in the central nervous system of patients with multiple sclerosis [4,58,63]. Although these aggregates of CD20⁺ B cells surrounded by T cells and follicular dendritic cells have been found in only a fraction of patients, it is not known whether their formation is related to disease activity or T cell-dependent or -independent activation or whether they have the full capacity to select antigen-reactive cells and delete autoreactive B cells appropriately as in typical GCs.

Even though ectopic GCs have been linked to local over-activation of autoimmune B cells and plasma cells, conclusive evidence that they are required for the development of autoimmune diseases or are secondary to the deranged internal environment characteristic of these conditions has not been provided. Thus, no firm conclusion on the differences in the nature of classic GC versus ectopic GC in autoimmunity can be drawn since even classic GCs in tonsils from SLE patients were found to be defective in selection against 9G4 B cells as an indicator of anti-dsDNA activity [16].

An area of interest within the last decade has been the role of T cell-independent activation of B cells. While this can occur *in vitro*, ligating one of the receptors for BAFF/BLyS and APRIL, the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) [64-68], in only a subset of memory B cells [69], conclusive evidence concerning the role of this pathway in SLE is lacking.

Another mechanism by which B cells can be activated in the absence of T cells is via TLR activation. TLRs are also known as 'pathogen-associated molecular pattern receptors' or 'pattern recognition receptors' and are expressed by nearly every cell in the body. TLR-7, TLR-8, and TLR-9 are the most important of these with respect to B-cell activation. Bacterial DNA is the natural ligand of TLR-9, and single-stranded RNA is the ligand of TLR-7 and TLR-8. All three receptor-ligand interactions apparently lead to activation of B cells by an NF- κ B (nuclear factor-kappa-B)-dependent mechanism [70,71]. So far, the most convincing data available for human SLE concern TLR-9 and B-cell activation. As a result of TLR-9 stimulation, B cells can differentiate into (auto)antibody-producing plasma cells or produce pro-inflammatory cytokines [71]. Signaling via TLRs uniquely affects memory B cells [72]. The available data, however, suggest the possibility that memory B cells in patients with SLE could easily be activated via TLR-9 without T-cell help and therefore could account for the expansion in peripheral plasmablasts.

In conclusion, a complex interplay between a constantly over-activated immune system and apparent multiple abnormalities of B-cell development can be assumed in SLE. This permanent overactivation (of whatever cause) might overwhelm all possible check capacities of the immune system. Consistent with that, early check points before antigen or T-cell influences have been identified to be defective [14,15], classic GCs do not select properly [16], and ectopic GCs with a potential lack of selection are found in SLE that may permit the emergence of autoreactive cells.

4. Lessons from immune intervention trials

After therapeutic trials of anti-CD4 therapy in RA [73] and SLE [74] failed to show substantial clinical benefit, questions about the central role of CD4⁺ T cells were raised. Recent success achieved by blocking T-cell co-stimulation with CTLA4Ig (abatacept) by antigen-presenting cells, including B cells, and the effects of blocking CD40/CD154 interactions on autoimmunity [57,59] have again implied a role for the regulatory interaction of immune cells in systemic autoimmune diseases. However, in SLE, the use of anti-CD154 (BG9588) led to some safety concerns with thromboembolic complications [59] in lupus nephritis, although clear immunologic effects were seen. Moreover, blockade of ICOS-L was shown in mice to reduce lupus nephritis [75].

The success of B cell-depleting therapy in ameliorating rheumatoid inflammation and joint destruction has documented a role for B cells in RA but also in other autoimmune diseases, such as idiopathic thrombocytopenic purpura and SLE [76]. Interestingly, a reduction of expressed co-stimulatory molecules such as CD80, CD86, and CD40L on T cells after B-cell depletion was observed in SLE [77], a reduced infiltration of CD68 macrophages was noted in the RA synovium [78], and an increase of regulatory T cells was seen in autoimmune thrombocytopenia [79]. It is clear from these clinical trial results that B cells together with other immune cells play important roles in autoimmunity. However, their role in the induction versus maintenance phase of the disease and the specific contributions of particular B-cell subsets have not been dissected in detail. Although previous data in lupus have shown that B-cell hyperactivity and the resulting autoantibody production are central elements of the immunopathogenesis of SLE, preliminary data of the use of rituximab as an anti-CD20 antibody in non-renal (EXPLORER trial) [80] and renal (LUNAR trial) SLE have reportedly failed the primary endpoints. Although it remains possible that anti-B-cell therapy is not sufficient to suppress lupus activity, a number of other confounding variables of SLE trial design may have substantially contributed (too short trial duration, allowance of substantial glucocorticoid usage, heterogeneity of the patient population, etc). Since a number of additional early trials in SLE, such as the use of abatacept and abetimus, have not provided convincing therapeutic effects, the most likely explanation is that lupus patients are heterogeneous with regard to organ manifestations and patho-

physiology and require customized therapeutic strategies. Patients need to be stratified and characterized in detail prior to the choosing of a certain therapeutic approach. Parameters like the 'interferon signature', BLYS levels, and serologic and clinical findings should be considered and validated regarding their predictive value as biomarkers. In this regard, frequently detectable autoantibodies against DNA are produced by short-lived plasma cells versus anti-extractable nuclear antigens produced by apparently long-lived plasma cells. Given that both produce pathogenic autoantibodies, therapies should target both. Future assessments will be necessary to determine whether B cell-directed therapy can be effective clinically in SLE but also to test the hypothesis that specific B-cell abnormalities are essential for the pathogenesis of this disease. A further explanation of recent trial failure could be that the British Isles Lupus Assessment Group (BILAG) scoring system is less sensitive to changes in the BILAG B, which may preclude a sufficient discrimination of therapeutic success. Overall, the results of recent lupus trials challenge the rheumatology community to improve trial approaches in SLE, a patient population in need of improved therapies.

A different B cell-directed approach is targeting CD22 on B cells; CD22 is uniquely expressed on mature B cells but not on plasma cells or pre-B cells. The humanized anti-CD22 monoclonal antibody epratuzumab causes partial B-cell reduction in the blood, inhibits B-cell proliferation, and probably interferes with intracellular signaling. Preliminary results from an open-label phase IIa study indicate that epratuzumab is efficacious in SLE [81], with BILAG index scores improving by more than 50% in 77% of treated patients at week 6. A central question yet to be answered is how partial inhibition of B-cell activation may affect the clinical risk-benefit ratio as compared with total depletion as mediated by anti-CD20 antibodies. Partial inhibition might mean that B cells can still be activated by certain pathogens in this setting while epratuzumab (anti-CD22) inhibits activation of autoreactive B cells. A preferential reduction of naïve B cells in the peripheral blood under epratuzumab



The Scientific Basis of Rheumatology: A Decade of Progress

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treatment has been seen in clinical study. It has not been resolved whether this was caused by preferential depletion or apoptosis of naïve B cells or by enhanced migration of these cells from the blood into the tissue. *In vitro* data, however, suggested that this agent acts also by blocking proliferation of B cells [46], an effect that has been observed in patients with SLE but not in normal controls [46].

The current data are consistent with the conclusion that B-cell abnormalities in SLE can be targeted by cellular approaches, such as anti-B-cell therapy, but also interventions on key cytokines, such as IFN α , or blocking co-stimulation. A critical question will be to identify a common denominator of B-cell activation as a target that allows sufficient and safe immune intervention.

Competing interests

TD declares associations with the following companies: Roche, Genentech, Immunomedics, UCB. AJ and PL declare that they have no competing interests.

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