B-cell tolerance checkpoint violations in systemic lupus erythematosus

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B cells play a crucial role in the pathogenesis of the prototypic autoimmune disease systemic lupus erythematosus. The presence of autoantibodies in lupus is indicative of a breach in B-cell tolerance to self. Studies conducted over the past 15 years indicate that tolerance checkpoints exist at various stages of B-cell development to ensure that self-reactive B cells are censored and do not produce autoantibodies. These include an early checkpoint operative at the immature B-cell stage in the bone marrow, with receptor editing and deletion as the two main mechanisms. Additional checkpoints exist in the periphery and include receptor revision and anergy at the mature naive stage, with further checkpoints being operative at the germinal center and memory B-cell stages. In a recent study, it has been shown that mice harboring the lupus susceptibility gene \( \text{Sle1}^{bz/Ly108z} \) display defective tolerance both at the immature as well as the mature B-cell stage in the periphery. This, along with other studies conducted both in murine and human lupus, indicates that several key tolerance checkpoints may be violated in lupus B cells. Put together, these have provided a possible explanation for the presence of autoantibodies in lupus. B cells are key components of the adaptive immune system and generate responses to potentially pathogenic foreign antigens. It is also important that the B cells do not respond to self-antigens as this would lead to autoimmune diseases. Numerous mechanisms, mostly developmentally encoded, exist to ensure that this does not happen. Recent literature reports point to the existence of at least two key developmental checkpoints that seem to be operative in preventing the survival of self-reactive B cells. Checkpoint I – operative at the immature B-cell stage, with deletion and receptor editing of self-reactive immature B cells as the two main mechanisms of tolerance induction, and checkpoint II – operative at several successive mature B-cell stages, with numerous contributing mechanisms, such as anergy, cell death and receptor revision. In this review, we will focus on these tolerance checkpoints and mechanisms, highlighting some of the recent advances in the field.

Tolerance checkpoint I

The primary immunoglobulin (Ig) repertoire is a result of random V(D)J rearrangement and this results in the generation of immature B cells with a wide variety of specificities. Our current understanding of how these self-reactive B cells are censored has been enriched manifold by the generation and study of several murine B-cell receptor (BCR) transgenic models [1–3]. The underlying strategy in these studies is to engineer mice that generate high frequencies of self-specific B cells in the preimmune repertoire by transgenic introduction of a prearranged Ig receptor gene. The antigen these transgenic B cells are specific for is either ubiquitously expressed as self-antigens (e.g., DNA), or can be artificially introduced using a second transgene so that it is expressed as a neo-self-antigen. The latter model has particular utility as it allows one to study these transgenic B cells in the presence or absence of the corresponding self-antigen. The advantage of using such BCR Tg mice is that they allow one to follow the development and selection of transgenic B cells in vivo, something that cannot be achieved in mice with a polyclonal B-cell repertoire. Some of the earliest BCR Tg studies revealed that self-reactive immature B cells are effectively tolerized in the bone marrow (BM) through two important mechanisms – deletion through cell death and receptor editing – resulting in a drastic decrease in mature self-reactive B cells in the periphery (4–6).

One of the first BCR Tg models reported was the antihen egg lysozyme (HEL) BCR Tg, developed by Goodnow and colleagues. These mice had an essentially monoclonal repertoire directed against a foreign antigen, HEL (7). HEL was introduced as a surrogate self-antigen through a second transgene. An important lesson from this model was that the tolerance mechanism predominantly depended on the avidity of the BCR to its cognate self-antigen. Whereas clonal deletion of all HEL-reactive B cells was the main mechanism of tolerance when membrane HEL was introduced as the surrogate self-antigen, the same did not hold true when soluble HEL was the self-antigen (4.7).
In the latter model, mature HEL-reactive B cells were present in the periphery, but these were effectively tolerized through anergy [7]. Further studies revealed that immature B-cell deletion in the membranous HEL/anti-HEL double transgenic model occurred in two steps—developmental arrest followed by cell death in 1–3 days [8]. The HEL/anti-HEL BCR Tg model has been particularly useful in delineating the role of several key genes, such as complement, the Bcl2 family of molecules CD19, CD45, Slp1, Lyn, Syk, CD5 and BAFF, in B-cell tolerance induction [8–19]. These studies have shown if and how several of these key signaling molecules impact B-cell tolerance at either the immature B-cell stage, by impacting receptor editing or deletion, or at the mature B-cell stage, by impacting anergy induction. However, the role of many of these molecules in spontaneously arising lupus is yet to be determined.

Clonal deletion of self-reactive B cells was documented in two other BCR Tg models. Whereas Nemazee and colleagues used a BCR Tg directed against MHC-I, Weigert and colleagues used an anti-DNA BCR Tg; but both concluded that developmental arrest or deletion of self-reactive B cells were important mechanisms of B-cell tolerance induction [5,6]. In addition, these models were also important in helping uncover the important phenomenon of receptor editing amongst immature B cells in the BM [20–22]. These studies revealed that potentially DNA-reactive immature B cells had the capacity to re-express recombinase activated gene (RAG) products so that the potentially auto-reactive heavy chain (HC) could pair up with alternative endogenous light chains (LCs) to avoid self-reactivity. In vitro, BCR ligation of immature B cells also results in similar upregulation of RAG, with subsequent receptor editing or cell death, confirming that these processes are the result of BCR ligation by self-antigens [23]. In fact, receptor editing is common even in non-Ig-Tg mice with a diverse BCR repertoire [24].

Recent studies using the 3–83 anti-MHC-I BCR Tg model have suggested that receptor editing, and not clonal deletion, is the main mechanism of tolerance induction amongst immature B cells, with clonal deletion occurring only when receptor editing is artificially inhibited by exhausting all available secondary recombination avenues [25]. In fact, these conclusions have also been extended to the anti-HEL BCR Tg model in which it was previously thought that clonal deletion was the main mechanism of tolerance [26]. Another BCR Tg mouse model in which the mouse κ constant region was replaced with a human counterpart also revealed that approximately 25% of the generated antibody repertoire is the result of gene replacement, further bolstering the importance of receptor editing in shaping the primary Ig repertoire [27].

On the other hand, receptor editing can be a double-edged sword, as it can potentially lead to the generation of immature B cells that express both a nonautoreactive BCR (i.e., receptor-edited) and the primary autoreactive BCR (i.e., nonedited allele), as has been shown to occur in the anti-DNA 56R BCR Tg model as well as the 3–83 anti-MHC BCR Tg model [28–30]. In support of these studies is another recent report in which mice expressing two BCRs were generated, one being autoreactive and the other non-autoactive [31]. In these mice, even though receptor editing was attempted at the locus with the autoreactive BCR in an effort to escape negative selection, it nevertheless led to the development of self-reactive mature B cells. These reports have suggested that dual specificity BCR-expressing B cells could potentially be responsible for autoantibody production. The signaling pathways involved in receptor editing are only now being understood. A recent report has identified NFκB/Rel transcription factors to be important regulators of RAG activity [32].

These BCR Tg models have also been useful in identifying B-cell tolerance breaches in lupus-prone genetic backgrounds. Probably the most well-studied of these is the MRL/lpr lupus strain onto which several BCR Tg models have been bred. However, the MRL/lpr lupus-prone background did not have any effect on central tolerance in the anti-HEL or 3–83 anti-MHC BCR Tg models [33,34]. A recent study indicates that mice bearing the NZW-derived Sle1b/lupus predisposing allele display immature B cells with abberant apoptosis and RAG re-expression following BCR ligation [35]. The candidate gene Ly108b within the Sle1b region was found to be responsible for these phenotypes. Importantly, the normal Ly108b allele sensitized immature B cells to cell death and RAG re-expression. Placed in the context of earlier findings, one can envision a scenario in which lupus-predisposing genes such as Ly108b may act to decrease cell death and receptor editing among immature B cells that have rearranged a self-reactive BCR. Persistence and subsequent maturation of such self-reactive B cells can produce autoantibodies in such lupus-prone mice.
Importantly, studies on B-cell tolerance checkpoints in humans have corroborated the above findings in mice. Nussensweig and colleagues demonstrated that, whereas approximately 80% of all antibodies cloned from 'normal' human immature B cells displayed high reactivity to various autoantigens, with the majority being nuclear antigen reactive, these numbers dropped to only 20% among mature B cells [36]. This seminal study established that developing B cells, even in normal humans, are largely self-reactive and that the immature B-cell stage represents the very first stage at which these B cells may be censored. Although this study did not explore the censoring mechanisms underlying this checkpoint, extrapolating from the murine studies one could anticipate receptor editing and deletion to be two key tolerance mechanisms in human B cells as well. This has been supported by another study that showed LC-receptor editing, especially the use of Ig\(\gamma\), as an important mechanism to silence autoreactivity in human B cells [37].

Recent studies have extended these findings to human systemic lupus erythematosus (SLE). In one study based on three adolescent untreated SLE patients, Nussensweig and colleagues found that approximately 41–50% of the antibodies cloned from peripheral mature naive B cells exhibited Hep-2 autonuclear antibodies (ANA) ELISA reactivity, as opposed to only 20% in normal controls [38]. Importantly, there was no difference in the proportion of ANA-reactivity amongst antibodies cloned from new emigrant B cells in SLE patients or healthy controls, suggesting that the tolerance checkpoint that marks the transition from new emigrant immature B cells to mature naive B cells may be aberrant in human SLE. Interestingly enough, another report examining the B-cell repertoire of SLE patients in clinical remission has found that even these patients possess high levels of autoantibody-encoding B cells in the naive mature B-cell compartment, albeit in lower numbers than that seen in patients with active disease [39]. These findings led the investigators to conclude that early B-cell tolerance checkpoint abnormalities are an integral part of the disease and cannot be altered by clinical treatment. Similar findings were also observed amongst untreated rheumatoid arthritis (RA) patients, wherein autoreactive B cells accounted for nearly 50% of the naive mature B-cell compartment, again indicating that self-reactive immature B cells are being allowed to mature in autoimmune diseases such as RA and SLE [40].

However, central tolerance is not a foolproof mechanism and can fail even in normal individuals. A subset of normal human B cells have been reported to coexpress surrogate and conventional LCs with an unusual antibody repertoire, with clear evidence of receptor editing [41]. In spite of this, these circulating peripheral B cells displayed a high degree of autoreactivity, suggesting that these B cells have escaped central tolerance mechanisms. Perhaps as a failsafe measure to deal with autoreactive new-emigrant and naive mature B cells that have leaked past the central checkpoint, further checkpoints exist in the periphery to police any such 'escapee' B cells.

**Tolerance checkpoint II**

The mere presence of mature self-reactive B cells in the periphery of normal humans alludes to the need for additional peripheral checkpoints that must be in place to thwart these B cells from generating autoimmune responses [36]. Studies have indicated that these peripheral tolerance checkpoints may be operative at three or more stages of B-cell development – mature naive, germinal center (GC) and memory B cells. Key tolerance mechanisms operative at the naive mature B-cell stage in the periphery include anergy and receptor revision. Anergy or cellular inactivation was first described by Goodnow and colleagues in the anti-HEL BCR Tg mouse model expressing low levels of surrogate self-antigen in the form of soluble HEL [7]. Although self-reactive (HEL-reactive) B cells did mature and reach the periphery, such B cells were effectively tolerized in that they did not generate responses to the surrogate self-antigen HEL and were also inhibited from entering follicles [42]. This process of anergy has also been described in the other BCR Tg models, including anti-Ars/A1, anti-DNA, anti-MHC-I, rheumatoid factor (RF) and anti-Sm BCR Tg models [5–7,43–45].

Three important mechanisms seem to contribute to anergy induction – reduced lifespan, downregulation of soluble (s)IgM and damped BCR signaling as a result of repeated stimulation of the BCR by its cognate antigen [46]. Anergic B cells can also be identified in non-BCR Tg mice and constitute approximately 5–8% of the peripheral B-cell population [47]. Many molecules seem to contribute to anergy induction and maintenance, including CD19, CD45, Shp1, Lyn, Syk, CD5 and B-cell survival factor (BAFF) [8–19]. BAFF appears to be particularly important in maintaining peripheral B-cell tolerance, as self-reactive
B cells in BAFF transgenic anti-HEL BCR Tg mice were no longer anergic [19]. It has been reported that mice bearing the NZW-derived Sle1β lupus susceptibility allele were defective in this process and produced antibodies to the surrogate self-antigen HEL in the anti-HEL BCR Tg model [55]. Also, such Sle1β bearing B cells from the double transgenic model maintained responses to BCR crosslinking in vitro, a feature not observed in anergic B cells from normal anti-HEL/HEL double transgenic mice, a further indication that Ly108β-bearing B cells are not functionally inactivated following repeated self-antigen exposure. Lupus-prone NZB mice bearing the anti-HEL/soluble HEL transgenes also revealed B cells that displayed aberrant survival, proliferation and generation of anti-HEL antibody-producing cells, suggestive of aberrant B-cell tolerance in the NZB background. However, analyses of lupus-prone BWF1 mice bearing the anti-DNA 3H9 Tg, revealed that B cells from such mice displayed normal tolerance to DNA in that they were adequately anergized [48]. It is presently unclear if the observed differences in the above models relate to differences in strain background or the antigen system used as a model.

The second process, termed receptor revision, is a process occurring in mature peripheral B cells, wherein these cells upregulate RAG and undergo secondary V(D)J recombination [49,50]. This process is quite different from the receptor editing that occurs amongst immature B cells in the BM in that receptor revision can be initiated in vitro by a combination of lipopolysaccharide and IL-4, and BCR ligation appears to inhibit this process [51]. Receptor revision involves both LC and HC replacement [50]. A subset of B cells termed B-1 cells are more prone to autoantibody production in some models, and this subset exhibits more RAG expression and receptor revision compared with conventional B-2 cells, suggesting that inhibition of receptor revision, rather than its promotion, may be an important tolerance maintenance mechanism [52]. In support of this conclusion, Weigert and colleagues found that in a chronic graft-versus-host disease model of lupus in anti-DNA BCR Tg mice, incomplete LC editing as a consequence of receptor revision led to polyreactivity towards a variety of additional self-antigens [29]. Recent studies in humans have also indicated that VH replacement products occur quite commonly in patients with SLE, Sjogrens syndrome and RA, suggesting that VH replacement could be associated with autoreactivity [53]. Another critical checkpoint in the periphery occurs in the GC [54]. The GC is the location where follicular B cells, along with appropriate T-cell help, generate responses to pathogens, becoming either memory or plasma B cells. A recent study examining the in vivo contribution of T cells in overcoming B-cell tolerance suggests that although anti-DNA specific B cells develop in 56R SCID mice, these mice develop IgG anti-DNA autoantibodies only in the presence of exogenously added T cells [55]. This study alludes to the importance of appropriate T-cell help in the development of autoimmunity. Following the development of self-reactive B cells, mounting evidence now indicates that the inappropriate selection of self-reactive B cells into the GC is a feature associated with SLE. VH4-34 HC variable region gene-encoded antibodies (VH4-34 antibodies), are intrinsically autoreactive even in normal individuals, without requiring somatic mutation and independent of the associated LCs, suggesting that such autoreactive B cells need to be tightly regulated to prevent autoantibody production [56]. These antibodies belong to a class of naturally occurring IgM antibodies secreted by the CD5+ B-1 cells. Importantly, although in normal individuals only 0.5% of total serum antibodies are VH4-34 encoded, these levels are increased in SLE patients. Sanz and colleagues found that such VH4-34 (9G4) B cells were under-represented in the memory and GC compartments in normal individuals, but present in large numbers in the naive B-cell compartment [57]. In addition, 9G4 B cells in normal individuals displayed anergic phenotypes, including reduced calcium flux on BCR crosslinking [58]. By contrast, the VH4-34 B cells were positively selected into the GC and plasma cells in SLE patients, suggesting that entry into the GC is an important checkpoint in preventing autoimmunity [58]. Autoreactive B cells that escape negative selection at this peripheral checkpoint can potentially enter the long-lived GC and memory B-cell pool, leading to autoantibody production. FcγRIIB seems to play an important role in the selection and maintenance of self-reactive GC B cells and plasma cells, as anti-DNA 56R mice lacking this molecule displayed excessive anti-DNA autoantibodies with an accumulation of DNA-reactive IgG+ B cells with a plasma-cell phenotype [59]. In line with these findings, partial restoration of inhibitory Fc receptor (FcγRIIB) levels on B cells in lupus-prone mice is sufficient to restore tolerance and prevent autoimmunity [60]. Moreover, it has been recently
demonstrated that FcγRIIB is frequently downregulated in memory B cells in human SLE [61]. Recent studies also indicate that Toll-like receptors (TLRs), especially TLR9 and -7 appear to play important roles in promoting autoimmune [62–65], although their impact on various tolerance checkpoints and mechanisms remain to be evaluated.

Studies using the RF-specific BCR Tg model have indicated that autoreactive B cells formed GCs in both autoimmune and normal mouse backgrounds, but became antibody forming cells (AFCs) only in autoimmune mice [66]. Although the RF+ B cells in nonautoimmune mice also underwent somatic hypermutation, the patterns were quite different from those seen in autoimmune mice, revealing a failure to select and expand potentially pathogenic B-cell clones in nonautoimmune mice. This would then suggest that an additional checkpoint may exist within the GC, wherein mere selection of self-reactive B-cells into the GC does not entail autoimmunity. Further activation of such autoreactive B cells within the GC may require additional cosignaling to convert these into AFCs, in the context of an autoimmune prone genome.

More recently, the existence of a novel tolerance checkpoint has been suggested in the normal human IgM+ memory B-cell compartment [67]. At this checkpoint, IgM+ B cells that display both self-reactive and broadly bacterial reactive profiles were removed from the repertoire in the transition from naive to memory B cells, leaving behind only the innocuous B cells that were reactive solely to pathogens. However, similar studies of IgG+ memory B cells revealed that these B cells frequently continued to express self-reactive antibodies; hence it has been suggested that dysregulation of this particular peripheral checkpoint amongst IgG+ B cells may be associated with autoimmunity [68]. In concordance with these findings a recent report analyzing the role of activation-induced deaminase in the MRL/lpr murine lupus model also identifies the importance of class-switched hypermutated autoreactive IgG and not IgM in the development of fatal kidney disease [69]. To summarize, these studies underscore the importance of the IgG class of autoantibodies in the development of autoimmune disease.

Conclusions & future perspective

At least two key tolerance checkpoints exist to ensure that self-reactive B cells do not contribute to the serum antibody pool – an early checkpoint at the immature B-cell stage and peripheral checkpoints operative at the mature naive, GC and memory B-cell stages. Although there are several examples from engineered mouse models of genes that could potentially breach B-cell tolerance [8–19], the genes and molecules that infringe B-cell tolerance in spontaneously arising lupus is only now becoming clear. Repertoire-monitoring studies in human SLE indicate that B-cell tolerance may be breached at the new emigrant and mature naive checkpoints. The very first clues of how lupus susceptibility genes might impact these different tolerance checkpoints have recently emerged. One such study aimed at understanding the role of the NZW-derived Sle1Bz/Ly108z lupus susceptibility gene in B-cell tolerance and demonstrated specifically that this lupus gene promoted a breach in B-cell tolerance, both at the immature B-cell and mature stages, by hampering receptor editing, cell death and anergy; three key tolerance processes. The exact mechanism through which Ly108z fine tunes this tolerance threshold warrants further study. The full complement of genes that are operative at each tolerance checkpoint awaits elucidation.

Studies over the next several years are likely to extend our perspective on which lupus susceptibility genes break B-cell tolerance, and also illuminate the specific molecular mechanisms that may be responsible.

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Executive summary

- At least two key tolerance checkpoints exist to ensure that self-reactive B cells do not contribute to the serum antibody pool – an early checkpoint at the immature B-cell stage and peripheral checkpoints operative at the mature naive, germinal center and memory B-cell stages.

- Recent studies in murine and human lupus indicate that several key tolerance checkpoints may be violated in lupus B cells.

- Future studies should be aimed at deciphering the key signaling pathways that are triggered in B cells at each successive tolerance checkpoint, as this will augment our understanding of the molecular basis of this disease.
One of the first reports, along with references 4 and 5, to demonstrate the role of central deletion of self-reactive B cells in the maintenance of B-cell tolerance.


• First report to demonstrate the role of anergy in the maintenance of B-cell tolerance.


• Identifies the role of B-cell survival factor (BAFF) in peripheral B-cell tolerance through anergy induction.


• First report, along with references 21 and 22, to identify the role of receptor editing in B-cell tolerance maintenance.


• First report, along with references 20 and 22, to identify the role of receptor editing in B-cell tolerance maintenance.


• First report, along with references 20 and 21, to identify the role of receptor editing in B-cell tolerance maintenance.


• Identifies the role of receptor editing in shaping the peripheral antibody pool.


B-cell tolerance checkpoint violations in systemic lupus erythematosus – REVIEW


65. Gaither TL, Nickerson K et al.: Toll-like receptor 7 and -9 in the MRL/lpr model of murine lupus.


- Interesting report identifying that antibody forming cells is an important B-cell tolerance checkpoint.


- Suggests that IgM+ memory B cells are not sufficient for development of autoreactivity.


- Suggests that IgG+ memory B cells are necessary for the development of autoreactivity.


- Interesting report identifying the role of IgG, and not IgM, in the development of fatal autoimmune disease.

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