# Balancing diversity and tolerance: lessons from patients with systemic lupus erythematosus

Annett M. Jacobi and Betty Diamond

**The autoimmune disease systemic lupus erythematosus (SLE) is caused by a failure of B cell tolerance. Recent studies in mouse models of SLE have identified several distinct tolerance checkpoints that must each function appropriately to protect against disease. However, studies of B cell repertoire selection in humans are essential to understand which checkpoints are defective in human autoimmune diseases.**

The major challenge of the immune system is to defend the host against microbial pathogens while protecting itself from its own arsenal. Meeting this challenge requires down-regulation of autoreactivity. For B cells, autoreactivity is generated at two stages during differentiation—first in the bone marrow and again in the germinal center response.

Whereas hyperactivity of B cells and increased antibody production in SLE have been extensively described, the mechanisms underlying the failure of tolerance in SLE remain uncertain. Mouse models have taught us two critical lessons with regard to B cell selection. First, there are many tolerance checkpoints during B cell maturation. Second, autoreactive B cells that escape tolerance may mature to be autoantibody-secreting cells with the phenotypic characteristics of any B cell subset: B1, marginal zone (MZ) B cells, short-lived plasma cells, or germinal center–matured long-lived plasma cells. Thus, one important implication from mouse studies is that we should anticipate extensive heterogeneity in patients with lupus, with respect to both defects in B cell tolerance and the differentiation state of the autoreactive B cells. Here we will discuss recent insights into the stages in development at which B cell autoreactivity can emerge, and new data that provide clues to the stages at which B cell tolerance breaks down in patients.

A.M.J. is at Department of Rheumatology and Clinical Immunology, Charite University Hospital, 10117 Berlin, Germany.
B.D. is at Departments of Medicine and Microbiology, Columbia University Medical Center New York, NY 10032.

CORRESPONDENCE
B.D.: bd2137@columbia.edu

## Checkpoints during B cell development

During B cell development a delicate balance of proliferation and apoptosis is required to shape a highly diversified antibody repertoire. The B cell receptor (BCR) is the central regulator of selection processes. Normally, inappropriate tonic signaling or cross-linking of the BCR by antigen results in receptor editing. This process involves the rearrangement of a new light chain gene to generate an antibody with a new antigenic specificity or clonal deletion of immature B cells in the bone marrow. Although there is evidence that receptor editing also occurs in SLE patients, whether it functions to eliminate autoreactivity appropriately is not known.

To develop from the immature state in the bone marrow to the mature naive state in the peripheral lymphoid organs, a B cell must survive three checkpoints (Fig. 1). The first checkpoint is between the immature cell in the bone marrow and the transitional T1 cell in the spleen. The second is between the T1 and more mature T2/3 state, and the third is between the T2/T3 stage and mature MZ and B1 and follicular B cells. Negative selection at each of these checkpoints is mediated by BCR signaling and is generally considered to be a B cell intrinsic property, although extrinsic factors like the female sex hormone estradiol can diminish the BCR signal and thereby diminish negative selection (1).

T2/T3 cells can be rescued from negative selection by costimulatory signals. CD40 engagement by CD40 ligand (CD40L) can rescue B cells destined to undergo BCR-mediated apoptosis. Several reports suggest that costimulatory molecules are overexpressed or dysregulated in SLE, which might explain the survival of autoreactive transitional B cells (2, 3). The hormone prolactin, which is elevated in ~25% of lupus patients, causes an increase in the number of autoreactive follicular B cells. This increase is T cell dependent and appears to be related to enhanced CD40–CD40L interactions (4). B cell activating factor of the tumor necrosis factor family (BAFF) can also enhance the survival of autoreactive transitional B cells in mice (5). As BAFF is found in high concentrations in sera of patients with SLE, it may contribute to defective B cell tolerance in these patients (6).

## Checkpoints involving mature B cell subsets

There are three important populations of mature immunocompetent B cells: B1, MZ, and follicular B cells. Understanding B cell defects in SLE may well require identifying which B cell subset is responsible for autoantibody production, as mouse models have clearly shown that all three subsets can produce pathogenic autoantibodies.

### B1 cells

B1 cells express CD5, are restricted in BCR diversity, and fail to generate a memory population. In nonautoimmune mice, autoreactive B cells bearing low-affinity BCRs usually home to the peritoneal cavity as B1 cells. The autoantibodies made by these cells are

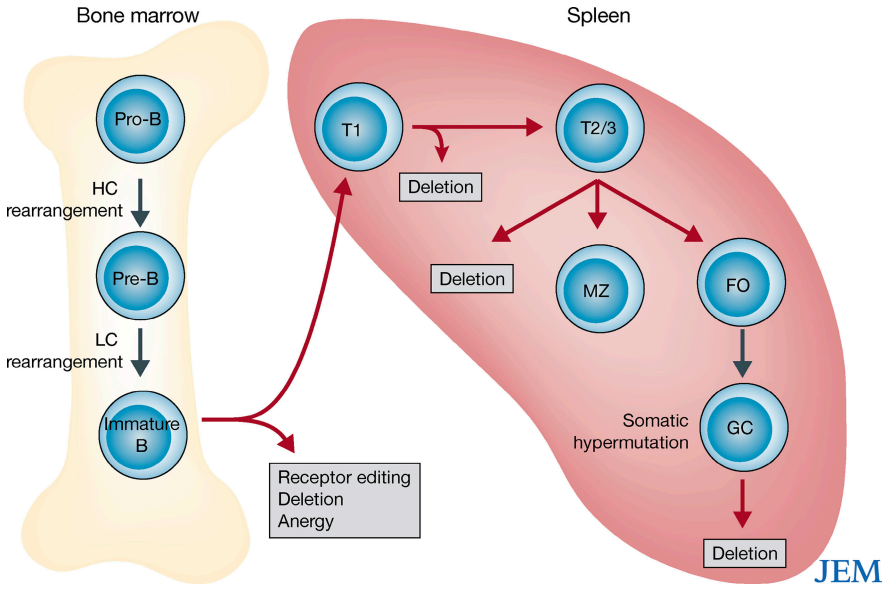


**Figure 1. Negative selection checkpoints in B cell development.** There are two points of BCR diversification—initial receptor rearrangement in the bone marrow and somatic hypermutation during the germinal center reaction. Each is followed by negative selection of autoreactive cells. Negative selection, receptor editing, anergy, or deletion occur in immature and transitional B cells after BCR engagement. The mechanisms for negative selection of autoreactive cells in GCs are not known. HC, heavy chain; LC, light chain.

thought to help avoid pathogenic autoreactivity by assisting in the clearance of apoptotic cells (7). In female, phytoestrogen-treated, lupus-prone BWF1 mice, B1 cells produce pathogenic autoantibodies (8). Likewise, in the moth-eaten mice, which are deficient in the inhibitory phosphatase SHP-1, high-affinity IgM antibodies that recognize double-stranded (ds)DNA are produced by the B1 cell population (9).

A comparable human B cell subpopulation also expresses CD5. Human $CD5^+$ B cells produce polyreactive, low-affinity IgM antibodies using germline-encoded V genes. In SLE, anti-DNA antibodies may be produced by both $CD5^+$ and $CD5^-$ B cells. $CD5^+$ B cells may not be the precursors of the $CD5^-$ antibody-secreting cells, which produce high-affinity, immunoglobulin (Ig) class-switched anti-DNA antibodies. Human $CD5^+$ B cells can be induced to differentiate into cells with features of germinal center (GC) cells (10) and autoreactive $CD5^+$ B cells carrying somatically mutated V genes have been described in rheumatoid arthritis (11), suggesting that there might be circumstances under which $CD5^+$ B cells enter GCs or undergo ectopic somatic mutation. These findings may indicate that impaired regulation of polyreactive $CD5^+$ B cells, or a B cell abnormality that begins as far back as the generation of the B1 cell repertoire, may be involved in the development of SLE. One caveat is that it is not clear if $CD5^+$ cells in human are the same as B1 cells in mice or whether they might also represent post-GC anergic B cells.

Autoantibody secretion by $CD5^+$ B cells is normally observed as a transient event that accompanies certain infections. Cold agglutinins are pathogenic IgM antilymphocyte antibodies that use *VH4-34* rearrangements in the germline configuration and are made by a $CD5^+$ population. If B cell tolerance is intact, *VH4-34*–expressing, $CD5^+$ B cells are present in the naive repertoire but are excluded from GC reactions. In SLE patients, however, *VH4-34*–expressing B cells enter GCs and contribute to the memory B cell pool (12). This observation suggests that the barrier which keeps autoreactive $CD5^+$ cells from entering the GC is an essential checkpoint in B cell development and that, for unknown reasons, this barrier may be broken in SLE.

### Marginal zone B cells

Some anti-DNA antibodies are produced by MZ B cells in NZB/WF1 mice (13) and in other mouse models of lupus (1). MZ B cell development depends on BAFF, and these cells are easily activated by dendritic cells and mature rapidly into plasmablasts (14). MZ B cells also act as antigen-presenting cells (APCs) and activate T cells, as they express costimulatory molecules on their surface (15). Recent studies in lupus-prone mice suggest that some B cells, possibly MZ B cells, are able to generate T cell–independent autoimmune responses and can undergo heavy chain class-switching and somatic mutation in extrafollicular regions of the spleen (16). MZ B cells have been shown to initiate GC formation (14). But what regulates their differentiation to either a GC or an extrafollicular focus of antibody production is not known. In humans, MZ B cells appear to be present in the circulation as $CD1c^+IgD^{low}IgM^+CD27^+$ cells (17) and can populate all secondary lymphoid organs. It remains to be elucidated to what extent MZ B cells contribute to the secretion of pathogenic autoantibodies in human SLE; however, these highly flexible actors have all the characteristics required to break T cell tolerance.

### Germinal center cells

The GC is another critical checkpoint in B development. After antigen encounter and T cell help, follicular B cells generate a GC to mount an affinity-matured antibody response and generate memory B cells. During somatic hypermutation and Ig class-switching, a stringent but poorly understood balance of proliferative and apoptotic signals is required to guarantee the expansion of B cells with high affinity to foreign antigens and prevent the survival of autoreactive B cells. During immune responses to foreign antigens in mice, as many as 40% of the GC B cells display potentially pathogenic autoreactivity (18). Since many

autoantibodies in SLE display extensive somatic mutation, there is a strong presumption of a defect in negative selection of GC-matured B cells. GC cells surviving negative selection enter the pool of long-lived memory or plasma cells, which is relatively refractory to many immunosuppressive therapies. Very little is understood about the process of negative selection in GCs, although there is some new evidence suggesting that receptor editing may also function at this point to eliminate autoreactivity (19). The Fc receptor Fcγ2RIIB may also mediate an important inhibitory pathway at this stage of B cell differentiation, as immune complexes may be critical in regulating B cell survival within the GC. Lack of expression of Fcγ2RIIB causes an accumulation of plasma cells secreting anti-DNA antibodies in mice (20), and polymorphisms of *Fcγ2RIIB* have been associated with autoimmunity in mice and man. It is also possible that the presence of autoreactive T cells in SLE may serve to mediate positive selection of autoreactive GC B cells.

### New insights from patient studies

Nussenzweig and colleagues have previously reported that more than half of all newly generated immature B cells in healthy individuals appear to be polyreactive and capable of binding self-antigen (21). However, only a small percentage of immature/transitional B cells survive the process of ligand-dependent selection and enter the pool of mature naive B cells.

In a recent issue of the *JEM*, the same group described the B cell repertoire at the transitional and naive stage in three newly diagnosed, untreated patients with SLE (22). In two patients, they did not find the anticipated diminution in the frequency of autoreactive B cells as the cells matured from the transitional to the naive stage. In the third patient, this checkpoint appeared to be partly intact. However, this patient had an higher frequency of self-reactive B cells among new B cell emigrants, consistent with a defect in early B cell tolerance in the bone marrow. All three patients displayed an increased frequency of autoreactive cells in the repertoire of immunocompetent naive B cells. Although the demonstration of a failure to eliminate autoreactive B cells from the bone marrow or the transitional compartment is important, it does not allow the precise identification of the tolerance defect. Furthermore, it remains to be investigated whether this failure is B cell intrinsic or a consequence of a factor extrinsic to the B cell itself that either modulates the strength of BCR signaling or engages a rescue pathway.

The definition of autoreactivity in this paper (22) is the binding of serum antibodies to a lysate of Hep 2 cells in an ELISA. The antigens are, therefore, both nuclear and cytoplasmic. When the autoreactive antibodies were studied in detail, almost all were found to be polyreactive and to bind multiple self-antigens including single-stranded (ss)DNA and insulin, but rarely to dsDNA, which is the characteristic autoantigen in SLE. Finally, the study examined V gene usage in autoantibodies and found it to be heterogeneous, suggesting a polyclonal response to antigen rather than the proliferation of a single dysregulated B cell clone. Because some studies have demonstrated long heavy chain CDR3 regions in lupus autoantibodies and an increased frequency of arginine residues in anti-DNA antibodies, the authors examined CDR3 regions in their panel of autoantibodies. They found these features to be quite variable. Samuels et al. have described a similar defect in negative selection of B cells in patients with rheumatoid arthritis (RA; reference 23). It will be important to learn at which state polyreactivity is lost, and, for example, when anti-insulin antibodies disappear from the repertoire of patients with either SLE or RA. It is possible in SLE that the naive B cells that are preferentially activated are those which bind RNA or DNA. For these cells, encounter with antigen would activate BCR signaling and Toll-like receptors—innate pathogen receptors that may also recognize self-DNA and RNA—and lead to cellular activation (24). RNA- and DNA-binding cells may, therefore, undergo positive selection and be rescued from deletion, whereas B cells binding self-antigens that do not trigger Toll-like receptors may be neither rescued nor activated. What mediates positive selection in RA is less clear.

It is not yet clear whether the polyreactive autoreactive B cells that are present at increased frequency in patients with SLE and RA reflect a defect in negative selection that correlates with the development of disease or whether they represent the precursors of the B cells that produce pathogenic autoantibodies. There is little to guide us in addressing this question, except the important observation that both murine and human antibodies to dsDNA in SLE can be derived from an antibody that in the germline configuration had no specificity for DNA and no apparent autoreactivity (25). This observation recalls the Jerne hypothesis that all germline antibodies are inherently autoreactive and mutation leads away from autoreactivity. The opposite hypothesis has also been posed, and the answer may be that both hypotheses are correct. It is highly likely that some pathogenic autoantibodies arise from naive B cells that are autoreactive and others do not.

### Future questions

Studies of B cells in human SLE are still in their infancy. There is already evidence that more than one defect in selection contributes to an increased frequency of autoreactive B cells. It is likely that the study of more patients will reveal an even wider array of regulatory defects. In fact, it is likely that lupus patients will be at least as heterogeneous as mouse models of SLE, and that further study will reveal both genetically determined and extrinsically induced alterations in the B cell repertoire. Of note, 10–20 percent of individuals receiving interferon therapy or TNF blockade produce lupus-like antibodies and some develop symptomatic lupus (26), suggesting that there can be externally induced changes in the homeostatic mechanisms that regulate B cell repertoire.

There are many unanswered questions regarding lupus autoantibodies. One question, for example, relates to the differential regulation of autoantibodies with different antigenic specificities. In SLE, the dominant autospecificities are to nuclear antigens. Antibodies

to dsDNA play a major role in disease, since they are found in many affected tissues and can clearly contribute to kidney disease. Their titer usually correlates with disease activity and can be used to monitor patients during immunosuppressive therapy. However, some patients maintain high titers of anti-dsDNA antibodies, despite immunosuppressive therapy. In addition, there are other autoantibodies characteristic of SLE, such as antiphospholipid antibodies or antibodies to ribonucleoproteins, that do not display fluctuating titers regulated either by disease activity or by immunosuppressive therapy. This clinical observation cannot be explained by current knowledge of B cell selection and activation. It seems clear that we will need additional studies that focus on particular autospecificities to fully understand B cell dysregulation in SLE. Furthermore, determining where and why the spectrum of autoantigenic specificities in SLE and RA, and perhaps other diseases characterized by autoantibody production, diverge will be a challenge for future studies.

Studies of GC selection are, likewise, crucial but are difficult to perform, as it is difficult to obtain access to the necessary human lymphoid tissue. Yet, there are many hints in the literature that a critical defect in lupus occurs during GC B cell selection. We need to learn whether B cell selection in individual lupus patients is aberrant only in the immature or naive repertoire, only in the GC repertoire, or whether defects in selection always affect both the pre- and post-GC B cell repertoire. More studies of B cell selection, combined with molecular analyses of BCR signaling pathways, may help phenotype SLE patients in ways that will permit an understanding of the heterogeneity of the disease. A description of the clinical phenotypic features that associate with each regulatory defect will lead us toward patient-specific therapies that may eliminate autoreactivity while preserving immunocompetence.

## REFERENCES


1. Grimaldi, C.M., D.J. Michael, and B. Diamond. 2001. Cutting edge: expansion and activation of a population of autoreactive marginal zone B cells in a model of estrogen-induced lupus. *J. Immunol.* 167:1886–1890.
2. Bijl, M., G. Horst, P.C. Limburg, and C.G. Kallenberg. 2001. Expression of costimulatory molecules on peripheral blood lymphocytes of patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* 60:523–526.
3. Koshy, M., D. Berger, and M.K. Crow. 1996. Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J. Clin. Invest.* 98:826–837.
4. Peeva, E., D. Michael, J. Cleary, J. Rice, X. Chen, and B. Diamond. 2003. Prolactin modulates the naive B cell repertoire. *J. Clin. Invest.* 111:275–283.
5. Thien, M., T.G. Phan, S. Gardam, M. Amesbury, A. Basten, F. Mackay, and R. Brink. 2004. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity.* 20:785–798.
6. Zhang, J., V. Roschke, K.P. Baker, Z. Wang, G.S. Alarcon, B.J. Fessler, H. Bastian, R.P. Kimberly, and T. Zhou. 2001. Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus. *J. Immunol.* 166:6–10.
7. Kim, S.J., D. Gershov, X. Ma, N. Brot, and K.B. Elkon. 2002. I-PLA(2) activation during apoptosis promotes the exposure of membrane lysophosphatidylcholine leading to binding by natural immunoglobulin M antibodies and complement activation. *J. Exp. Med.* 196:655–665.
8. Yurino, H., S. Ishikawa, T. Sato, K. Akadegawa, T. Ito, S. Ueha, H. Inadera, and K. Matsushima. 2004. Endocrine disruptors (environmental estrogens) enhance autoantibody production by B1 cells. *Toxicol. Sci.* 81:139–147.
9. Westhoff, C.M., A. Whittier, S. Kathol, J. McHugh, C. Zajicek, L.D. Shultz, and D.E. Wylie. 1997. DNA-binding antibodies from viable motheaten mutant mice: implications for B cell tolerance. *J. Immunol.* 159:3024–3033.
10. Caligaris-Cappio, F., M. Riva, L. Tesio, M. Schena, G. Gaidano, and L. Bergui. 1989. Human normal CD5+ B lymphocytes can be induced to differentiate to CD5− B lymphocytes with germinal center cell features. *Blood.* 73:1259–1263.
11. Mantovani, L., R.L. Wilder, and P. Casali. 1993. Human rheumatoid B-1a (CD5+ B) cells make somatically hypermutated high affinity IgM rheumatoid factors. *J. Immunol.* 151:473–488.
12. Pugh-Bernard, A.E., G.J. Silverman, A.J. Cappione, M.E. Villano, D.H. Ryan, R.A. Insel, and I. Sanz. 2001. Regulation of inherently autoreactive VH4-34 B cells in the maintenance of human B cell tolerance. *J. Clin. Invest.* 108:1061–1070.
13. Zeng, D., M.K. Lee, J. Tung, A. Brendolan, and S. Strober. 2000. Cutting edge: a role for CD1 in the pathogenesis of lupus in NZB/NZW mice. *J. Immunol.* 164:5000–5004.
14. Song, H., and J. Cerny. 2003. Functional heterogeneity of marginal zone B cells revealed by their ability to generate both early antibody-forming cells and germinal centers with hypermutation and memory in response to a T-dependent antigen. *J. Exp. Med.* 198:1923–1935.
15. Attanavanich, K., and J.F. Kearney. 2004. Marginal zone, but not follicular B cells, are potent activators of naive CD4 T cells. *J. Immunol.* 172:803–811.
16. William, J., C. Euler, S. Christensen, and M.J. Shlomchik. 2002. Evolution of autoantibody responses via somatic hypermutation outside of germinal centers. *Science.* 297:2066–2070.
17. Weller, S., M.C. Braun, B.K. Tan, A. Rosenwald, C. Cordier, M.E. Conley, A. Plebani, D.S. Kumararatne, D. Bonnet, O. Tournilhac, et al. 2004. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. *Blood.* 104: 3647–3654.
18. Ray, S.K., C. Putterman, and B. Diamond. 1996. Pathogenic autoantibodies are routinely generated during the response to foreign antigen: a paradigm for autoimmune disease. *Proc. Natl. Acad. Sci. USA.* 93:2019–2024.
19. Rice, J.S., J. Newman, C. Wang, D.J. Michael, and B. Diamond. 2005. Receptor editing in peripheral B cell tolerance. *Proc. Natl. Acad. Sci. USA.* 102:1608–1613.
20. Fukuyama, H., F. Nimmerjahn, and J.V. Ravetch. 2005. The inhibitory Fcgamma receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G+ anti-DNA plasma cells. *Nat. Immunol.* 6:99–106.
21. Wardemann, H., S. Yurasov, A. Schaefer, J.W. Young, E. Meffre, and M.C. Nussenzweig. 2003. Predominant autoantibody production by early human B cell precursors. *Science.* 301:1374–1377.
22. Yurasov, S., H. Wardemann, J. Hammersen, M. Tsuiji, E. Meffre, V. Pascual, and M.C. Nussenzweig. 2005. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J. Exp. Med.* 201:703–711.
23. Samuels, J., Y.S. Ng, C. Coupillaud, D. Paget, and E. Meffre. 2005. Impaired early B cell tolerance in patients with rheumatoid arthritis. *J. Exp. Med.* 201:1659–1667.
24. Leadbetter, E.A., I.R. Rifkin, and A. Marshak-Rothstein. 2003. Toll-like receptors and activation of autoreactive B cells. *Curr. Dir. Autoimmun.* 6:105–122.
25. van Es, J.H., F.H. Gmelig Meyling, W.R. van de Akker, H. Aanstoot, R.H. Derksen, and T. Logtenberg. 1991. Somatic mutations in the variable regions of a human IgG anti–double-stranded DNA autoantibody suggest a role for antigen in the induction of systemic lupus erythematosus. *J. Exp. Med.* 173:461–470.
26. Debandt, M., O. Vittecoq, V. Descamps, L. Le, and O. Meyer. 2003. Anti-TNF-alpha-induced systemic lupus syndrome. *Clin. Rheumatol.* 22:56–61.