Regulation of Anti-DNA B Cells in Recombination-activating Gene–deficient Mice

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Summary

Anti-DNA antibodies are regulated in normal individuals but are found in high concentration in the serum of systemic lupus erythematosus (SLE) patients and the MRL lpr/lpr mouse model of SLE. We previously studied the regulation of anti–double-stranded (dsDNA) and anti–single-stranded (ssDNA) B cells in a nonautoimmune background by generating mice carrying immunoglobulin transgenes coding for anti-DNAs derived from MRL lpr/lpr. Anti-dsDNA B cells undergo receptor editing, but anti-ssDNA B cells seem to be functionally silenced. Here we have investigated how anti-DNA B cells are regulated in recombination-activating gene (RAG)-2−/− mice. In this setting, anti-dsDNA B cells are eliminated by apoptosis in the bone marrow and anti-ssDNA B cells are partially activated.

Key words: anti-DNA antibody • B cell deletion • B cell anergy • recombination-activating gene deficiency • apoptosis

A striking feature of Lupus is that individual SLE patients express unique subsets of the many anti-nuclear antibodies (ANAs) seen in the population of individuals with this disease (1). As pointed out by Hardin (2), these idiosyncratic spectra of ANAs would not be expected by non-specific activation of the repertoire and suggest instead that autoantibodies arise by immunization. The oligoclonal nature of autoantibodies and the evidence for positive selection of somatic mutants of autoantibodies provides support for this notion (3, 4). Craft and Hardin (5) also noted that these subsets of ANAs include antibodies to "physically-linked epitopes", e.g., anti-DNA with anti-histone(s). They interpreted such linked sets to mean that the activating antigen must be a nucleoprotein particle. An elegant extension of this insight is the demonstration that the blebs on the surface of apoptotic cells contain the targets of Lupus autoantibodies such as DNA, Ro, La, and ribonucleoprotein and could thereby be the delivery vehicles of self-antigen (6).

The observation that self-antigens activate autoreactive B cells in disease implies that such B cells are normally under active, negative regulation. Indeed, B cells specific for both facultative and constitutive self-antigens have been shown to be deleted or inactivated (7–10). The exact site and stage of these manifestations of tolerance have been obscured by receptor editing, a process that rescues autoreactive B cells by revising their receptors (11–16). It has been possible to study deletion directly in mice that are either unable to edit (SCID or recombination-activating gene [RAG]−/−) or lack a substrate for editing (H or L chain-deficient mice) (17–20). Mature B cells can be generated by crossing H and L chain transgenes onto either RAG−/− or SCID mice. These immunodeficient mice can then delete or arrest autoreactive B cells, and thereby provide a powerful tool for studying tolerance in the absence of rearrangement.

We have generated transgenic (tg) mice expressing anti-DNA antibodies characteristic of those found in autoimmune disease (10). In this study we have crossed these anti-double-stranded (dsDNA) (3H9RVk4) and anti-ssDNA (3H9RVk8) tg mice onto RAG−/− mice. This model allows us to determine whether anti-DNA B cells are deleted by programmed cell death, whether apoptotic cells present the immunogen that drive DNA-specific B cells, and whether negative regulation of these autopathies is sustained in the absence of T cells. We find that in RAG−/− mice, dsDNA B cells are eliminated by apoptosis but ssDNA B cells appear to be activated. These data show that deletion efficiently regulates anti-dsDNA and suggest a role for T cells in B cell anergy but not central deletion.

*Abbreviations used in this paper: ANA, anti-nuclear antibodies; BCR, B cell receptor; dsDNA, double-stranded DNA; RAG, recombination-activating gene; ssDNA, single-stranded DNA; tg, transgenic.
Materials and Methods

Mice. The generation of 3H9 site-directed tg (3H9 R), Vk4, and Vk8tg mice has been described previously (15, 16). All three lines were crossed to RAG-2−/− mice on an inbred 129/SvEv background (Taconic Farms, Inc., Germantown, N.Y.). 3H9R/ RAG-2−/− and 3H9 R mice were crossed to Vk4/RAG-2−/− and Vk8R AG-2−/− to produce 3H9R Vk4/RAG-2−/− and 3H9R Vk8/R AG-2−/− or double tg RAG-2−/− mice, respectively. The genotypes of mice were determined by PCR as described previously (15, 16). All experimental mice were 10–16 wk of age.

Flow Cytometric Analysis. Single-cell suspensions were prepared from the bone marrow and spleen as described previously (20). Cell staining and four-color FACSort analysis were conducted as described previously (20). Pro-B, pre-B, immature B, and mature B cells were visualized by incubating bone marrow and splenic cells with a combination of FITC-α11, an antibody against CD43 (21); allophycocyanin-682, an anti-B220 antibody (PharMingen, San Diego, CA); Texas red goat anti-mouse IgM (Southern Biotechnology Associates, Birmingham, AL); and biotin-conjugated rat anti-mouse IgD (Southern Biotechnology Associates, Inc.). PE-streptavidin (Molecular Probes, Inc., Eugene, OR) was used to reveal the biotin reagents. An anti-dsDNA antibody, 3H9 Vk4, and an antidiotyptic antibody, 1.209, specific for various 3H9 heavy and κ light chain combinations (8), were conjugated with FITC following the manufacturer's instruction (Molecular Probes). Dead cells were excluded by staining with propidium iodide and cell samples were analyzed using a FACSort Vantage® (Becton Dickinson, San Jose, CA).

ELISAs. Ig levels in serum and supernatants were measured as described previously (20). Binding to ssDNA and dsDNA was tested by a two-step solution phase ELISA as previously described (13). The cell suspensions were prepared following the procedures provided by the company, except that the cells were stained with allophycocyanin-B220 before fixation. Apoptotic cells were analyzed by flow cytometry.

Induction of Apoptosis and Gel Electrophoresis DNA Fragmentation Analysis. Isolated splenic cells were incubated with ionomycin, a Ca2+ agonist (Sigma Chemical Co., St. Louis, MO), at 1 μg/ml at 37°C overnight. Apoptosis of splenic cells was confirmed by gel electrophoresis. DNA was prepared for gel electrophoresis as follows: cell pellets obtained by centrifugation at 13,000 rpm for 30 s were lysed with 400 μl of lysis buffer containing 0.2% Triton X-100, 10 mM Tris, and 1 mM EDTA, pH 8.0. DNA fragments were precipitated from 200 μl of cell lysates with 0.5 M NaAc and 1 vol isopropanol at −20°C overnight. DNA fragments were washed with 70% ETOH and resuspended in 100 μl of water. Electrophoresis was performed on 1% agarose gels.

Cell Depletion. T cells were depleted using anti-Thy 1.2 antibody (1:10; American Type Culture Collection, Rockville, Md.) and rabbit complement (Accurate Chemical & Scientific Co., Westbury, N.Y.). Spleen cells were incubated with anti-Thy 1.2 (1:100) and complement (1:10) for 45 min at 37°C. Dead cells were removed by centrifugation through Lympholyte-M (Accurate Chemical & Scientific Corporation) for 20 min at 1,200 g at room temperature.

Cell Proliferation. Cell proliferation was monitored by [3H] thymidine incorporation. 2 × 105 T cell-depleted splenocytes were cultured in 96-well microtiter plates with various doses of LPS (0–10 μg/ml), IL-4 (5 μl/ml); R&D Systems, Minneapolis, MN), and anti-IgM F(ab)2 (0–50 μg/ml; Jackson Immunoresearch Labs., Inc. West Grove, PA) in RPMI 1640 medium containing 10% FCS for 72 h. Cells were pulsed with [3H] thymidine (1 μCi/well) for 18 h before harvesting. Determinations were performed in triplicate and the results are expressed as mean of triplicate. Experiments were repeated at least three times with one to two mice in each group.

Results

A ntidsDNA B C els A re A bsent, but A ndsDNA B C els M ate in RAG-2−/− M ice. B cell development was assessed in 3H9R Vk4/RAG-2−/− or 3H9R Vk8/RAG-2−/− mice based on the expression of the cell surface markers B220, CD43, IgM, and IgD. According to B220 and CD43, B cell development was arrested at the pre-B cell stage in RAG-2−/− mice (B220+, CD43−, IgM−; Fig. 1 a). These results are similar to those in H chain tg SCID or RAG−/− mice (Table 1). The introduction of both 3H9H chain and Vk8L chain genes into RAG-2−/− mice completely rescued B cell development as evidenced by the generation of surface IgM + IgD + B cells (Fig. 1 b). Mature B cells extensively populated the periphery and expressed normal levels of sIgM (Fig. 1 c). These results show that functional anti-ssDNA antibody genes are able to rescue B cell development in RAG-2−/− mice. Similar results have been reported for 3H9Vk8 transgenes in SCID mice (Table 1).

The H and L chain gene combination 3H9R Vk4 that codes for anti-dsDNA failed to promote full maturation of RAG-2−/− B cells. No IgM+ or IgM−IgD + B cells were detectable in the bone marrow (Fig. 1, a and b) or spleen (Fig. 1 d). We assume that these anti-dsDNA B cells are deleted at the transition from pre-B to immature B cells, at a point at which the BCR density is below our level of detection.

A ntidSNA B C els A re D eleted by A poptosis. To ask whether anti-dsDNA B cells are eliminated by apoptosis, we assayed for programmed cell death in the bone marrow using the TUNEL (Tdt-mediated dUTP nick-end labeling) assay. Apoptotic cells were detected at frequencies of ~10% in RAG-2−/− and 3H9R Vk4/RAG-2−/− mice and 4–5% in non-tg RAG−/− and 3H9R Vk8/RAG-2−/− B cells (Fig. 2). These apparently apoptotic cells had very low levels of B220 on the surface (Fig. 2), which was nevertheless clearly higher than the signal given by nonspecific Ig. Therefore, we believe that these cells are B cell precursors and that the low level of B220 results from the membrane changes of lymphocytes undergoing apoptosis (23, 24). Furthermore, other bone marrow cells such as macrophages and stem cells are unlikely to contribute to the increased apoptosis in 3H9R Vk4 RAG−/−tg mice.

The Regulation of Anti-dsDNA B Cells May Be Initiated by Binding to A Poptotic C els. Deletion of anti-dsDNA B cells in the bone marrow implies that the antigen(s) specific for the 3H9R Vk4 BCR is present in the bone marrow. A
likely source of DNA and/or DNA–protein complexes are the blebs present on the surface of apoptotic cells (6).
Therefore, we examined the binding of 3H9Vk4 antibody to apoptotic cells. Spleen cells were treated with ionomycin and apoptosis was confirmed by DNA fragmentation patterns (Fig. 3 a). 3H9Vk4 antibody bound to ~60% of splenic cells incubated with ionomycin, as compared with 25% of cells without ionomycin. A control antibody of

Table 1: B Cell Development of tg Mice Crossed on RAG−/− or SCID Background

| RAG−/− | Specificity | B cell development | Reference |
|--------|-------------|---------------------|-----------|
| 3H9Vk4 | dsDNA       | Deleted at the transition from pre-B to immature B cells | 20        |
| 3H9Vk8 | ssDNA       | Mature B cells in the periphery | 10        |
| Sp6    | TNP/DNA    | Arrested at the transition from pre-B to immature B cells | 22        |
| Anti–MHC I | H-2k | In H-2h background; deleted at the transition from B220hl to B220hl cells | 17        |
|         |            | In H-2h background; mature B cells in the periphery |           |
| HC186  | NP         | Mature B cells in the periphery | 18        |
| 3H9Vk8/SCID | ssDNA | Mature B cells in the periphery | 19        |

Figure 1. Flow cytometric analysis of B cell development. Cells from the bone marrow (a and b) and spleen (c) were resolved by four-color analysis for surface expression of B220/CD45R, CD43/S7, IgM, and IgD. Correlative expression of CD43, B220 (a, top) and IgM (a, bottom) based on lymphocyte gate. Cells from the B220−CD43− window of (a, top) were analyzed for expression of IgM and IgD (b). Pre-B (B220−CD43−IgM−), immature B (B220−CD43−IgM−IgD−), and mature B (B220−CD43−IgM−IgD−) cells are shown in different quadrants. Splenic cells were analyzed with anti-B220 and anti-IgM antibodies based on lymphocyte gate (c).
same isotype, MOPC141, did not bind to apoptotic cells (Fig. 3 b). These results are similar to those observed for SLE sera specific for DNA, Ro, and La antigens (6).

We also examined the binding of 3H9Vk4 antibody to bone marrow cells of various mice. We found that 3H9Vk4 antibody bound to ∼14% of bone marrow cells from RAG-2⁻/⁻, but to only 1% of bone marrow cells from RAG-2⁻/⁻ mice (Fig. 3 c). The binding of 3H9Vk4 antibody to bone marrow cells was also high in 3H9Vk4/RAG-2⁻/⁻ mice (∼13%, data not shown). The comparable level of apoptosis in RAG-2⁻/⁻ and 3H9Vk4/RAG-2⁻/⁻ bone marrow suggests that autoreactive B cells in 3H9Vk4/RAG-2⁻/⁻ mice are deleted by apoptosis.

Anti-ssDNA B Cells Do Not Secrete Antibodies In Vivo. RAG-2⁻/⁻ mice with anti-ssDNA transgenes (3H9RVk8) have nearly normal numbers of B cells. All these B cells are idiotype positive, as demonstrated by the reaction with 1.209 antibody specific for the 3H9 heavy chain paired with a k light chain (Fig. 4). These peripheral B cells do not secrete antibody as 3H9RVk8/RAG-2⁻/⁻ mice have 100-500-fold lower levels of serum Ig than do normal mice (Fig. 5).

To understand the status of these B cells, we analyzed splenic cells for additional markers. Staining with anti-IgM and anti-IgD showed two populations of B cells, IgMhi-IgDlo and IgMlo-IgDhi B cells, in 3H9RVk8/RAG-2⁻/⁻ mice, but only IgMhi-IgDhi B cells in 3H9RVk8/RAG-2⁻/⁻ mice (Fig. 6, top). The extra IgMhi-IgDlo population in the RAG-2⁻/⁻ mice may either be activated B cells (25) or maturation-blocked B cells (26). To distinguish these alternatives, CD43 expression of the IgMhi-IgDlo B cells was examined. CD43 is expressed on pro-B cells and activated B cells (27), but not mature resting B cells (28). We found that IgMhi-IgDlo B cells express high levels of CD43 (IgMlo-IgDhi B cells had the characteristic low level of CD43) (Fig. 6, bottom). Thus, we can define two populations of B cells in 3H9RVk8/RAG-2⁻/⁻ mice. One population consists of activated B cells as defined by high expression of IgM and CD43, but low IgD (IgMhi-IgDlo-CD43hi), and a second population consists of B cells with no CD43, low IgM, but high IgD (IgMlo-IgDhi-CD43-). Few, if any, of the activated B cells are found in 3H9RVk8/RAG-2⁻/⁻ mice or in non-tg littermates (Fig. 6).

Are Anti-ssDNA B Cells from RAG-2⁻/⁻ and RAG-2⁻/⁻ Mice Functional? We measured the response of 3H9RVk8 B cells to LPS and found that cells from both 3H9RVk8/RAG-2⁻/⁻ and 3H9RVk8/RAG-2⁻/⁻ mice responded to LPS.
LPS stimulation in a dose-dependent manner (Fig. 7a), but 3H 9R Vkb/RAG-2−/− B cells are hyperresponsive to LPS relative to non-tg RAG-2−/− B cells. This hyperactivity may be derived from the “activated” B cells seen in vivo.

B cells from 3H 9R Vkb/RAG-2−/− mice had a reduced response to low doses of LPS (Fig. 7a), consistent with the idea that these B cells are anergic. The difference between RAG-2−/− and RAG-2−/− mice may reflect the presence or absence of T cells. We studied the possible influence of T cells by addition of IL-4 to our in vitro proliferation assay. Proliferation of B cells in response to a submitogenic concentration of LPS (0.1 μg/ml) was enhanced by IL-4 in 3H 9R Vkb/RAG-2−/− mice (Fig. 7d), indicating that an IL-4-mediated signal was capable of acting in synergy with LPS. 3H 9R Vkb/RAG-2−/− B cells responded poorly to low doses of LPS even in the presence of IL-4. The failure of IL-4 to promote 3H 9R Vkb/RAG-2−/− B cell proliferation in synergy with LPS supports the view that these B cells have a defect in response to LPS and that this defect cannot be overcome by this T cell–derived cytokine.

We also tested the proliferative response of 3H 9R Vkb B cells to anti-IgM. 3H 9R Vkb B cells from RAG-2−/− mice showed a better response to anti-IgM cross-linking than did those from non-tg RAG-2−/− mice, providing further evidence for in vivo activation of these anti-ssDNA B cells. Surprisingly, B cells from 3H 9R Vkb/RAG-2−/− mice responded as well as non-tg RAG-2−/− mice to anti-IgM stimulation (Fig. 7b). Why 3H 9R Vkb/RAG-2−/− B cells have a normal response to anti-IgM cross-linking, but an impaired response to LPS is unclear.

In addition, we determined the effect of IL-4 on B cell proliferation stimulated by anti-IgM. In all three mouse lines, the combination of anti-IgM and IL-4 induced a strong proliferative response that was greater than the sum of that induced by either stimulus alone (Fig. 7c). This suggests that the 3H 9R Vkb B cells have an intact signal transduction pathway through both their Ig and IL-4 receptors.

**Discussion**

It is generally accepted that of the pool of progenitor B cells, only a few B cells manage to mature and the rest die at various stages of development. Yet it has been difficult to actually detect apoptotic B cells in the bone marrow, probably because of rapid phagocytosis of dying cells by macrophages (29). Recently, cell death has been detected at different stages of B lineage differentiation when precursor B cell proliferation is perturbed, as in Eμ-myc tg (30) and SCID (31) mice. Presumably, phagocytosis cannot keep pace with the rate of cell death in these cases. In keeping with these findings, we detect elevated levels of apoptosis in the bone marrow of RAG-2−/− mice. RAG-2−/− B cell development is arrested at the pro-B stage (Fig. 1), but why cell death should occur at this stage is not known.

Rearrangement and H chain expression is one of the requirements for entry into cell cycle and survival of these cells, and indeed, crossing heavy and light chain transgenes into RAG−/− mice rescues B cell development (17, 18) and reduces apoptosis to wild-type levels (Fig. 2). On the other hand, autoantibody tg’s have a limited capacity for restoring functional B cells in RAG−/− mice. 3H 9R Vkb4 transgenes advance B cell development to the B220−CD43−IgM− pre-B stage, but immature B cells (B220+, IgM+) are unde-
detectable. For this autospecificity, a B cell early in the transition from pre-B to immature B cell appears to be the target for deletion, and consequently the level of apoptosis in 3H9 RVk4/RAG-2−/− mice is as high as in RAG-2−/− mice. We assume that the expression of the self-reactive IgM on the cell surface initiates signaling events that result in apoptosis. The fact that we cannot detect appreciable levels of IgM or B220 on the apoptotic TUNEL-positive cells—even though they are immature B cells—might be due to membrane disintegration and concomitant loss of cell surface molecules during the apoptotic process (23, 24).

A second model for anti-DNA-reactive B cells is the Sp6 tg mouse. Here the anti-TNP/DNA cross-reactive B cells are also deleted at the pre-B to immature B transitional stage (22). Such a short survival time of immature B cells predicts a narrow time window for editing in anti-dsDNA B cells. Indeed, we find that editing in 3H9 RVk4 B cells is predominantly on the targeted allele, suggesting that these B cells. Indeed, we find that editing in 3H9 RVk4 B cells is predominantly on the targeted allele, suggesting that these B cells.

Anti-dsDNA B cells fail to edit their receptors (e.g., in the absence of RAG) die via apoptosis. These dying cells provide an enriched source of autoantigens to anti-dsDNA B cells. Weaker IgM cross-linking by sDNA allows the B cells to mature and populate the periphery with an activated phenotype. We propose that these activated B cells are subsequently inactivated or eliminated by T cell–dependent mechanism(s).

Figure 8. A model for the regulation of anti-DNA B cells. Pre-B cells that have functional rearranged heavy and light chain genes express surface IgM that can bind to autoantigens. The strong IgM cross-linking by dsDNA from blebs of apoptotic cells induces secondary rearrangement (receptor editing) and altered binding specificity. Anti-dsDNA B cells that fail to edit their receptors (e.g., in the absence of RAG) die via apoptosis. These dying cells provide an enriched source of autoantigens to anti-dsDNA B cells. Weaker IgM cross-linking by ssDNA allows the B cells to mature and populate the periphery with an activated phenotype. We propose that these activated B cells are subsequently inactivated or eliminated by T cell–dependent mechanisms.

Regulation of Anti-DNA B Cells in RAG-deficient Mice

| Pre-B | Immature | Mature |
|-------|----------|--------|
| CD43+ IgM+ IgD+ B220 low | CD43- IgM high IgD low B220 low | CD43+ IgM+ IgD+ B220 high |
| CD43- IgM low IgD+ B220 low | CD43- IgM low IgD high B220 low | CD43+ IgM+ IgD+ B220 high |
| Activation | Deactivation (anergy) | Anti-DNA |

Cells in RAG-2−/− mice contain few if any B cells with an activation phenotype. Rather they are anergic. We show that they are hyporesponsive to LPS stimulation, whereas strong IgM cross-linking with intact anti-IgM antibody results in normal proliferation of the tg B cells. Similar results have been obtained by Nguyen et al. (34) using conventional VH3H9/Vk8 tg mice. Proliferation to LPS
and suboptimal IgM cross-linking with anti-IgM F(ab')2 antibody were reduced, whereas IgM cross-linking in the presence of T help was normal in most animals (34). Interestingly, the VH3H9/Vk8 anti-ssDNA anergic B cells differ from anti-HEL B cells that are exposed to soluble HEL (9). The latter show a 20-fold reduction of surface IgM (9), whereas the overall IgM levels in conventional and site-directed 3H9/Vk8 tg B cells were similar to non-tg littermates (Fig. 6, reference 34). This finding might also explain where the normal Ca2+ flux in the VH3H9/Vk8 mice (34), compared with transient Ca2+ flux in anti-HEL/soluble HEL double tg mice (35). In addition, anti-HEL B cells have a shortened life span in the presence of soluble HEL (36), whereas anti-ssDNA B cells live as long as non-tg B cells (34). In summary, it appears that B cell tolerance not only consists of the two distinct stages of deletion and anergy, but that qualitative differences also exist in the level of energy. These differences are probably due to variations in the strength and/or quality of antigen–BCR interactions (as discussed earlier for deletion). What mechanism(s) are responsible for the anergy induction? Goodnow (37) discussed that B cell anergy may be induced by (a) the encounter with self-antigen early in development (clonal energy), (b) interaction with antigen-specific regulatory T cells, and (c) regulatory elements of an idiotype network (37). Our data clearly favor the hypothesis that regulatory T cells silence self-reactive B cells, since self-reactive 3H9R Vk8 B cells in RAG-2-/- mice are activated rather than anergized. We presume that the "self" milieu of all mice is similar, although quantitative difference are apparent in tg or mutant mice (see Fig. 2) and self-activated B cells are likely to be a feature of all mice, not just RAG-2-/- tgs. How then do normal mice inactivate 3H9R Vk8 or other self-reactive B cells? T cells seem the most likely regulator and the activated status of B cells exposed to antigens at early stages of development may be the target for regulatory T cells. How T cells affect B cell anergy (or accelerated cell death) and whether direct suppression by cell–cell contact occurs or whether direct suppression by cell–cell contact occurs or soluble factors produced by T cells are involved is not known. Furthermore, the phenotype of regulatory T cells is also unclear. TCR-γ/δ cells seem to downregulate the autoimmune process in MRL mice since TCR-γ/δ-deficient mice show a much more severe disease (38). In the system of organ-specific autoimmunity, e.g., after neonatal thymectomy, CD4+ IL-2Rα cells (which probably express TCR-γ/δ) prevent autoimmunity (39). Although it seems likely that the regulatory T cells are activated by the same antigen as the self-reactive B cells, the question of specificity of regulatory T cells in either organ-specific or systemic autoimmunity has not yet been resolved. In addition, future work will have to address if B cells become energized by one single event (antigen–B cell interaction/antigen–B cell–T cell interaction) or if they must constantly receive anergizing signals.

In summary, we propose the following model for B cell selection (Fig. 8): B cells that are unable to express a BCR die by apoptosis at the pre-B cells stage (RAG-2-/- mice). The expression of a functional BCR allows maturation to immature B cells. As soon as IgM is expressed on the cell surface it can receive signals due to antigen binding. If this is a strong binding resulting in a strong signal, then receptor editing is initiated. If a cell continues to express a self-reactive receptor (either because it can not be directly edited in case of a conventional tgs or because the BCR is still autoantigenic after editing), the amount of signal will increase and induce apoptosis. Cells that manage to revise their BCR will be released from the death signal and continue to mature. If such mature B cells then encounter antigen in the periphery, they will be stimulated to produce antibody. Alternatively, if B cells bind self-antigen weakly, they will receive a qualitatively different signal that results in cell activation rather than editing/deletion. Such activated B cells persist in the periphery of T cell–deficient mice, but are energized by regulatory T cells in RAG-competent mice.

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