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Autoreactive B Cells Escape Clonal Deletion by Expressing Multiple Antigen Receptors[1]

James J. Kenny,[2] Louis J. Rezanka, Ana Lustig, Randy T. Fischer, Jeffrey Yoder, Shannon Marshall, and Dan L. Longo

IgH and L chain transgenes encoding a phosphocholine (PC)-specific Ig receptor were introduced into recombinase-activating gene (Rag-2$^{-/-}$) knockout mice. The PC-specific B cells that developed behaved like known autoreactive lymphocytes. They were 1) developmentally arrested in the bone marrow, 2) unable to secrete Ab, 3) able to escape clonal deletion and develop into B1 B cells in the peritoneal cavity, and 4) rescued by overexpression of *bcl-2*. A second IgL chain was genetically introduced into Rag-2$^{-/-}$ knockout mice expressing the autoreactive PC-specific Ig receptor. These dual L chain-expressing mice had B cells in peripheral lymphoid organs that coexpressed both anti-PC Ab as well as Ab employing the second available L chain that does not generate an autoreactive PC-specific receptor. Coexpression of the additional Ig molecules rescued the autoreactive anti-PC B cells and relieved the functional anergy of the anti-PC-specific B cells, as demonstrated by detection of circulating autoreactive anti-PC-Abs. We call this novel mechanism by which autoreactive B cells can persist by compromising allelic exclusion receptor dilution. Rescue of autoreactive PC-specific B cells would be beneficial to the host because these Abs are vital for protection against pathogens such as *Streptococcus pneumoniae*. *The Journal of Immunology,* **2000, 164: 4111–4119.**

Central to current models of B/T lymphocyte development as well as the immunobiology of response to foreign Ag is the axiom that a single B/T cell expresses a single Ag receptor with a single specificity. This phenomenon is the outcome of the process referred to as allelic exclusion. Allelic exclusion is generally regulated at the level of rearrangement and expression of the genes coding for the TCR and Ig molecules (1–8). Once an intact immune receptor is inserted into the cell membrane, additional recombination is halted by feedback inhibition of the recombinase system (9–11). The single-cell/single-receptor rule is fundamental to the clonal selection model (12) of lymphocyte development and Ag-driven selection. Expression of a single-Ag-specific receptor on a lymphocyte ensures first that each lymphocyte will be effectively monitored and tolerized during ontogeny if its receptor efficiently binds self Ags, and second that Abs specific to the foreign Ags will be produced by the host following exposure to infectious agents.

We have been studying the B cell response to phosphocholine (PC),[3] an Ag expressed on the cell wall of *Streptococcus pneumoniae*. PC-specific Abs have been shown to be highly protective against infection with *S. pneumoniae*, (13–17). Concordantly, the absence of anti-PC Ab-producing B cells in X-linked immune-deficient (Xid) mice leaves this host highly susceptible to infection with *S. pneumoniae* (16, 17). Analysis of B cell development in Xid mice indicated that PC-specific B cells behave like autoreactive B cells, in that they are clonally deleted (18, 19). Paradoxically, in normal mice, these PC-specific B cells are positively selected into peripheral lymphoid tissues. These data suggest that it is advantageous to the host to retain these potentially autoreactive PC-specific B cells rather than delete them.

During normal B cell development, B lymphocytes that recognize self Ags in the bone marrow (BM) are arrested at the transition from pre-B to immature (sIgM$^+$) cells and are subsequently clonally deleted (20–25). The interaction between the B cell receptor and membrane-displayed self-Ags or polymeric self-Ags (i.e., DNA) signals the reactivation and up-regulation of the recombinase-activating genes (Rag) and results in efficient receptor editing of IgL chain genes (26–30). This editing process replaces the original L chain with one that can pair with the IgH chain to form a receptor that binds an Ag other than a self Ag (24, 28). However, in Rag knockout (KO) mice, B cells expressing rearranged H and L chain transgenes encoding autoreactive Ig receptors cannot edit their transgene-encoded Ig receptors because they cannot rearrange their endogenous Ig genes. Therefore, virtually all the B cells expressing autoreactive Ag receptors on a Rag KO background are developmentally arrested at the pre-B/immature B cell transition stage and undergo apoptosis (31).

Additionally, B lymphocytes that recognize self-Ags can undergo tolerance induction. Different states of anergy can result depending on the nature of the autoantigen. In the soluble HEL/anti-HEL system developed by Goodnow and colleagues (32, 33), the anergic B cells down-regulate their IgM receptors and are unable to proliferate in vitro following stimulation with anti-IgD or anti-IgD-dextran (Dex). On the other hand, Xu et al. (34) found that anergic ssDNA-binding B cells did not produce Ab in vivo, but proliferated in vitro in response to anti-μ plus IL-4, and Andersson et al. (31) found that anergic DNA-binding B cells could be induced to differentiate into plasma cells in response to

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[3] Abbreviations used in this paper: PC, phosphocholine; BM, bone marrow; Dex, dextran; HEL, hen egg lysozyme; KO, knockout; Rag, recombinase-activating gene; sIg, secretory Ig; TG, transgene; TI, thymus-independent; Xid, X-linked immune-deficient.

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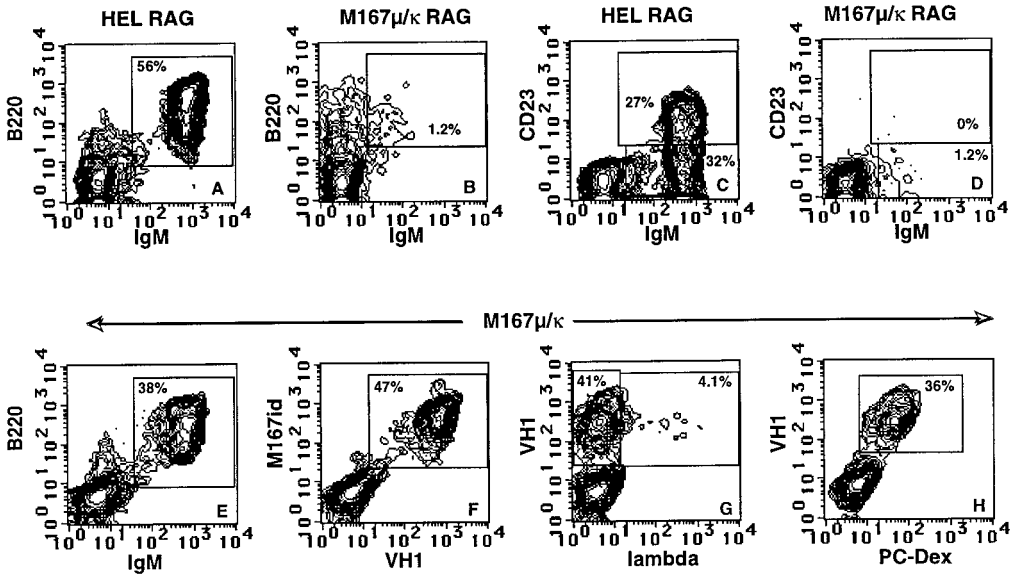

FIGURE 1. B cell surface immunophenotype of spleen cells from transgenic mice expressing H and L chain transgene-encoding autoreactive anti-PC Abs in Rag-2-deficient and phenotypically normal genetic backgrounds. *A* and *C*, and *B* and *D*, FACS profiles of splenic B cells in Rag-$2^{-/-}$ KO mice expressing anti-HEL (32) and anti-PC M167H:κ24L transgenes (35), respectively. *E–H*, Immunophenotype of spleen cells from wild-type mice expressing anti-PC M167H:κ24L transgenes. Data are shown as 5% contour plots of total spleen cells and are representative of three or more experiments per genetic background, with each experiment consisting of analysis of five or more mice per genotype. Cells were stained with FITC-and PE-conjugated Abs in the presence of the Fc receptor-blocking Ab 2.4G2 as described in *Materials and Methods*.

a cross-reactive thymus-independent (TI)-type 2 Ag, trinitrophenyl-Ficoll.

We present evidence that B cells expressing transgenes encoding PC-specific Abs are indeed autoreactive; however, rather than being developmentally arrested and clonally deleted, these autoreactive PC-specific B cells persist by expressing more than one Ab specificity, thereby compromising allelic exclusion and the paradigm of one Ag-specific Ig receptor per lymphocyte.

Materials and Methods

Animals

Transgenic mice carrying the MOPC-167 (M167) myeloma-derived μ plus κ transgenes (line 207-4, designation Tg[Igh + Igk]Bri12) (35) were obtained from Dr. U. Storb (University of Chicago, Chicago, IL) through Dr. R. L. Brinster (School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA). λ1 transgenic mice were obtained from K. Rajewsky, University of Cologne (Cologne, Germany), through F. Alt, Children's Hospital (Boston, MA) and were backcrossed to Rag-2 KO mice in our laboratory. Rag-2 KO mice were obtained from Taconic Farms (San Francisco, CA) and were crossed to Ig transgenic mice in our animal facility. κ KO mice were obtained from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 μMT/μMT mice (36, 37) were obtained from Dr. K. Rajewsky through Dr. C. Sidman (University of Cincinnati, Cincinnati, OH).

Flow cytometric analysis

Spleen, BM, and peritoneal (PerC) cells were prepared and stained as previously described (38, 39). PE conjugation of anti-V_H1, anti-M167-id (clone 28-6-20) (38), and anti-CD23 (40) was conducted by Molecular Probes (Eugene, OR). FITC-conjugated PC-Dex was a gift from Dr. H. Dintzis (The Johns Hopkins University, Baltimore, MD) and was used at a final concentration of 2.5 μg/10^6 cells. The synthesis of PC-Dex was previously described (19). Stained cells were analyzed using a FACScan, and 5% probability contour profiles were generated using CellQuest Graphics Software (Becton Dickinson, San Jose, CA). Images presented in the figures are representative of three or more experiments per background. In each experiment five or more mice per genotype were analyzed.

ELISA analysis of serum

Blood was collected by retro-orbital puncture, and serum was titrated on PC-BSA-coated plates as previously described (41). The plates were washed three times with Tris-buffered saline and Tween 20 and were tested for the presence of the transgene-encoded μ^a allotype, the endogenous μ^b allotype, and total Ig using biotinylated anti-μ^a (clone DS-1), anti-μ^b (clone AF6), and anti-κ (clone HB58), respectively. The plates were washed and developed using streptavidin alkaline phosphatase (Calbiochem, La Jolla, CA) for 1 h at room temperature. The plates were washed, and substrate (*p*-nitrophenyl phosphate at 1 mg/ml in 1 M diethanolamine; 104–100, Sigma, St. Louis, MO) was added for 30 min at room temperature. Substrate cleavage was read on a Bio-Tek ELx800 ELISA reader (Bio-Tek, Burlington, VT) at 405 nm, and Ab values were determined from internal IgM anti-PC standards included on each plate.

Proliferation assays

Spleen cells were stimulated in vitro with the TI type 2 Ags, PC-Sepharose, PC-Dex, and anti-id conjugated to Sepharose as previously described (19, 42).

Results

PC-specific B cells are autoreactive

We used the Rag-2 KO mouse to determine whether PC-binding Ag receptors were autoreactive in vivo. This was previously predicted from the finding that these PC-specific B cells appear to clonally delete in Xid mice (18, 19). Mice carrying the M167 V1 H chain variant and the κ-24 L chain as transgenes (M167H:κ24L) (35) encoding an anti-PC Ab were crossed onto the Rag-$2^{-/-}$ (43) background, and B lymphocyte development was analyzed by characterizing the surface immunophenotype of cells isolated from the spleens of these mice. Fig. 1 (*B* and *D*) illustrates that there were virtually no mature B cells present in the spleens of the M167H:κ24L:Rag-$2^{-/-}$ mice. What few B cells were observed expressed very low levels of sIgM (Fig. 1*B*), and no detectable mature $CD23^+$/$sIgM^+$ B cells were present (Fig. 1*D*). Similar analyses of Rag-$2^{-/-}$ KO mice expressing μκ anti-hen egg lysozyme (anti-HEL) transgenes (32) are presented as a control for development of B cells expressing non-self Ig receptors. In contrast to the M167H:κ24L:Rag-$2^{-/-}$ mice, large numbers of mature, $sIgM^{high}B220^{bright}$ B cells (Fig. 1*A*) and $CD23^+$/$sIgM^+$ B cells (Fig. 1*C*) were observed in the anti-HEL:Rag-$2^{-/-}$ mice.

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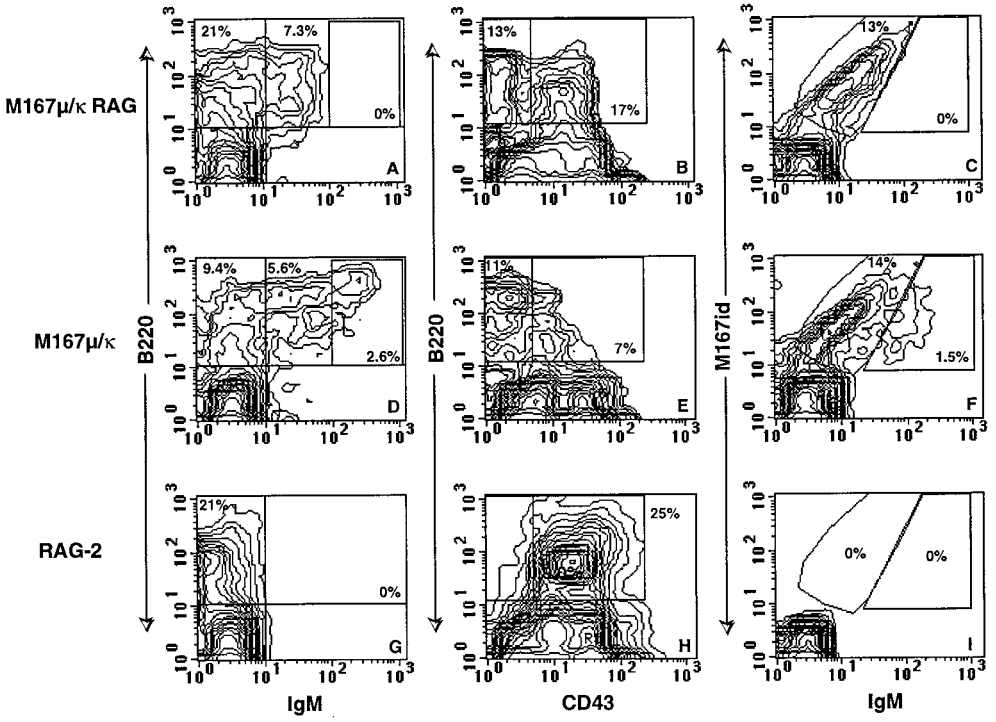

FIGURE 2. B cell development in the bone marrow of M167H:κ24L Rag-$2^{-/-}$ and Rag-$2^{+/-}$ mice. *A–C* and *D–F*, FACS profiles of BM cells from M167H:κ24L Rag-$2^{-/-}$ and Rag-$2^{+/-}$ mice, respectively. *G–I*, FACS profiles from a Rag-$2^{-/-}$ control. Data are shown as 5% contour plots of total BM cells and are representative of three or more experiments per genetic background, with each experiment consisting of analysis of five or more mice per genotype. Cells were stained with FITC-and PE-conjugated Abs in the presence of the Fc receptor-blocking Ab 2.4G2 as described in *Materials and Methods*.

These data demonstrate that B cells expressing the M167H:κ24L transgene receptor are arrested at the pre-B/immature B cell transition point typical of B cells expressing autoreactive Abs. In the phenotypically normal M167H:κ24L:Rag-$2^{-/+}$ littermates, large numbers of $sIgM^+$:$B220^+$ cells (Fig. 1*E*) were observed. All the splenic B cells were V_H1^+:M167-id^+ (Fig. 1*F*) and bound PC-Dex (Fig. 1*H*); therefore, they express the autoreactive B cell receptor. The 28-6-20 Ab used to identify the M167-Id recognizes the κ24L chain when paired with a heavy chain and therefore identifies $κ24^+$ cells.

M167H:κ24L PC-specific B cells develop in the BM of TG^+ Rag-$2^{-/-}$ mice

The developmental profile of B cells in the BM of M167H:κ24L:Rag-$2^{-/-}$ and their phenotypically normal M167H:κ24L:Rag-$2^{-/+}$ littermates is presented in Fig. 2. B cell development was clearly arrested at the $B220^+/S7^+$, pro-B cell stage in the Rag-$2^{-/-}$ mice (Fig. 2*H*), and there were no detectable $B220^+/sIgM^+$ or M167$id^+/sIgM^+$ B cells (Fig. 2, *G* and *I*, respectively). Expression of the M167H:κ24L transgenes in Rag-$2^{-/-}$ mice allows the B cells to make the pro-B to pre-B transition ($B220^+/S7^-$; Fig. 2*B*). Similar percentages of $B220^+/sIgM^+$ and M167$id^+/sIgM^+$ B cells were observed in the M167H:κ24L:Rag-$2^{-/-}$ as well as the TG^+Rag-$2^{-/+}$ littermates (Fig. 2, *A*/*C* and *D*/*F*, respectively). However, a distinct immature B cell population, $sIgM^{bright}$, was clearly observed in the M167H:κ24L Rag-$2^{-/+}$ mice (Fig. 2*D*), but was missing in the M167H:κ24L:Rag-$2^{-/-}$ (Fig. 2*A*). Additionally, both the M167H:κ24L:Rag-$2^{-/-}$ mice and their TG^+ Rag-$2^{-/+}$ littermates expressed the transgene-encoded κ24L chain, as shown by the expression of M167-id (Fig. 2, *C* and *F*); however, a small population of more mature $sIgM^{bright}$ B cells was observed in the phenotypically normal TG^+ Rag-$2^{-/+}$ mice (Fig. 2*F*), which was clearly absent in the TG^+ Rag-$2^{-/-}$ mice (Fig. 2*C*).

Autoreactive B cells escape deletion in the peritoneal cavity and differentiate into B1 B cells

In addition to receptor editing, homing to anatomically privileged sites such as the peritoneal cavity has been shown to be another way that autoreactive B cells escape clonal deletion (44, 45). In the transgenic system devised by the Honjo laboratory, transgenic B cells expressing anti-autoerythrocyte Abs were present in the peritoneal cavity, but were clonally deleted in the BM. These peritoneal autoreactive erythrocyte-specific B cells developed into self-renewing B1 ($CD5^+$) B cells. In our transgenic system of anti-PC Abs, cells isolated from the peritoneal cavity of Rag-$2^{-/-}$ KO mice expressing the M167H:κ24L transgenes exhibited large numbers of V_H1^+ B cells (Fig. 3, *A–C*). Approximately 42% (20% of the total events) of these V_H1^+ peritoneal B cells expressed CD5 on their surface and 100% expressed Mac-1 (CD11b; Fig. 3, *A* and *B*, respectively), while 20% (9.1% of the total events) of the V_H1^+ B cells present in the peritoneal cavity of Rag-$2^{+/-}$ anti-PC transgenic mice expressed CD5, and ~50% (24% of the total events) expressed Mac-1 (Fig. 3, D and *E*, respectively). Forty percent (17 and 14% of the total events) of peritoneal V_H1^+ B cells in M167H:κ24L:Rag-$2^{-/+}$ mice and IgM^+ C57BL/6 control mice expressed CD23 (Fig. 3, *F* and *I*, respectively), whereas the TG^+ Rag-$2^{-/-}$ mice lacked these $CD23^+$ B2 B cells (Fig. 3*C*). All the peritoneal B cells were both M167-id^+ and bound PC-Dex (data not shown). Thus, like autoreactive erythrocyte-specific B cells (44, 45), PC-specific B cells can escape deletion by homing into the peritoneal cavity and differentiating into B1 B cells.

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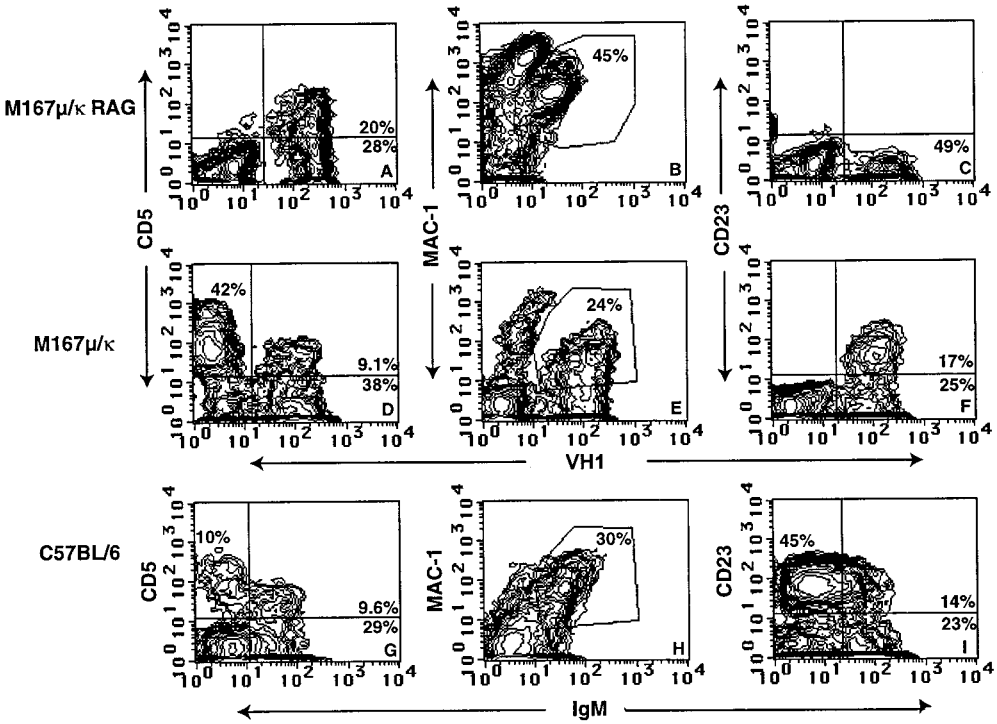

FIGURE 3. B1 B cells develop in the peritoneal cavity of M167H:κ24L Rag-$2^{-/-}$. Peritoneal B cells were harvested in HBSS containing 10 mM EDTA. Samples containing RBC were eliminated, and the remaining samples were stained and analyzed as described in Fig. 1. FACS analysis was performed on an extended small cell gate to eliminate large monocytes and macrophages, and data are shown as 5% contour plots. Data presented are representative of three or more experiments per genetic background, with each experiment consisting of analysis of five or more mice per genotype. *A–C* and *D–F*, FACS profiles of BM cells from M167H:κ24L Rag-$2^{-/-}$ and Rag-$2^{+/-}$ mice, respectively. *G–I*, FACS profiles from a C57BL/6 control.

Overexpression of bcl-2 *prevents clonal deletion*

In several model systems in which potentially autoreactive B cells are generated, overexpression of *bcl-2* does not alter their susceptibility to induction of central tolerance in the BM, but can prevent induction of peripheral clonal deletion (20, 46–48). We crossed Rag-$2^{-/-}$ M167H:κ24L, anti-PC transgenics to Rag-$2^{-/-}$ *bcl-2* transgenic mice to determine the effect overexpression of *bcl-2* would have on B cell development in these mice. As we previously observed in Xid mice (19), overexpression of *bcl-2* prevented the clonal deletion of PC-specific cells in the Rag-$2^{-/-}$ M167H:κ24L/bcl-2 double-transgenic mice (Fig. 4, *D–F* compared with *A–C*). The absolute number of M167-id$^+$/sIgM$^+$-staining B cells increased 10-fold in the spleen of many of these mice, corresponding to an ~10-fold increase in the percentage of sIgM$^+$ cells (compare Fig. 4, *A* and *B* to *D* and *E*). All these B cells were PC specific when stained with FITC-conjugated PC-Dex (Fig. 4*F*). However, the level of sIgM was lower on B cells from the Rag-$2^{-/-}$ *bcl-2*$^+$ M167H:κ24L B cells compared with B cells from the Rag-$2^{+/+}$ M167H:κ24L mice (compare Fig. 4, *D* and *E* to *G* and *H*). Although introduction of the *bcl-2* transgene resulted in increased numbers of sIgM$^+$ B cells compared with those in *bcl-2*$^-$/TG$^+$ Rag-$2^{-/-}$ mice (Fig. 4, *A* and *B* vs *D* and *E*), these B cells were still anergic, as reflected by the absence of anti-PC Abs in their serum (Table I).

Escape from tolerance by coexpression of multiple sIg receptors

While the specific autoantigen recognized by PC-binding Abs is still undefined, it is clear from previous data in Xid mice and the above data in Rag-$2^{-/-}$ KO mice that B cells expressing M167H:κ24L, PC-binding Abs are characteristically autoreactive, i.e., they undergo developmental arrest in the BM and home to privileged sites in the periphery. However, normal mice carrying these transgenes generated near normal numbers of B220$^+$/sIgM$^+$ cells (Fig. 1*E*). These B cells expressed the V1 H chain gene product and were positive for the M167-id, which indicates that they are expressing the κ24 L chain in association with the M167 H chain variant (Fig. 1*F*). Additionally, all these V_H1^+ B cells also bound PC-Dex (Fig. 1*H*). The first insight into a possible explanation for the persistence of these B cells, which were obviously expressing the autoreactive anti-PC Ig receptor, was gained by examining Fig. 1*G*. This figure illustrates that ~10% of the V_H1^+ B cells (4.1% of the total events) also expressed the λ L chain. All the V_H1^+ cells also expressed κ24 (identified by anti-M167-id (28-6-20), which recognizes κ24L when associated with an IgH chain) and bound PC-Dex. These data suggested that these PC-specific B cells coexpress more than one Ig receptor.

The above data suggest that coexpression of multiple Ig receptors, which compromises the central tenet of allelic exclusion, may provide a mechanism by which autoreactive B cells can elude negative selection. To directly confirm that coexpression of multiple Ig receptors would rescue autoreactive PC-specific B cells from developmental arrest, M167H:κ24L Rag-$2^{-/-}$ mice were crossed to λ1 L chain Rag-$2^{-/-}$ mice. The offspring of this cross have one heavy chain and two L chains from which to make an Ab. One H:L combination is autoreactive, and one is not. In these double L chain transgenic mice, substantial numbers of B220$^+$/sIgM$^+$ B cells developed (Fig. 5*A*). These V_H1^+ B cells expressed the autoreactive M167-id (Fig. 5*B*), bound PC-Dex (Fig. 5*E*), and expressed the λ L chain (Fig. 5*C*). Detection of M167-id and binding to PC-Dex both demonstrate the expression and association of the κ24L chain with the M167H chain. Finally, coexpression of both κ24L chain (as shown by the presence of the M167-Id) and λL chain in association with the V_H1 H chain is demonstrated in Fig. 5*D*.

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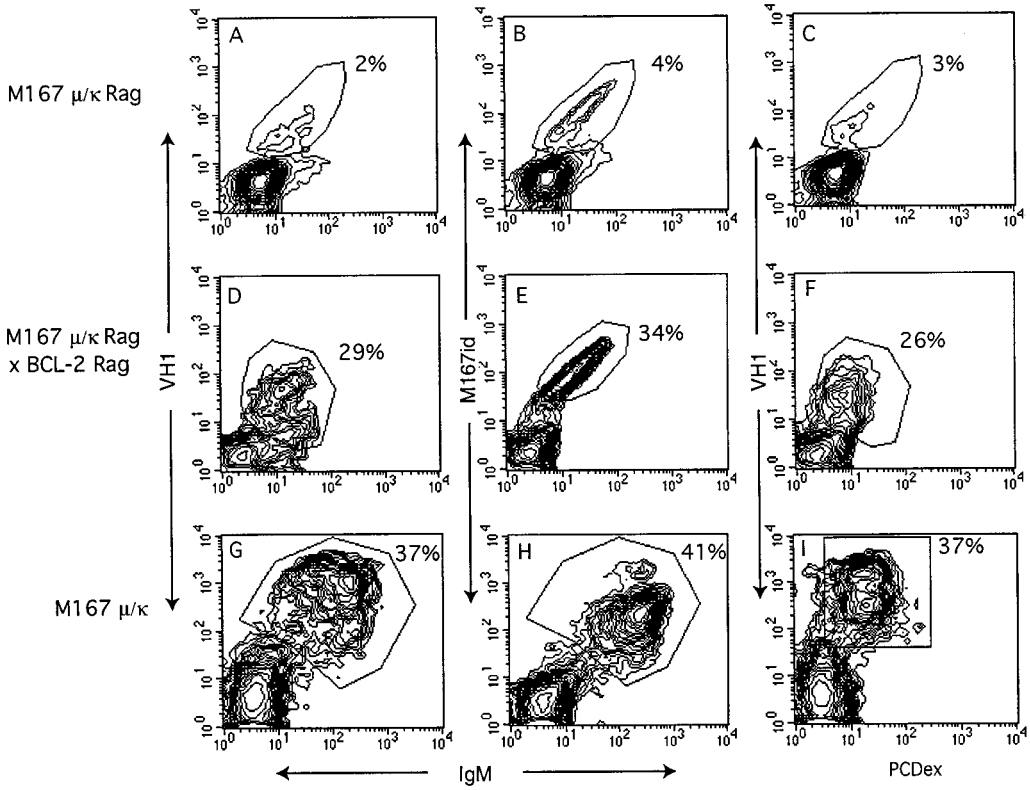

FIGURE 4. Overexpression of *bcl-2* rescues B cells in M167H:κ24L Rag-$2^{-/-}$ mice. Spleen cells from M167H:κ24L Rag-$2^{-/-}$ (*A–C*), M167H:κ24L Rag-$2^{-/-}$ × Bcl-2 Rag-$2^{-/-}$ (*D–F*), and M167H:κ24L Rag-$2^{+/+}$ mice (*G–I*) were stained and analyzed as described in Fig. 1. Data presented are representative of three or more experiments per genetic background, with each experiment consisting of analysis of five or more mice per genotype.

In wild-type mice expressing the anti-PC M167H:κ24L transgenes, 20% of the B cells coexpress an endogenous μ^b-allotype H chain along with the transgene-encoded μ^a -allotype H chain (41). Many H chain transgenic mice have been described as leaky or poor at allelic exclusion (5, 49–51), in that they coexpress endogenous μ^b-allotype H chain along with the transgene-encoded μ^a H chain. To ascertain what contribution coexpression of endogenous H chains makes to facilitating the escape of autoreactive B cells from clonal deletion, we backcrossed M167H:κ24L, anti-PC transgenic mice with μMT H chain KO mice (36, 37). Because the transmembrane exons of the μ H chain gene are eliminated in these mice, the B cells cannot insert endogenous H chains into the cell membrane; thus, the loss of endogenous H chain in this rescue process in μMT mice would force the B cell to increase coexpression of endogenous L chains to rescue the autoreactive PC-specific B cells. As shown in Fig. 5*F*, normal numbers of $B220^+$/$sIgM^+$ B cells were observed in the spleens of M167H:κ24L μMT mice. All the autoreactive $sIgM^+$ B cells expressed the M167-id (Fig. 5*G*) and bound PC-Dex (Fig. 5*J*). In this genetic background where the endogenous H chains cannot be used to modify the autoreactive receptor, 40–50% of the autoreactive, PC-binding, M167H:κ24L-expressing B cells coexpressed λ L chains (Fig. 5, *F–J*). Additionally, Fig. 5*I* clearly demonstrates that 40–50% of the κ L chain-expressing B cells also express sIg receptors containing the λ L chains. Presumably, the remainder of the autoreactive PC-binding, M167H:κ24L-expressing B cells have rearranged and coexpressed additional endogenous κ L chains that do not form an autoreactive Ab when paired with the M167 H chain.

Table I. *Lack of serum anti-PC Abs in M167 μκ Rag-$2^{-/-}$ mice*

Animal Strains	Anti-PC Abs (μg/ml)[a]	
	μ^a	μ^b
M167μκ	162	23
M167μκ Rag-$2^{-/-}$	0	0
M167μκ *bcl-2* Rag-$2^{-/-}$	0	0
M167μκ/λ Rag-$2^{-/-}$	5	0
M167μκ μMT/μMT	64	0
M167μκ/$\kappa^{-/-}$	48	7
M167μκ nude	44	18
C57BL/6	0	9

[a] Serum anti-PC levels were determined by ELISA as described in *Materials and Methods*.

Coexpression of endogenous κ L chains is difficult to analyze, inasmuch as available reagents cannot discriminate between the κ24 transgene product and endogenous κ L chains at the protein level. To determine whether coexpression of endogenous κ L chains plays a major role in modulating the developmental fate of autoreactive, anti-PC-expressing B cells, the M167H:κ24L anti-PC transgenes were backcrossed onto the $\kappa^{-/-}$ KO (52) mouse background. Fig. 5*K* reveals that in the absence of endogenous κ L chain expression, 100% of the autoreactive, M167-id^+ ($\kappa24L^+$) B cells coexpressed endogenous λ L chains while still binding low levels of PC-Dex (Fig. 5*L*). These data are consistent with the observations presented in the other genetic backgrounds described above and contrast dramatically with the ~10% of M167-id^+/λ^+-coexpressing B cells (4.0 and 4.1% of the total events, respectively) observed in either $\kappa^{+/-}$ M167H:κ24L (Fig. 5*M*) or $\kappa^{+/+}$ M167H:κ24L (Fig. 1*G*) mice. All these data indicate that the developmental fate of these autoreactive, PC-specific, M167H:κ24L-expressing B cells is being altered by coexpression of additional H and/or L chains.

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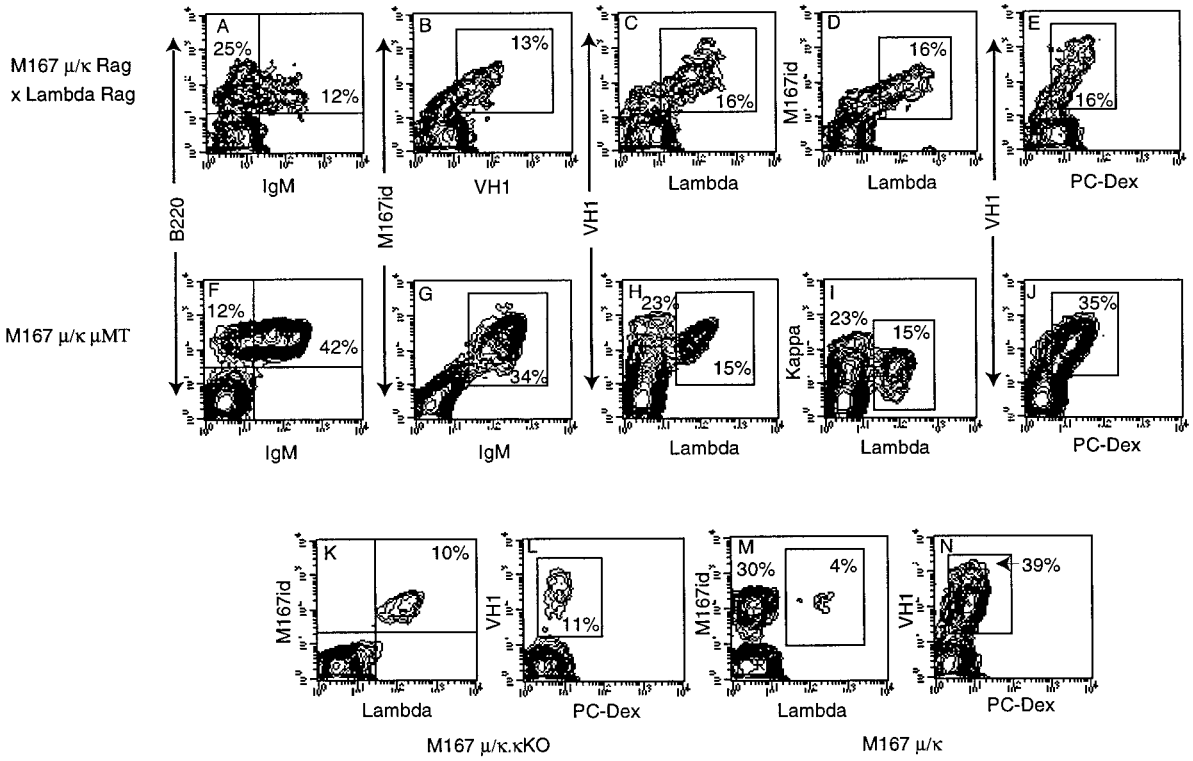

FIGURE 5. Rescue of autoreactive B cells by coexpression of multiple L chains. *A–E*, Rescue of autoreactive, PC-specific splenic B cells in M167H:κ24L Rag-$2^{-/-}$ mice crossed to λL Rag-$2^{-/-}$ mice. *F–J*, Immunophenotype of spleen cells from mice expressing M167H:κ24L transgenes crossed onto the μMT genetic background and coexpression of κ and λ L chains on autoreactive, PC-binding B cells. *K* and *M*, Expression of endogenous λ L chains on M167-id$^+$ splenic B cells from $\kappa^{-/-}$ KO and $\kappa^{-/+}$ KO mice, respectively, carrying M167H:κ24L transgenes. *L* and *N*, Binding of FITC-conjugated PC-Dex to these same cells.

Receptor coexpression reverses anergy and permits serum Ig production

M167-id$^+$ B cells that developed in both M167 μκ Rag$^{-/-}$ and *bcl-2*$^+$ M167 μκ Rag$^{-/-}$ mice were anergic. This is illustrated by the data in Table I, which show that no anti-PC Abs were detected in the serum of these mice. When the tolerant M167 μκ Rag$^{-/-}$ B cells were rescued by coexpression of a λ L chain, low levels of TG$^+$ anti-PC Abs were produced (Table I). High levels of anti-PC Abs were detected in all strains of M167 μκ mice with functional Rag genes, including M167 μκ nude mice. This suggests that not only does receptor coexpression reverse tolerance, but the in vivo production of anti-PC Abs is driven by endogenous or environmental TI type 2 Ags. H chain coexpression is once again demonstrated by the presence of mixed μ^a/μ^b anti-PC molecules in all M167 μκ mice with functional Rag genes, except the μMT KO mice, which cannot insert the endogenous μ^b H chain into the B cell membrane (Table I). We previously demonstrated mixed μ^a/μ^b anti-PC molecules in normal M167 μκ transgenic mice (41).

M167 μκ anergic B cells proliferate in vitro

The data in Fig. 6 show that the PC-specific B cells in M167 μκ Rag$^{-/-}$ and *bcl-2*$^+$ M167 μκ Rag$^{-/-}$ mice appear to be developmentally arrested at a stage similar to anergic ssDNA-binding B cells (34). When whole spleen cells were stimulated in vitro with PC-conjugated to Dex or Sepharose or with anti-M167id-conjugated Sepharose, these anergic B cells proliferated. When one normalizes for the number of B cells present in the spleens, the magnitude of proliferation seen in the B cells from M167 μκ Rag$^{-/-}$ and *bcl-2*$^+$ M167 μκ Rag$^{-/-}$ mice would be equivalent to that in the M167 μκ Rag$^{+/+}$ controls. Spleen cells from Rag-$2^{-/-}$ mice did not proliferate in response to any of these TI-type 2 Ags (Fig. 6).

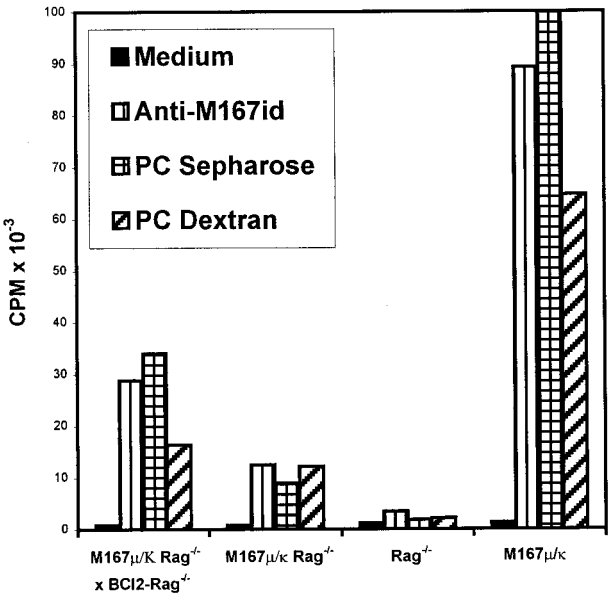

FIGURE 6. Anergic B cells from M167H:κ24L Rag-$2^{-/-}$ mice proliferate in vitro following stimulation with TI type 2 Ags. Spleen cells (5×10^5/well) were stimulated in 96-well plates as previously described (19, 42). The M167 μκ Rag-$2^{-/-}$ spleen had 5% sIgM^{dull} B cells; 11% B cells were present in the bcl-2 M167 μκ Rag-$2^{-/-}$ spleen, and 30% were present in the M167 μκ control. When data were normalized for numbers of B cells present, proliferation was similar in all groups expressing the M167 μκ transgenes.

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Discussion

The findings presented above demonstrate that transgenic M167 $\mu\kappa$ B cells expressing anti-PC Abs exhibit characteristics of autoreactive B cells when these cells develop in a Rag-2 deficient genetic background. Thus, the BM and splenic B cells express low levels of the transgene-encoded H and L chains and no mature B cells are present. These M167 $\mu\kappa$ B cells are clearly anergic, as judged by the lack of serum anti-PC Abs in either the M167 $\mu\kappa$ Rag$^{-/-}$ mice or the *bcl-2*$^{+}$ M167 $\mu\kappa$ Rag$^{-/-}$ mice (Table I). However, these low affinity PC-specific B cells are not as completely tolerized as those found in sHEL/anti-HEL double-transgenic mice, since they proliferate in vitro when stimulated with PC-conjugated TI type 2 Ags (Fig. 6). In this respect these B cells are similar to those found in transgenic mice with Ig receptors specific for ssDNA (34).

We have previously shown that B cells expressing anti-PC transgene Ig receptors in the Xid background are clonally deleted in the absence of a functional btk signaling pathway. Btk appears to play a critical role in the positive selection of long-lived B cells following low affinity receptor engagement by autoantigens that behave like TI type 2 Ags (53, 54). Here we have demonstrated that on the Rag-2$^{-/-}$ KO background these same anti-PC transgene-expressing B cells are developmentally arrested at the pre-B to immature B cell stage. This is similar to B cells expressing other known autoreactive Ig receptors, such as anti-DNA, anti-MHC, or anti-erythrocyte receptors. We have also shown that these autoreactive anti-PC-Ig-expressing B cells home to the peritoneal cavity, an immunologically privileged site, where they develop into B1 B cells. When the PC-specific receptors are modulated by coexpression of endogenous H or L chains, the subsequent signaling intensity is presumably lowered so that the B cells are not clonally deleted, and they no longer become anergic. Furthermore, the B cells rescued by receptor coexpression develop into CD23^{+} B2 B cells as well as B1 B cells (Fig. 3). Clarke and Arnold (55) demonstrated a similar shift from B1 to B2 cell development in the phosphate dylcholine-binding B cells that develop in V_H12 κ4 transgenic mice in the absence of a functional btk signaling pathway. It would therefore appear that the signal intensity from the Ig receptor regulates which B cell subset will develop. B1 B cells dominate when receptor engagement and signaling are high, while lower signaling promotes B2 subset development.

We have also demonstrated that autoreactive M167H:κ24L anti-PC-expressing B cells can be rescued from clonal deletion by overexpression of *bcl-2* in both the Xid and Rag$^{-/-}$ KO backgrounds. However, the B cells that develop in *bcl-2*$^{+}$ M167 $\mu\kappa$ Rag$^{-/-}$ mice remain anergic, appear to down-regulate their sIgM, and do not develop into mature CD23^{+} B cells, whereas the B cells rescued in Xid M167 $\mu\kappa$ mice are predominantly CD23^{+} and are not anergic (19). Once again, the intensity of the signal and the signaling pathway activated by the sIg receptor alter both the subset and the functional state of the B cell. Although the B cells from *bcl-2*$^{+}$ M167 $\mu\kappa$ Rag$^{-/-}$ mice were anergic in vivo, they proliferated in vitro in response to TI type 2 Ags (Fig. 6).

Introduction of the λ1 L chain into M167H:κ24L Rag-2$^{-/-}$ mice was able to rescue B cells expressing the autoreactive Ig receptor. However, the total number of B cells in the spleen was still significantly below normal. A similar decrease in the total number of B cells was observed in the experiments with $\kappa^{-/-}$ KO mice. The decrease in splenic cell numbers in these two models persists even though near normal numbers of B220^{+}/sIgM^{+} cells were observed in the BM. Additionally, normal numbers of splenic B cells were observed in the μMT experiments. These data could be explained if the capacity to populate and proliferate within the spleen varied between B cells expressing different sIg receptors. For B cells at the same developmental stage, the capacity to populate and proliferate within the spleen could reflect the level of cell surface expression of the Ig receptor, the affinity and avidity of the sIg receptors for some positive-selecting Ag, or the quantity and quality of the signal induced by sIg receptor engagement.

Surprisingly, in a M167H:κ24L transgenic Rag^{+} mouse, the number of anti-PC-expressing B cells observed was similar to the number of normal B cells observed in transgenic or normal mice that express nonautoreactive Ig receptors. Membrane-displayed PC in the BM should lead to the clonal deletion of immature PC-specific B cells during their ontogeny (22). However, PC-specific B cells not only escape clonal deletion in the BM, but appear to be positively selected via an Ag-mediated, receptor-driven process (19, 38). In TG^{-} mice, T15-id^{+} B cells get selected into the self-renewing, autoreactive, CD5^{+} subset of B cells (56). This apparent incongruity was explained by demonstrating that these autoreactive anti-PC-expressing B cells escaped clonal deletion by coexpressing more than one functional Ig receptor.

In other models of B cell development that have examined the fate of B cells that express autoreactive Ig receptors, coexpression of multiple Ig receptors has been described (26, 28, 31, 57). The coexpression of multiple Ig receptors has been interpreted within the context of the autoreactive B cells undergoing the receptor-editing process, suggesting that they are transitional. It is evident from our data that 1) B cells expressing anti-PC receptors are autoreactive; 2) coexpression of multiple Ag receptors by the introduction of multiple transgenes can rescue the autoreactive B cells; 3) in a permissive system (i.e., Rag-2$^{+/+}$) these autoreactive B cells express more than one functional Ag receptor on their cells; and 4) this population of autoreactive B cells, which expresses more than one functional Ag receptor, is a stable population of B cells and can constitute a significant portion of the B cell population. The data presented here are certainly concordant with the model of receptor editing; however, it would relax the stringency of application of the principle of allelic exclusion.

It should be noted that the immunological consequence of rescuing autoreactive B cells by coexpressing multiple sIg receptors is entirely different from that achieved by receptor editing. Following receptor editing, the initial Ag specificity of the rescued B cell is lost. However, by coexpressing multiple sIg receptors, one autoreactive and one not autoreactive, B cells retain the autoreactive, yet potentially beneficial, sIg receptor and still escape negative selection by clonal deletion. One possible mechanism by which coexpression of multiple receptors may effect this escape from negative selection is by lowering the number of autoreactive receptors. This could functionally raise the threshold for tolerance induction and allow the B cell to be positively selected into the peripheral B cell repertoire. Experiments in normal mice are currently underway to test this hypothesis.

Coexpression of multiple Ig receptors is not limited to transgenic mouse systems. Gollahon et al. (58) have shown that 20% of λ^{+} B cells coexpress κ L chains. This observation could not be adequately explained by either a stochastic model of V gene rearrangement or a feedback model for controlling V gene rearrangement. It is possible that these endogenous $\lambda\kappa$ double L chain B cells represent cells in which the original $\mu\kappa$ receptor was autoreactive, and the coexpression of λ allowed the B cell to escape tolerance. If our hypothesis is correct, these double L chain-positive B cells will exhibit a high degree of autoreactivity. Additionally, B cells that express two H or L chains generally lose one of these chains after hybridoma formation and in vitro passage (50, 58). This may account for the fact that few anti-PC hybridomas or myelomas express more than one L chain. However, there is at

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least one T15-id myeloma, the HOPC-8 anti-PC, in which two different functional κ L chains are expressed (59).

Generally, the escape of autoreactive B cells from clonal deletion or anergy is considered to be detrimental to the host. However, in our system compromising the tenet of allelic exclusion and rescuing the autoreactive anti-PC binding B cells confers a clear immunological advantage to the host. The germline-encoded T15-id$^+$, anti-PC Abs dominate the immune response in most strains of mice following vaccination with avirulent *S. pneumoniae* or PC-conjugated protein Ags (60–64). Anti-PC Abs are highly protective against pathogens such as *S. pneumoniae* and filariae, and the inability to produce anti-PC Abs results in a dramatic increase in susceptibility to infection by *S. pneumoniae* (13–17). It is likely that this mechanism of escape from clonal deletion and tolerance will account for the frequent observation of potentially beneficial, low affinity autoantibodies in normal individuals. This mechanism is not likely to be used in B cells with high affinity for self Ags; thus, it should not occur when the receptor affinity is $>10^9$, as it is in anti-HEL transgenic mice. High affinity autoreactive B cells would be forced to use receptor editing to escape clonal deletion.

References

1. Yancopoulos, G. M., and F. W. Alt. 1986. Regulation of the assembly and expression of variable-region genes. *Annu. Rev. Immunol. 4:339.*
2. Alt, F. W., T. K. Blackwell, and G. D. Yancopoulos. 1987. Development of the primary antibody repertoire. *Science 238:1079.*
3. Alt, F. W., E. M. Oltz, F. Young, J. Gorman, G. Taccioli, and J. Chen. 1992. VDJ recombination. *Immunol. Today 13:306.*
4. Storb, U., K. A. Ritchie, R. O'Brien, B. Arp, and R. Brinster. 1986. Expression, allelic exclusion and somatic mutation of mouse immunoglobulin κ genes. *Immunol. Rev. 89:85.*
5. Storb, U. 1987. Transgenic mice with immunoglobulin genes. *Annu. Rev. Immunol. 5:151.*
6. Storb, U. 1995. Ig gene expression and regulation in Ig transgenic mice. In *Immunoglobulin Genes*. T. Honjo and F. Alt, eds. Academic Press, New York, p. 345.
7. Uematsu, Y., S. Ryser, Z. Dembic, P. Borgulya, P. Krimpenfort, A. Berns, H. von Boehmer, and M. Steinmetz. 1988. In transgenic mice the introduced functional TCR β gene prevents expression of endogenous β genes. *Cell 52:831.*
8. Malissen, M., J. Trucy, J. Jouvin, E. Marche, P. A. Cazenave, R. Scollay, and B. Malissen. 1992. Regulation of TCR α and β gene allelic exclusion during T-cell development. *Immunol. Today 13:315.*
9. Nussenzweig, M. C., A. C. Shaw, E. Sinn, D. B. Danner, K. L. Holmes, H. C. Morse III, and P. Leder. 1987. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin. *Science 236:816.*
10. Nussenzweig, M., A. Shaw, E. Sinn, J. Campos-Torres, and P. Leder. 1988. Allelic exclusion in transgenic mice carrying mutant human IgM genes. *J. Exp. Med. 167:1969.*
11. Manz, J., K. Denis, O. Witte, R. Brinster, and U. Storb. 1988. Feedback inhibition of immunoglobulin gene rearrangement by membrane μ, but not by secreted μ heavy chains. *J. Exp. Med. 168:1363.*
12. Burnet, F. M. 1959. *The Clonal Selection Theory of Acquired Immunity*. Vanderbilt University Press, Nashville.
13. Kenny, J. J., G. Guelde, R. T. Fischer, and D. L. Longo. 1994. Induction of phosphocholine-specific antibodies in X-linked immune deficient mice: in vivo protection against a *Streptococcus pneumoniae* challenge. *Int. Immunol. 6:561.*
14. Fischer, R. T., D. L. Longo, and J. J. Kenny. 1995. A novel phosphocholine antigen protects both normal and X-linked immune deficient mice against *Streptococcus pneumoniae*: comparison of the 6-*O*-phosphocholine hydroxyhexanoate-conjugate with other phosphocholine-containing vaccines. *J. Immunol. 154: 3373.*
15. Guo, W., A. M. Burger, R. T. Fischer, D. G. Sieckmann, D. L. Longo, and J. J. Kenny. 1997. Sequence changes at the V-D junction of the V_H1 heavy chain of anti-phosphocholine antibodies alter binding to and protection against *Streptococcus pneumoniae. Int. Immunol. 9:665.*
16. Briles, D. E., M. Nahm, K. Schroer, J. Davie, P. Baker, J. F. Kearney, and R. Barletta. 1981. Antiphosphocholine antibodies found in normal mouse serum are protective against intravenous infection with type 3 *Streptococcus pneumoniae. J. Exp. Med. 153:694.*
17. Briles, D. E., C. Forman, S. Hudak, and J. L. Claflin. 1982. Anti-phosphorylcholine antibodies of the T15 idiotype are optimally protective against *Streptococcus pneumoniae. J. Exp. Med. 156:1177.*
18. Kenny, J. J., A. M. Stall, D. G. Sieckmann, M. C. Lamers, F. Finkleman, L. Finch, and D. L. Longo. 1991. Receptor-mediated elimination of phosphocholine-specific B cells in X-linked immune deficient mice. *J. Immunol. 146: 2568.*
19. Kenny, J. J., R. T. Fischer, A. Lustig, H. M. Dintzis, M. Katsumata, J. C. Reed, and D. L. Longo. 1996. *bcl-2* alters the antigen-driven selection of B cells in $\mu\kappa$ but not in μ-only Xid transgenic mice. *J. Immunol. 157:1054.*
20. Hartley, S. B., M. P. Cooke, D. A. Fulcher, A. W. Harris, S. Cory, A. Basten, and C. C. Goodnow. 1993. Elimination of self-reactive B lymphocytes proceeds in two stages: arrested development and cell death. *Cell 72:325.*
21. Melamed, D., R. J. Benschopk, J. C. Cambier, D. Nemazee, and R. J. Benschop. 1998. Developmental regulation of B lymphocyte immune tolerance compartmentalizes clonal selection from receptor selection. *Cell 92:173.*
22. Goodnow, C. C., J. G. Cyster, S. B. Hartley, S. E. Bell, M. P. Cooke, J. I. Healy, S. Akkaraju, J. C. Rathmell, S. L. Pogue, and K. P. Shokat. 1995. Self-tolerance checkpoints in B lymphocyte development. *Adv. Immunol. 59:279.*
23. Nemazee, D., and K. Buerki. 1989. Clonal deletion of autoreactive B lymphocytes in bone marrow chimeras. *Proc. Natl. Acad. Sci. USA 86:8039.*
24. Palanda, R., S. Schwers, R. M. Torres, D. Nemazee, and K. Rajewsky. 1997. Receptor editing in a transgenic mouse model: site, efficiency, and role in B cell tolerance and antibody diversity. *Immunity 7:765.*
25. Chen, C., Z. Nagy, M. Z. Radic, R. R. Hardy, D. Huszar, S. A. Campers, and M. Weigert. 1995. The site and stage of anti-DNA B-cell deletion. *Nature 373: 252.*
26. Gay, D., T. Saunders, S. Camper, and M. Weigert. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J. Exp. Med. 177:999.*
27. Radic, M. Z., J. Erikson, S. Litwin, and M. Weigert. 1993. B lymphocytes may escape tolerance by revising their antigen receptors. *J. Exp. Med. 177:1165.*
28. Tiegs, S. L., D. M. Russell, and D. Nemazee. 1993. Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med. 177:1009.*
29. Hertz, M., and D. Nemazee. 1997. BCR ligation induces receptor editing in IgM$^+$IgD$^-$ bone marrow B cells in vitro BCR ligation induces receptor editing in IgM$^+$IgD$^-$ bone marrow cells in vitro. *Immunity 6:429.*
30. Melamed, D., and D. Nemazee. 1997. Self-antigen does not accelerate immature B cell apoptosis, but stimulates receptor editing as a consequence of developmental arrest. *Proc. Natl. Acad. Sci. USA 94:9267.*
31. Andersson, J., F. Melchers, and A. Rolink. 1995. Stimulation by T cell independent antigens can relieve the arrest of differentiation of immature auto-reactive B cells in the bone marrow. *Scand. J. Immunol. 42:21.*
32. Goodnow, C. C., J. Crosbie, S. Adelstein, T. B. Lavioe, S. J. Smith-Gill, R. A. Brink, H. Pritchard-Briscoe, J. S. Wotherspoon, R. H. Loblay, K. Raphael, et al. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature 334:676.*
33. Cooke, M. P., A. W. Heath, K. M. Shokat, Y. Zeng, F. D. Finkelman, P. S. Linsley, M. Howard, and C. C. Goodnow. 1994. Immunoglobulin signal transduction guides the specificity of B cell-T cell interactions and is blocked in tolerant self-reactive B cells. *J. Exp. Med. 179:425.*
34. Xu, H., H. Li, E. Suri-Payer, R. R. Hardy, and M. Weigert. 1998. Regulation of anti-DNA B cells in recombination-activation gene deficient mice. *J. Exp. Med. 188:1247.*
35. Storb, U., C. Pinkert, B. Arp, P. Engler, K. Gollahon, J. Manz, W. Brady, and R. Brinster. 1986. Transgenic mice with μ and κ genes encoding anti-phosphorylcholine antibodies. *J. Exp. Med. 164:627.*
36. Kitamura, D., J. Roes, R. Kuhn, and K. Rajewsky. 1991. A B-cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature 350:423.*
37. Kitamura, D., and K. Rajewsky. 1992. Targeted disruption of μ chain membrane exon causes loss of heavy-chain allelic exclusion. *Nature 356:154.*
38. Kenny, J. J., C. O'Connell, D. G. Sieckmann, R. T. Fischer, and D. L. Longo. 1991. Selection of antigen-specific, idiotype positive B cells in transgenic mice expressing a rearranged M167-μ heavy chain gene. *J. Exp. Med. 174:1189.*
39. Kenny, J. J., A. M. Stall, R. T. Fischer, E. Derby, M. W. Yang, P. W. Tucker, and D. L. Longo. 1995. Ig(γ)2b transgenes promote B cell development but alternate developmental pathways appear to function in different transgenic lines. *J. Immunol. 154:5694.*
40. Rao, M., W. Lee, and D. Conrad. 1987. Characterization of a monoclonal antibody directed against the murine B lymphocyte receptor for IgE. *J. Immunol. 138:1845.*
41. Kenny, J. J., F. Finkleman, F. Macchiarini, W. C. Kopp, U. Storb, and D. L. Longo. 1989. Alteration of the B cell surface phenotype, immune response to phosphocholine and the B cell repertoire in M167 μ plus κ transgenic mice. *J. Immunol. 138:1363.*
42. Sieckmann, D. G., K. Holmes, P. Hornbeck, E. Martin, G. Guelde, S. Bondada, D. L. Longo, and J. J. Kenny. 1994. B cells from M167 $\mu\kappa$ transgenic mice fail to proliferate after stimulation with soluble anti-Ig antibodies. *J. Immunol. 152: 4873.*
43. Shinkal, Y., G. Rathburn, K. Lam, E. M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A. M. Stall, et al. 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V-(D)-J rearrangement. *Cell 65:855.*
44. Murakami, M., T. Tsubata, M. Okamoto, A. Shimizu, S. Kumagai, H. Imura, and T. Honjo. 1992. Antigen-induced apoptotic death of Ly-1 B cells responsible for autoimmune disease in transgenic mice. *Nature 357:77.*
45. Okamoto, M., M. Murakami, A. Shimizu, S. Ozaki, T. Tsubata, S. Kumagai, and T. Honjo. 1992. A transgenic model of autoimmune hemolytic anemia. *J. Exp. Med. 175:71.*
46. Nisitani, S., T. Tsubata, M. Murakami, M. Okamoto, and T. Honjo. 1993. The *bcl-2* gene product inhibits clonal deletion of self-reactive B lymphocytes in the periphery but not in the bone marrow. *J. Exp. Med. 178:1247.*

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47. Lang, J., B. Arnold, G. Hammerling, A. W. Harris, S. Korsmeyer, D. Russell, A. Strasser, D. Nemazee, G. J. Hammerling, S. J. Korsmeyer, et al. 1997. Enforced *bcl-2* expression inhibits antigen-mediated clonal elimination of peripheral B cells in an antigen dose-dependent manner and promotes receptor editing in autoreactive, immature B cells. *J. Exp. Med. 186:1513.*
48. Lam, K., and K. Rajewsky. 1998. Rapid elimination of mature autoreactive B cells demonstrated by Cre-induced change in B cell antigen receptor specificity in vivo. *Proc. Natl. Acad. Sci. USA 95:13171.*
49. Stall, A. M., F. G. Kroese, F. T. Gadus, D. G. Sieckmann, and L. A. Herzenberg. 1988. Rearrangement and expression of endogenous immunoglobulin genes occurs in many murine B cells expressing transgenic membrane IgM. *Proc. Natl. Acad. Sci. USA 85:3546.*
50. Lam, K., L. A. Herzenberg, and A. M. Stall. 1993. A high frequency of hybridomas from M54 μ heavy chain transgenic mice initially co-express transgenic and rearranged endogenous μ genes. *Int. Immunol. 5:1011.*
51. Roth, P. E., L. Doglio, J. T. Manz, J. Y. Kim, D. Lo, and U. Storb. 1993. Immunoglobulin γ2b transgenes inhibit heavy chain gene rearrangement, but cannot promote B cell development. *J. Exp. Med. 178:2007.*
52. Chen, J., M. Trounstine, C. Kurahara, F. Young, C. Kuo, Y. Xu, J. F. Loring, F. W. Alt, and D. Huszar. 1993. B cell development in mice that lack one or both immunoglobulin κ light chain genes. *EMBO J. 12:821.*
53. Oka, Y., A. G. Rolink, J. Andersson, M. Kamanaka, J. Uchida, T. Yasui, T. Kishimoto, H. Kikutani, and F. Melchers. 1996. Profound reduction of mature B cell numbers, reactivities and serum Ig levels in mice which simultaneously carry the *xid* and CD40 deficiency genes. *Int. Immunol. 8:1675.*
54. Khan, W. N., A. Nilsson, E. Mizoguchi, E. Castigli, J. Forsell, A. K. Bhan, R. Geha, P. Sideras, and F. W. Alt. 1997. Impaired B cell maturation in mice lacking Bruton's tyrosine kinase (Btk) and CD40. *Int. Immunol. 9:395.*
55. Clarke, S. H., and L. W. Arnold. 1998. B-1 cell development: evidence for an uncommitted immunoglobulin (Ig) M^+ B cell precursor in B-1 cell differentiation. *J. Exp. Med. 187:1325.*
56. Masmoudi, H., T. Mota-Santos, F. Huetz, A. Coutinho, and P.-A. Cazenave. 1990. All T15 antibodies (but not the majority of V_HT15^+ antibodies) are produced by peritoneal $CD5^+$ B lymphocytes. *Int. Immunol. 2:515.*
57. Iliev, A., L. Spatz, S. Ray, and B. Diamond. 1994. Lack of allelic exclusion permits autoreactive B cells to escape deletion. *J. Immunol. 153:3551.*
58. Gollahon, K., J. Hagman, R. Brinster, and U. Storb. 1988. Ig-producing B cells do not show feedback inhibition of gene rearrangement. *J. Immunol. 141:2771.*
59. Rudikoff, S. 1983. Immunoglobulin structure-function correlates: antigen binding and idiotypes. *Contemp. Top. Mol. Biol. 9:169.*
60. Cosenza, H., and H. Kohler. 1972. Specific inhibition of plaque formation to phosphorylcholine by antibody against antibody. *Science 176:1027.*
61. Claflin, J. L., R. Lieberman, and J. Davie. 1974. Clonal nature of the immune response to phosphorylcholine. I. Specificity, class, and idiotype of phosphorylcholine-binding receptors on lymphoid cells. *J. Exp. Med. 139:58.*
62. Claflin, J. L., and J. Davie. 1974. Clonal nature of the immune response to phosphorylcholine. IV. Idiotypic uniformity of binding site antigenic determinants among mouse phosphorylcholine antibodies. *J. Exp. Med. 140:673.*
63. Claflin, J. L. 1976. Uniformity in the clonal repertoire for the immune response to phosphorylcholine in mice. *Eur. J. Immunol. 6:669.*
64. Gearhart, P. J., N. H. Sigal, and N. R. Klinman. 1977. The monoclonal antiphosphocholine antibody response in several murine strains: genetic implications of a diverse repertoire. *J. Exp. Med. 145:876.*

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