A B-cell receptor-specific selection step governs immature to mature B cell differentiation

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Seventy percent of peripheral immature conventional (B2) B cells fail to develop into mature B cells. The nature of this cell loss has not been characterized; the process that governs which immature B cells develop into long-lived peripheral B cells could be either stochastic or selective. Here, we demonstrate that this step is in fact selective, in that the fate of an immature B cell is highly dependent on its Ig receptor specificity. A significant skewing of the B cell receptor repertoire occurs by the time cells enter the mature B cell fraction, which indicates that there is selection of only a minority of immature B cells to become mature B cells. Because only a few heavy-light chain pairs are enhanced of the diverse available repertoire, we favor the idea that selection is positive for these few heavy-light chain pairs rather than negative against nearly all others. Because most immature B cells are lost at this transition, this putative positive selection event is likely to be a major force shaping the mature B cell receptor repertoire available for adaptive immune responses.

As B cells develop, they follow an ordered process of differentiation, first in the bone marrow and later in the periphery, where fully mature naive B cells are found. During early stages of B cell development in the bone marrow, Ig gene rearrangement occurs with heavy chain gene rearrangement usually preceding κ or λ light chain gene rearrangement. Productively rearranged heavy chains pair with the surrogate light chains to form the pre-B cell receptor, which may signal the cell to stop rearrangement at the heavy chain locus and begin rearrangement at the κ or λ light chain locus (1, 2). Failure to make productive rearrangements of both heavy and light chains that can pair with each other and be expressed on the cell surface results in loss of that cell.

Bone marrow B cells with functional heavy and light chains express B cell receptors (BCRs) on the cell surface and are called immature B cells (Hardy fraction E). These cells are surface IgM^{hi}, IgD^{-}, $B220^{int}$, HSA^{hi} (3, 4). Before these immature cells leave the bone marrow for the periphery, it has been shown, they undergo several types of negative selection to avoid autoreactivity. These processes, including clonal deletion (5), receptor editing (6, 7), and clonal anergy (8), are thought to be largely complete before the exit of these cells into the periphery, where a fraction of them subsequently differentiate into mature B cells (Hardy fraction F) (9, 10), which are surface IgM^{int}, IgD^{hi}, $B220^{hi}$, HSA^{int} (4). Seventy percent of peripheral immature B cells that bear functional surface IgM (fraction E) do not survive to maturity (fraction F), although the nature of the process that causes this cell loss is unknown (3). This cell loss either could occur by a stochastic process in which cell fate is determined without regard to the specificity of the BCR or could occur by a selective process in which the specificity of the BCR determines which cells can survive.

To better understand the selective processes that determine which immature B cells survive to comprise the mature BCR repertoire, we examined the BCR repertoire of immature peripheral B cells and compared it to the BCR repertoire of mature peripheral B cells. A stochastic process would predict identical BCR repertoires in the immature and mature B cell populations; a selective process would be reflected by substantial differences between the two repertoires. We find that there is indeed significant selection of the BCR repertoire at the immature to mature conventional B cell transition in the periphery of the mouse. The nature of this selection is most indicative of positive selection of certain cells that comprise a small fraction of the BCR repertoire.

Materials and Methods

Mice. Heavy chain transgenic mice of Meg and Daisy line were constructed as described (11) (L.G.H., A.M.H., and M.J.S., unpublished work) and were bred to the CB-17 background.

FACS Analysis. Single-cell suspensions of spleen were incubated on ice with the antibodies IgM^a-PE, B220-Red670, and HSA-FITC for 30 min, were washed, and were sorted on a FACStar Plus (Becton Dickinson). Sorting of Daisy mouse spleen cells not backcrossed to $J_H^{-/-}$ also involved IgM^b-biotin antibody (PharMingen). All biotinylated antibodies were revealed with streptavidin-Texas Red. Additional staining with anti-B7.2-biotin (clone GL1) (12), rat anti-mouse-CD1d purified antibody (PharMingen) followed by a goat anti-rat-IgG-biotin secondary (Kirkegaard & Perry Laboratories), anti-CD5 (clone 53.7), and anti-CD44 (clone PgP-1) (11) was performed to assess surface expression of these markers.

PCR Amplification. Genomic DNA was extracted with the DNAzol reagent (GIBCO/BRL) and was amplified by using a degenerate V_κ consensus primer (13) and one of two $J_\kappa 2$ intronic primers. The internal $J_\kappa 2$ primer (14) was utilized for the first Meg line mouse and all three Daisy line mice analyzed. An external $J_\kappa 2$ primer, TCCCTCCTTAACACCTGATCTGAGAATGG, was used for the analysis of the second two Meg line mice and the wild-type control mouse. PCR conditions were 28 cycles of 94°C for 30 sec, 60°C for 90 sec, and 72°C for 60 sec, with a 5-sec extension per cycle (14).

Cloning and Sequencing. Consensus V_κ-$J_\kappa 2$ PCR products ($\approx$190 bp for the internal $J_\kappa 2$ primer and $\approx$300 bp for the external $J_\kappa 2$

Abbreviation: BCR, B cell receptor.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF076238).

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primer) were visualized on 1.7% agarose gels (Bethesda Research Laboratories). PCR products were directly cloned by using the TopoTA cloning vector (Invitrogen). Individual bacterial colonies were picked, and the inserts were amplified by PCR using the M13(-20) and M13 reverse primers. All PCR products from plasmids containing inserts were purified (GFX PCR Purification Kit, Pharmacia) and were sequenced with the T7 primer using the *Taq* DyeDeoxy Terminator Sequencing Kit (Applied Biosystems) and an Applied Biosystems 373A DNA sequencer.

Sequence Analysis. Sequences were analyzed with MACVECTOR software (Oxford Molecular Group, Oxford, U.K.). A database of κ light chains by the method of Strohal *et al.* was generated in MACVECTOR to assign individual sequences to specific V_κ members (15). Sequences with 1-bp mismatches were considered to be attributable to *Taq* substitution error as described (14). Sequences with >1 bp of mismatch from the Strohal collection were compared with GenBank and in all but one case exactly matched a previously characterized sequence. These sequences are labeled N1–N9 (accession nos.: N1, M84442; N2, U55660; N4, Z25829; N5, M64160; N6, U18598; N7, U18571; N8, U18561; N9, U22902). The single novel sequence, N3, has been submitted to GenBank (accession no. AF076238).

Statistical Analysis. We utilized the distribution of the sum of three hypergeometric random variables (one for each of three mice) at each gene locus (16), and the Bonferroni correction (17) for the number of loci to achieve 5% experimentwise type I error, to test the hypothesis of overexpression in F cells over that in E cells. Six genes in the Daisy experiment were found in either E or F cells from all three mice and hence were testable. After Bonferroni adjustment for multiple comparisons, an experimentwise type I error of 5% was achieved by testing at the $0.05/6 = 0.0083$ level for the Daisy line. Thirteen genes were testable in the Meg line, and so experimentwise type I error 5% was achieved by testing at the $0.05/13 = 0.0038$ level. A fourteenth gene in the Meg experiment was found in all three mice but in insufficient numbers to possibly yield a significant result and, hence, was not tested.

Results

Strategy. We have designed a system to compare the BCR repertoire in sorted immature (population E phenotype) (3) and mature (population F phenotype) (4) B cell populations of an individual mouse. This strategy is based on a previous study that examined TCR α chain CDR3 length and sequence in mice with a fixed transgenic TCR β chain. In mature thymocytes, the α chain CDR3 was highly constrained in the context of a transgenic β chain in the presence of a limited peptide repertoire, providing direct evidence for positive selection of T cells on self peptides (18). Here, by analogy, we have used IgH μ transgenic mice to fix one half of the BCR. This allowed us to focus on the light chain repertoire as an indicator of the specificity of the BCR. If the E to F maturation step occurs by a stochastic process, the E and F populations will have a similar light chain repertoire whereas if this maturation step occurs by a selective process acting upon the BCR, then there will be significant differences in the light chain repertoires of the two populations.

Immature (3) and naive mature (4) splenic B cells from these transgenic mice were sorted by flow cytometry on the basis of surface B220, IgM, and HSA levels (Fig. 1 *a–d*). IgD was unavailable as a marker for mature cells in this system because of the heavy chain transgene construct, but HSA levels have been shown to effectively separate peripheral immature from naive mature B cells (3). The identity of these cells was confirmed by FACS analysis of the sorted populations for B7.2, CD5, and CD1 surface markers. In all cases, the stains were positive for less than

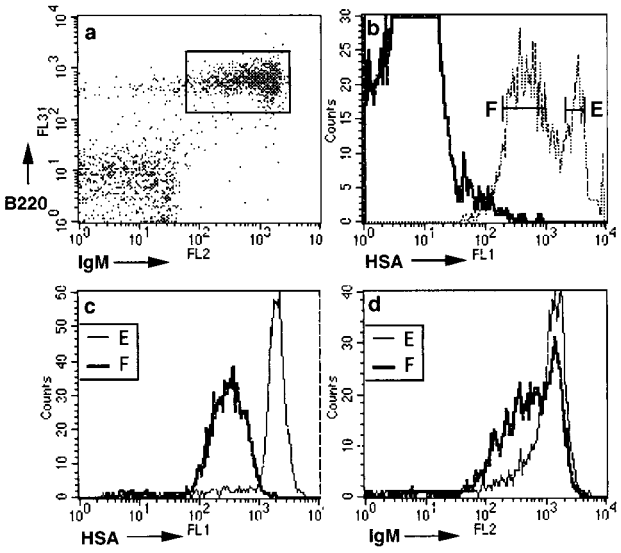

Fig. 1. FACS sorting of immature (population E) and mature (population F) B cells. $B220^+/IgM^{a+}$ splenocytes from Meg mice were gated to separate immature and mature B cells from other cells (*a*), and then sorting of immature B cells (E, HSA^{hi}) from mature B cells (F, HSA^{int}) was performed by using the indicated gates (*b*). The bold line shows unstained cells. FACS reanalysis of immature (thin line) and mature (bold line) B cells from *a* and *b* was performed. (*c*) Reanalysis of the separation of population E and F cells by surface HSA levels as shown in *b*. (*d*) Reanalysis of surface IgM levels in population E and F. Note the uniformly high IgM levels in population E.

2% of cells, indicating that neither sorted population harbored significant numbers of activated, B-1, or marginal zone (19) B cells, respectively (data not shown).

Using a 5′ V_κ primer that amplifies at least 80% of V_κ genes (13), the join between V_κ and $J_\kappa 2$ was amplified by PCR from the genomic DNA extracted from these populations (14). Joins to $J_\kappa 2$ were selected because they are more easily sequenced by our protocol than the longer $J_\kappa 1$ joining products, yet $J_\kappa 1$ and $J_\kappa 2$ are used at a similar high frequency in primary V_κ-J_κ joins (20). The PCR products were used to construct a plasmid library for the E and F populations of each mouse analyzed. Individual clones were randomly selected for sequencing of the V_κ region, and the in-frame joins were tabulated. In this way, the V_κ repertoire was assessed in both the immature and mature B cell populations in the periphery within a single mouse.

Selection at the E to F Transition in the First Transgenic Line. We studied mice from two independent transgenic lines. The first line, on the CB-17 background, bore a V_H186.2 (J558 family) transgene originally isolated from a λ light chain bearing anti-NP hybridoma (21) and was backcrossed to a mouse lacking J_H (22) to ensure expression of only the transgenic heavy chain; these mice were named Meg (L.G.H., A.M.H., and M.J.S., unpublished work). We analyzed three individual mice from the Meg line and compared the in-frame κ light chain repertoires of immature and mature B cells within each individual mouse. In all three mice of this line, the immature B cell κ light chain sequences were diverse (Fig. 2 *a–c*). However, in the mature B cells of all three mice, the frequency of a single member of the $V_\kappa 24/25$ family, sequence 80 as denoted by Strohal *et al.* (15) was significantly enhanced (Fig. 2 *a–c*). The frequency of the sequence 80 light chain was 3- to 7-fold higher in the mature B cell population compared with the immature B cell population within the same mouse. The magnitude of difference in all mice analyzed was similar, and in all cases the sequence 80 light chain

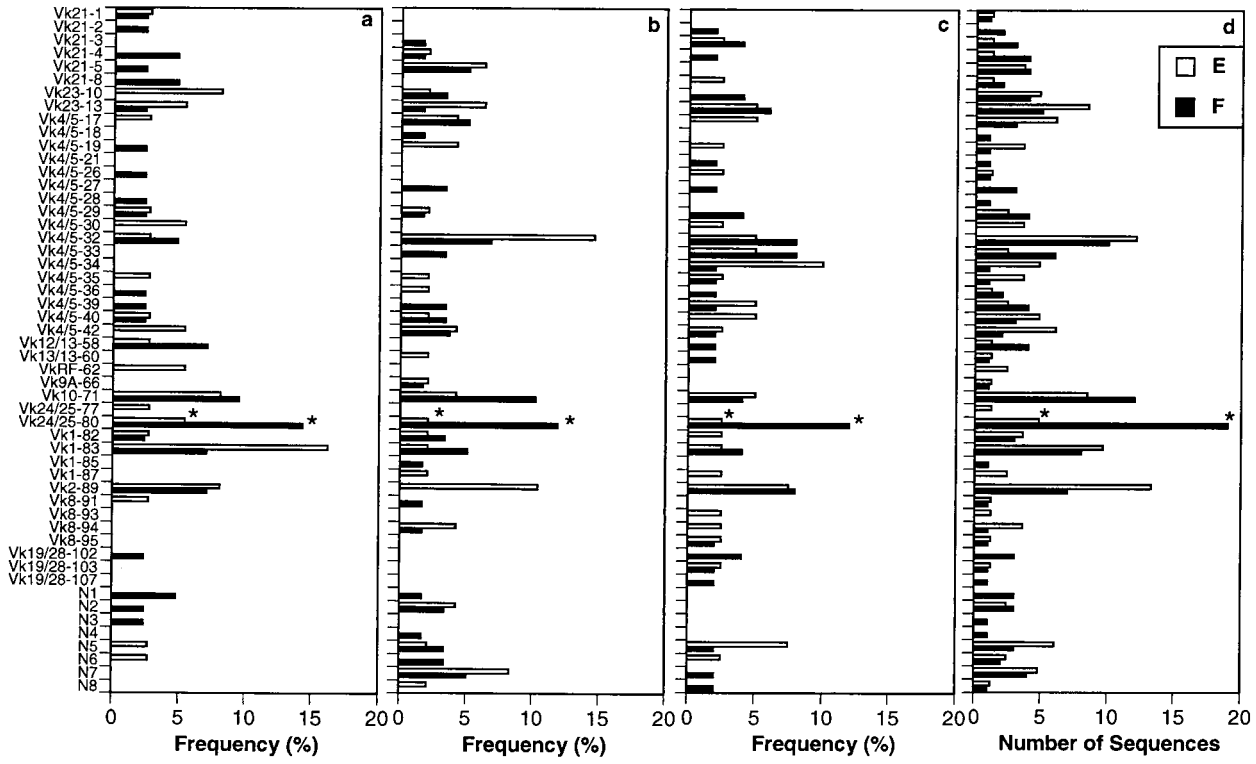

Fig. 2. In-frame κ light chain repertoire is more restricted in mature naive B cells than in immature B cells from three Meg (V_H186.2 transgenic $\times$ $J_H^{-/-}$) mice. κ light chain joins to J_κ2 from immature (population E, white bars) and mature (population F, black bars) B cell populations were amplified, cloned, and sequenced. Each panel compares the in-frame light chain repertoire of immature and mature splenic B cells from an individual mouse. The horizontal axis is labeled with V_κ families, and chain numbers as assigned by Strohal *et al.* (15). Chains not identified by Strohal are labeled N1–N9. The vertical axis depicts standardized κ light chain expression frequency as a percentage of total κ chains sequenced. (*a*) V_κ24/25 family member number 80 (marked with an asterisk) was found to be at a three-fold greater percentage in mature (black bars) than immature (white bars) B cells within the first mouse (E, $n = 37$; F, $n = 42$). This Vκ24/25 family member also represents the most common κ light chain in the mature B cell population. (*b*) In the second mouse, the same V_κ24/25 family member number 80 was found to be enhanced at the E to F transition, representing 12% of total in-frame V_κ chains in the mature B cell population and only 2% in the immature population (E, $n = 48$; F, $n = 59$). (*c*) In a third mouse analyzed, the same V_κ24/25 family member number 80 once again appeared at a much greater frequency in the mature B cell population (12%) than the immature B cell population (2.5%) (E, $n = 40$; F, $n = 50$). (*d*) Tabulation of all V_κ-J_κ2 sequences from the Meg line for immature (E) and mature (F) cells is shown. The horizontal axis is labeled with as above whereas the vertical axis depicts total number of sequences with the immature numbers normalized to be equivalent to the mature numbers ($n = 151$ for both populations). V_κ24/25 sequence 80 (marked with an asterisk) represents just under 5 sequences of 151 in immature B cells (3.2%) and represents 19 of 151 sequences (12.6%) in mature B cells. This enhancement is highly significant ($P < 0.003$).

was the most prevalent light chain isolated from the mature B cells. To ensure against PCR contamination or unsuspected primer-based bias, a different primer set was used for the second and third mouse analyzed with no change in the enhancement of the sequence 80 light chain. The difference in the frequency of the sequence 80 light chain between immature and mature B cell populations is highly significant ($P = 0.003$). More than 85% of sequence 80 isolates from the mature B cells in Meg mice were in frame, also consistent with BCR-specific selection (data not shown). These data, summed in Fig. 2*d*, demonstrate a selection event in the Meg line at the E to F transition, which depends on the expressed V_L and presumably on the V_H/V_L pair.

A Similar Repertoire Restriction at the E to F Transition in a Second Transgenic Line. If the transition of cells from E to F is mediated by the specificity of the V_H/V_L pair of the BCR, a different light chain repertoire would be expected to be selected in a mouse carrying a different heavy chain transgene. To test this idea, we analyzed the V_κ-J_κ2 light chain repertoires of a second distinct J558 family IgH μ transgenic line, also on the CB-17 background. This heavy chain, originally found to be paired with a light chain V_κ8 family member having rheumatoid factor specificity, was used to construct a heavy chain transgenic mouse line named Daisy (11). Immature and mature splenic B cells were sorted from this line by using a similar protocol to that used for the Meg line.

The analysis of three mice of the Daisy line again revealed that skewing of the in-frame light chain repertoire occurs at the E to F transition. Changing the heavy chain transgene, however, resulted in the enhancement of a different light chain than that detected in the Meg line—a member of the V_κ1 family, sequence 82 (Fig. 3 *a–c*). This light chain was significantly enhanced at the E to F transition in every Daisy line mouse analyzed ($P < 0.003$). Additionally, the closest relative to this selected light chain, V_κ1 family, sequence 83, was enhanced in two of three mice analyzed, although it was not detected in either population of the other mouse, possibly because of sample size (P was not significant). Finally, the sole member of the V_κ2 family, sequence 89, was detected frequently in both populations in the Daisy line and in fact was quite prevalent in the Meg line (Fig. 2) and the control (Fig. 3*e*) as well. This may indicate that this light chain recombines frequently in this strain. The V_κ24/25 family member number 80 that was so prominent in the Meg mouse was detected only once in the mature cells of the Daisy line. The data from the Daisy line, summed in Fig. 3*d*, show that a heavy chain that is distinct from that utilized in the Meg mouse selects a distinct set of κ light chains.

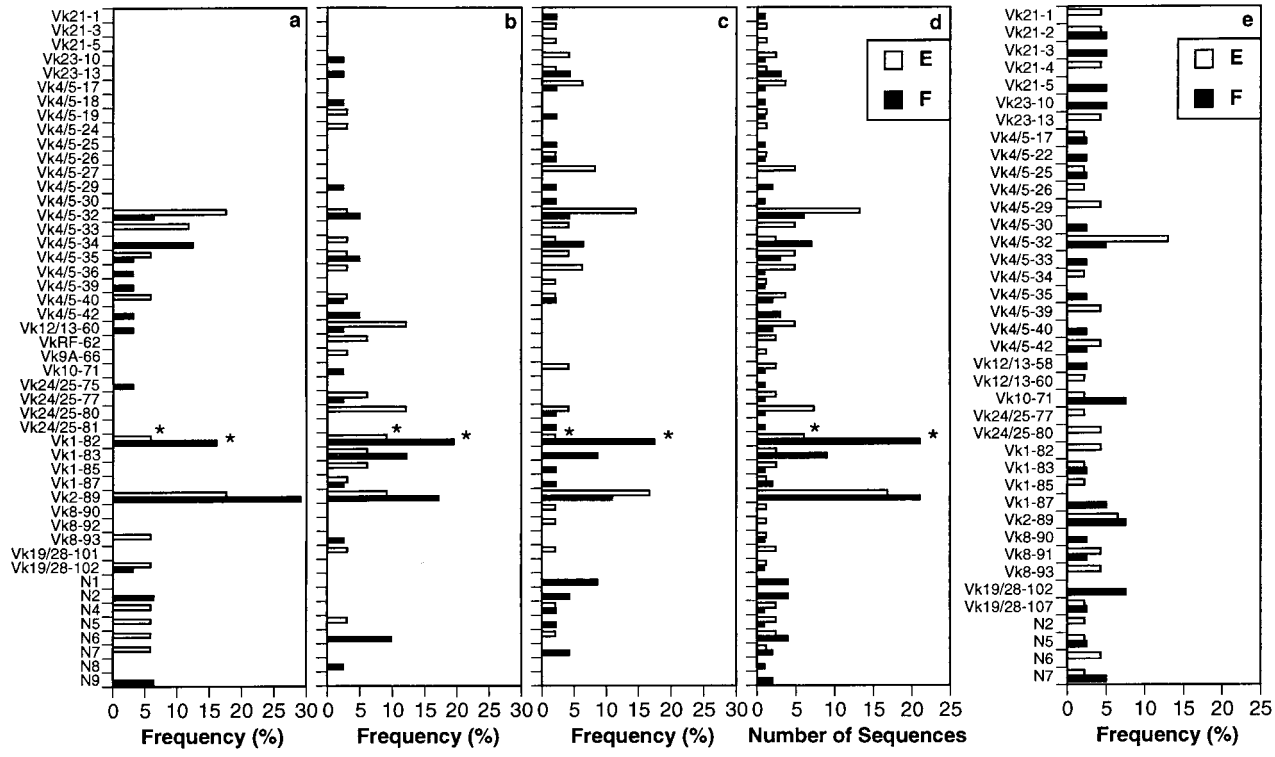

Fig. 3. In-frame κ light chain repertoire is more restricted in mature naive B cells than in immature B cells from three daisy (V_HJ558 Transgenic) mice. In-frame κ light chain joins to $J_\kappa 2$ from immature (population E, white bars) and mature (population F, black bars) B cell populations were amplified, cloned, and sequenced. This figure is as described for Fig. 2 *a–c*. (*a–c*). In all three Daisy line mice analyzed, $V_\kappa 1$ family member number 82 (marked with an asterisk) was more prevalent in the mature B cell population than the immature B cell population, and this enhancement was significant ($P < 0.003$) (15). (*a*) E, $n = 17$; F, $n = 31$. (*b*) E, $n = 33$; F, $n = 41$. (*c*) E, $n = 48$; F, $n = 46$. (*d*) Tabulation of all V_κ-$J_\kappa 2$ sequences from the Daisy line for immature (E) and mature (F) cells is shown. The horizontal axis is labeled with as above whereas the vertical axis depicts total number of sequences with the immature numbers normalized to be equivalent to the mature numbers ($n = 118$ for both populations). $V_\kappa 1$ sequence 82 (marked with an asterisk) represents 6 out 118 sequences (5.1%) in immature B cells and represents 21 of 118 (17.8%) sequences in mature B cells. This enhancement is highly significant ($P < 0.003$). (*e*) The same sequencing protocol as in Fig. 2 was applied to a nontransgenic control mouse of the same background as the Meg line. Population E, white bars; population F, black bars. E, $n = 46$; F, $n = 40$.

Control Nontransgenic Mouse Shows No Repertoire Bias. A control mouse of similar genetic background bearing no heavy chain transgene was analyzed by the same method. We found similar diversity in both immature and mature B cell populations of the control mouse. The sequence 80 light chain (Meg) and the sequence 82 light chain (Daisy) were detected only in the immature population of this control mouse (Fig. 3*e*). This result argues against unsuspected PCR bias as an explanation for the skewing in population F of the Meg or Daisy line. The fact that no significant difference in light chain usage was detected between immature and mature B cell populations shows that fixing one half of the BCR with the IgH transgene is crucial in unmasking this selective event that otherwise would be lost in the complexity of all possible heavy-light chain pairs.

Discussion

Only 30% of the immature B cells that reach the spleen mature into the long-lived pool of recirculating mature B cells (3). The reasons for the enormous cell loss and what controls it have not been clear. We wanted to test the hypothesis that the survival of cells in fraction E as they mature into fraction F is controlled by the specificity of the receptor. Alternatively, this developmental step could be dictated stochastically, without regard for the specificity of the BCR. Our analysis has revealed that receptor-based selection governs this transition from immature to mature B cells in the periphery. It is therefore clear that a stochastic process alone cannot account for the loss of immature B cells at this maturation step.

The data provide some insight into the nature of this selective process. It is not attributable to traditional antigen-mediated activation and clonal expansion. The number of antigen-activated ($B7.2^+$) B cells is less than 2% in each sorted B cell population isolated from the spleen (data not shown), and this number cannot explain the greater than 12% of mature B cells from the Meg line that express a single light chain (Fig. 2). Importantly, a near absence of germinal centers in these unimmunized mice (L.G.H., A.M.H., and M.J.S., unpublished work) and a lack of somatic hypermutation in the light chain sequences from either population was noted (data not shown). Because stimulation with a protein antigen would yield germinal centers, B cell surface B7.2 expression, and somatic hypermutation, whereas stimulation with T-independent antigens would yield B cell surface B7.2 expression (23), we must conclude that antigen-driven activation cannot explain the selection that we have described. It was also determined that the cells analyzed in this study were conventional $CD5^-$ B cells and had no substantial contribution by the repertoire-restricted B1 cell fraction (data not shown), which has previously been shown to be selected by self antigens (24).

Chain-pairing effects are also very unlikely to account for the selection because all immature B cells were uniformly IgM^{hi}—as expected for nonautoreactive immature B cells that have made stable IgH-IgL pairs on the cell surface (Fig. 1*d*) (25). This strongly suggests that any chain pairing biases in the repertoire would have occurred before the peripheral immature B cell stage

of development and would therefore not affect the subsequent repertoire shift noted here.

This leaves either negative selection or positive selection as possibilities to explain the enrichment of specific B cells at the immature to mature transition in the periphery. B cell negative selection has been far better studied than positive selection, primarily by the use of transgenic systems to fix both BCR specificity and antigen availability to force negative selection to occur. Negative selection generally involves the elimination or inactivation of B cells that are specific for self components by processes including clonal deletion, receptor editing, and clonal anergy.

Clonal deletion has been shown to be complete by the immature B cell stage in the bone marrow (5, 9, 26), except in cases where a self antigen has been confined to peripheral tissues by transgenesis and is not available in the bone marrow (27). Even in this restricted case, peripheral autoreactive B cells are not detected, and thus, clonal deletion of these cells likely occurs immediately within the newly emerging immature B cell fraction (27) and not at the developmental step that we have analyzed here. Receptor editing is a process by which self-reactive B cells arrest development and thus continue to rearrange light chains until the self-reactivity of the BCR is mitigated and development resumes, or rearrangement opportunities are exhausted and the cell dies (6, 7). This process also has been shown to be complete by the immature B cell stage in the bone marrow for naive cells (9, 28). Clonal anergy results in the desensitization of self-reactive cells to stimulation through the BCR (8, 26). Cells undergoing anergy down-modulate IgM levels on their cell surface in the immature stage of development (29). We did not note such down-modulation in immature B cells in this work (Fig. 1*d*).

Because all known negative selection events that could explain the receptor skewing are complete by the time that immature B cells emerge in the periphery, we considered the possibility that we have found a novel negative selection event at the immature to mature peripheral B cell transition. However, for this phenomenon to be caused by negative selection, one would have to invoke a set of exclusively peripheral self antigens that induce deletion or receptor editing of most V_κ-$J_\kappa 2$ light chains other than the single member of the $V_\kappa 24/25$ family noted in the Meg line or the $V_\kappa 1$ family member, sequence 82 in the Daisy line. For this explanation to be valid, greater than 50 independent, simultaneous negative selection events would have to be occurring in each line. This seems unlikely to be the case.

Also arguing against negative selection in mature B cells, the residual light chains other than the selected $V_\kappa 24/25$ light chain differ in family and individual chain number between the individual syngeneic mice, suggesting that they are not absolutely selected against. In fact, nearly one-half of all κ light chain V regions were present at some frequency within the mature B cell population of the Meg mice, even with a sample size of 151 sequences. A negative selection event powerful enough to yield such enhancement of a single light chain would by necessity be quite stringent and would not leave such a diverse mature population.

Rather than negative selection, the model that best fits the data is that positive selection on the BCR is crucial in determining the surviving peripheral BCR repertoire. A current review of B cell positive selection states that, although the BCR is required for the generation of naive B cells, it is unclear whether these cells are actually positively selected (30). Previous studies compared either the fetal liver V_H repertoire (31) or the bone marrow preB cell V_H or V_κ repertoire (32–34) to the mature splenic B cell V_H or V_κ repertoire. It is now appreciated that multiple selection steps lie between the populations compared in these studies: positive and/or negative selection on the pre-BCR (35, 36) and negative selection of autoreactive cells in the bone marrow (5, 6, 8, 25). Each of these studies found that the repertoire of both V_H and V_κ is more limited in mature B cells than in early precursors, and in all cases these studies concluded that positive selection was possibly acting on B cells in the multiple intervening steps of development between the populations studied. However, because these earlier studies spanned all of these selection checkpoints, the V_H or V_κ repertoire skewing noted in earlier experiments (31–34) could not distinguish negative or positive selection on the pre-BCR (35, 36) and/or negative selection on the BCR (5, 6, 8, 9, 25–27). An additional recent study analyzed the V_λ repertoire in mature B cells from an adult Ig heavy chain transgenic mouse that also lacked expressed κ light chains (37). In the context of this very limited repertoire, skewing was noted, although negative and positive selection could not be distinguished, and the developmental step of selection was not determined. By focusing on the consecutive E to F populations, our studies differ from those previously described. Moreover, because this transition follows all known selective events, our approach has revealed a novel selection event—consistent with positive selection—at a clearly defined developmental step that is associated with significant cell loss (3).

While our study has utilized conventional B2 cells, it is known that the development of B1 cells involves some degree of positive selection, in that particular V_H families are found at high frequency within the mature B1 BCR repertoire. The development of B1 cells is quite distinct from conventional B2 cells; they appear early in ontogeny and thereafter seem to survive by repetitive partial activation by common antigens in the peritoneal cavity (38). Recent work has shown that the survival of IgH transgenic B1 cells depends on the coexpression of a self antigen that binds strongly to the half-transgenic BCR (24). This more directly shows positive selection of this particular restricted class of B cells. Although there may be some parallels between the peripheral selection of surviving mature conventional B2 cells and the selection of B1 cells, they are clearly distinct processes, and the resultant cells have distinct phenotypes.

Our findings are consistent with and extend several recent reports concerning the role of the BCR in signaling survival of mature B cells. The first study utilized an inducible gene targeting system to demonstrate that continued BCR expression is necessary for survival of mature B cells (39). This report indicates that the BCR has some function in keeping preimmune mature B cells alive apart from its role in antigen-mediated stimulation. However, it did not investigate the specificity of the BCR and therefore does not determine how the BCR plays that role (40). In fact, the authors conclude that the BCR may be delivering a signal even in the absence of antigen: for example, by simply assembling the BCR complex and tonic signaling (39). Our results add that, at least for selecting the mature BCR repertoire that develops beyond Fraction E, the specificity of the BCR, rather than tonic signaling *per se*, is important. It is possible that this selective process continues on in mature B cells as noted by Lam *et al.* (39).

Components of the BCR signaling complex, the *Syk* molecule (41), and the CD45 molecule (42) are involved in B cell maturation beyond the peripheral immature B cell stage. In $CD45^{-/-}$ anti-HEL transgenic mice, it was shown that in the presence of HEL these cells progressed to maturity whereas in the absence of HEL they tended to arrest at the immature stage. This may indicate that by weakening a strong antigenic signal, a weak survival signal can be generated that is sufficient to drive maturation but insufficient to drive activation or deletion (42). *Syk*-deficient mice, while having proximal B cell development inhibition, have a total developmental block at the immature to mature B cell transition in the periphery (41). Our data are consistent with the idea that a physiological

survival signal transduced through *Syk* is delivered in a receptor-specific fashion.

The fundamental significance of our data is that they demonstrate that the loss of the majority of B cells at the fraction E to fraction F transition is selective rather than stochastic. Although not proved directly by these studies, as discussed we favor the interpretation that certain cells are positively selected. If positive selection is occurring at the E to F transition, the question is raised: Why must B cells be positively selected, and what is the nature of the selecting ligand? It is possible that environmental antigens and nonpathogenic flora may be "preselecting" a naive recirculating B cell repertoire that is predisposed to recognize pathogenic antigens it may later encounter (32–34, 43). Positive selection of B cells that can survive as memory cells because of a similar endogenous ligand-receptor interaction may also be occurring (40). Regardless of the purpose of receptor-specific selection of immature B cells into the mature compartment, the present work adds another dimension to the mounting evidence that receptor-ligand interactions signal for the ongoing survival of lymphocytes in the periphery (24, 39, 44–48).

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