Receptor Editing Occurs Frequently during Normal B Cell Development

By Marc W. Retter* and David Nemazee‡

From the *National Jewish Medical and Research Center, Division of Basic Sciences, Department of Pediatrics, Denver, Colorado 80206; and the ‡University of Colorado Health Science Center, Department of Immunology, Denver, Colorado 80220

Summary

Allelic exclusion is established in development through a feedback mechanism in which the assembled immunoglobulin (Ig) suppresses further V(D)J rearrangement. But Ig expression sometimes fails to prevent further rearrangement. In autoantibody transgenic mice, reactivity of immature B cells with autoantigen can induce receptor editing, in which allelic exclusion is transiently prevented or reversed through nested light chain gene rearrangement, often resulting in altered B cell receptor specificity. To determine the extent of receptor editing in a normal, non-Ig transgenic immune system, we took advantage of the fact that λ light chain genes usually rearrange after κ genes. This allowed us to analyze κ loci in IgM + cells to determine how frequently in-frame κ genes fail to suppress λ gene rearrangements. To do this, we analyzed recombinated Vκ-Jκ genes inactivated by subsequent recombining sequence (RS) rearrangement. RS rearrangements delete portions of the κ locus by a V(D)J recombinase-dependent mechanism, suggesting that they play a role in receptor editing. We show that RS recombination is frequently induced by, and inactivates, functionally rearranged κ loci, as nearly half (47%) of the RS-inactivated Vκ-Jκ joins were in-frame. These findings suggest that receptor editing occurs at a surprisingly high frequency in normal B cells.

Key words: receptor editing • recombining sequence recombination • immune tolerance • B lymphocytes • V(D)J rearrangements

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Abbreviations used in this paper: FWR, framework region; JCκD/+, heterozygous κ deficient germline genotype; RS, recombining sequence; Tg, transgenic.
downstream of the Cκ exon (25) and has no coding function (26), but undergoes V(D)J recombinase-dependent rearrangement that inactivates the k locus by deletional rearrangements in ds (26–28) (see Fig. 1). In an autobody knock-in model system, R S rearrangements can inactivate functional k genes (20), but the extent of R S-mediated receptor editing in normal B cells remains unknown.

One approach to estimate the extent of receptor editing in normal B cells is to analyze V(D)J recombinational remnants that are the predicted residue of editing. In mouse B cells, which contain both k and L chain loci, k gene rearrangement almost always occurs after k rearrangement (for review see references 29, 30). Thus, if an appropriate k gene is not assembled, rearrangement at the k locus often follows. In λ+B cells, R S rearrangements usually have deleted the Cκ loci (27, 28, 31) either by recombining to Vκ through the well-characterized heptamer–nonamer recombination signal sequences (Fig. 1 B), or to heptamer sites in the Jκ–Cκ intron (Fig. 1 C) (27, 28, 32). Besides destroying the function of the k locus, this latter mode of R S recombination has two important effects: first, unlike nested Vκκ recombinations, it eliminates the Cκ-associated ds-acting enhancer elements that are critical for Vκκ expression and rearrangement (33–36), and second, it retains any Vκκ join that was previously adjacent to Cκ. This physiological knockout of regulatory sequences required for k gene rearrangement thus “freezes” the locus, allowing an analysis of the Vκκ gene that was assembled adjacent to the Cκ exon just before R S and L gene rearrangement.

In this study, we have isolated such Vκκ joins from a large number of individual IgM +λ+B cells and determined their nucleotide sequences in order to ascertain the extent to which R S inactivates functional k genes in a normal, non-Ig Tg immune system. The results indicate that in normal IgM +B cells R S-mediated receptor editing is induced by and frequently inactivates functionally rearranged k genes, probably because of immune tolerance.

Materials and Methods

Mice. Mice homozygous for the targeted deletion of the Jk–Cκ locus (JκD/JκD; a gift from D. Huszar, GenPharm International, San Jose, CA; reference 33) were maintained under specific pathogen-free conditions in the animal care facility at National Jewish Medical and Research Center. JκD/JκD mice were bred with B10.D2Sn/J mice to generate B10.D2nSn+/JκD+/+ mice (JκD+鼓舞), which were used at 6-8 wk of age.

Cell Sorting and Genomic DNA Isolation. Spellic cells from JκD+/+ mice were isolated and stained with goat anti–mouse IgM–PE (Caltag Labs., San Francisco, CA) and goat anti–mouse λ-FITC (Fisher Scientific Co., Pittsburgh, PA) and sorted on an ELITE flow cytometer (Coulter Corp., Miami, FL) to collect normal IgM+κ+ B cells. Genomic DNA was isolated from cells by over-night proteinase K digestion in lysis buffer (100 mM NaCl, 10 mM Tris Cl, pH 8, 25 mM EDTA, 0.5% SDS) at 55°C, followed by phenol/chloroform extraction and ETOH precipitation.

Analysis of Deleted PCR Amplified Ig K Rearrangements. Genomic DNA from sorted cells was used as a template to amplify Vκκ-intron-R S rearrangements. As shown in Fig. 1, primers A (degenerate Vκ framework region [FW R] [reference 37] and B (RS-101, 5’ ACATGGAGTTTTCCCAGGAATATG 3’; amplifies a product of ~1,450 bps for kκ) using an amplification profile of 1 min at 94°C, 1 min at 58°C and 1 min at 72°C for 30–35 cycles. The resulting Vκκ-intron-R S products were gel isolated and cloned into the TA II vector (Invitrogen, Carlsbad, CA) and colonies were screened by hybridization using the IVS probe (1). PCR clones were sequenced by the dideoxy termination method (Sequenase; United States Biochemical Corp., Cleveland, OH) using vector-specific forward and reverse primers, as well as antisense kκ-specific (5’ CTAACATGAAAC-CTGTGTCTTACACA 3’ and R S-specific (5’ AAGCTA- CATTAGGCTCAAATTAG 3’) primers. Both DNA strands from the PCR clones were sequenced over the Vκκ joins to verify the reading frame.

Production of λ+B hybridomas. Splenocytes from B10.D2Sn/JκD+/+ mice were cultured in DM EM supplemented with 10% fetal bovine serum plus 50 μg/ml LPS (E. coli LPS; Sigma Chemical Co., St. Louis, MO) for 3 d, then fused with N 50–bcl2 (38) myeloma cells for fusion 1 or SP2/0 (39) myeloma cells for fusions 2 through 5. Hybridoma supernatants were screened by ELISA for secretion of micrometer/liter Ig and the lack of k Ig secretion.

Analysis of λ+B hybridoma Ig R Rearrangements. λ+B hybridoma genomic DNA was digested with EcoR I or BamH I, then fractionated on 0.8% agarose gels, blotted to nylon membrane, and hybridized with the R S 0.8 (27) or IVS probes (Fig. 1 A). Vκκ-R S rearrangements were identified by genomic Southern blot analysis using the R S probe and/or by PCR amplification using primer A and primer B to yield a PCR product of ~255 bps. Vκκ-intron-R S rearrangements were identified by genomic Southern blot analysis using both the R S and IVS probes and/or by PCR amplification using primer C (kκ intron, 5’ CTAACATGAAAC-CTGTGTCTTACACA 3’) and primer B, and amplified for isolation and sequencing using primers A and B. The resulting products were isolated from 1.8% low-melt agarose gels, cycle sequenced directly (Dye Terminator Cycle Sequencing Ready Reaction kit; PE Applied Biosystems, Norwalk, CT) and analyzed using an ABI 377 DNA Sequencer (PE Applied Biosystems). To obtain near-full-length sequences of hybridoma 1-2A7, 1-2E11, 2-2H11, 3-15D6, 3-15C4 and 3-17B10 Vκκ genes, a consensus FWR1 oligo (amino acids –1 through 8; 5’ GGTGACATTGTCGTTCCAGTCTTCA 3’) was used with antisense kκ-intron oligos for PCR amplification, followed by cycle sequencing of the products. For hybrids 1-3E8, 2-15E11, 3-7G5, 4-1D2, 1-10A11 and 1-11A4, Vκκ leader-specific oligos (Ig Prime kit; Novagen, Madison, WI) were used for amplification.

Cloning and Expression of V(D)J R Rearrangements for Analysis of K Chain Pairing. The H chain V(D)J and the L chain Vκκ-R S rearrangements from hybridoma 2H11 were genomically cloned as previously described (40), with the modifications that λ2apo (Stratagene, La Jolla, CA) was the cloning vector and the R S and IVS probes were used to screen clones for the Vκκ-R S rearrangement. The 6.5-kb EcoR I fragment containing the V(D)J rearrangement and the 4.0-kb EcoR I-Xbal fragment containing the Vκκ rearrangement were gel isolated and ligated to pr μSal, a Cμ expression vector (41) and pSV2-neo-Cκ, a Cκ expression vector (42) respectively. The H chain of hybridoma 15E11 was cloned by PCR amplification using a leader intron oligo (5’ GAACTGCGACCTGGTGAATAATGACA 3’) and an oligo that spanned the Xbal site downstream of 3μ (5’ CAGTCTCCAGACCTTCTTCA 3’). The resulting product was digested with EcoO109I and Xbal and ligated into EcoO109I and Xbal digested 8-1Cκ expression vector.
V_{k^\text{R}}-RS rearrangement from hybridoma 15E11 was also cloned by PCR using a leader intron oligo (5' TGGAGTTCCAGGT-TCTACTGGGAGCATTTG-3') and an oligo which spanned the Xbal site downstream of jk5 (5' ACGAGTTGCTC-TAAGAGCCAGCTACCT 3'). The resulting product was digested with EcoRI and subcloned into a shuttle vector containing V_{k^\text{C}} leader and promoter elements. An Xbal fragment that contained the promoter elements, leader, and the 15E11 V_{k^\text{R}} rearrangement was isolated and cloned into the XbaI site in pSV2-neo-C_{\text{k}} (42). The 2H11 and 15E11 H and L chain constructs were then cotransfected into SP2/0 myeloma cells and selected by PCR using a leader intron oligo (5' 9 C leader and promoter elements. An XbaI fragment that contained the J_{k^5} enhancer and the XbaI site downstream of J_{k^5} was added in McIlvain's buffer (84 mM Na_{2}PO_{4}/48 mM citrate) and incubated for 2 h. After a final wash, the chromogenic substrate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co.) was added in PBS/Tween 20, then incubated with blocking buffer (PBS, 0.5% BSA, 0.4% Tween 20) for 1 h at room temperature. Serial dilution of transfected and parental hybridoma supernatants were added and incubated at room temperature for 2 h. Plates were washed and horseradish peroxidase–conjugated goat anti–mouse IgM (Southern Biotechnology Associates, Inc., Birmingham, AL) was added and incubated at room temperature for 3 h. Plates were washed five times with PBS/Tween 20, then incubated with blocking buffer (PBS, 0.5% BSA, 0.4% Tween 20) for 1 h at room temperature. Serial dilution of transfectedoma and parental hybridoma supernatants were added and incubated at room temperature for 2 h. Plates were washed and horseradish peroxidase–conjugated goat anti–mouse IgM (Southern Biotechnology Associates, Inc.) was added and incubated for 2 h. After a final wash, the chromogenic substrate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co.) was added in McIlvain’s buffer (84 mM Na_{2}PO_{4}/48 mM citrate, pH 4.6) with 0.005% H_{2}O_{2}, and O.D. 410 nm was read using an automated plate reader (Dynatech, Alexandria, VA). Transfectedoma and hybridoma antibody concentrations were estimated by comparison to a TEPC 183 (\mu k) standard curve.

### Results

Strategy for the Isolation of Editing Remnants. To determine the extent to which RS recombination inactivates functional, in-frame V_{k^\text{R}}-RS joins in the preimmune B cell repertoire, IgM \lambda^+ splenic B cells were isolated by fluorescence activated cell sorting and their genomic DNA was analyzed by the PCR strategy outlined in Fig. 1C. This cell sorting strategy should exclude from the template pool cells that are \kappa^-, H chain isotype switched, surface (s)Ig^-, or cells of a sIg^6, germinal center phenotype. In a second series of experiments, IgM \lambda^+ secreting hybridomas were isolated and their \kappa loci analyzed in detail. To simplify these analyses, all B cells analyzed were heterozygous for a targeted deletion of the jk-C_{\text{k}} locus (JK D/+) and RS allele could rearrange (33). The potential \kappa gene and RS element rearrangements are depicted in Fig. 1.

A analysis of IgM \lambda^+ C cells reveals frequent receptor editing. Genomic DNA from sorted IgM \lambda^+ cells was used as template for a PCR using a panspecific V_{k^\text{C}} FWR 3 oligonucleotide primer, which recognizes ~80% of V_{k} genes (37), together with an RS-specific primer to amplify V_{k^\text{R}}-intron-RRS rearrangements (Fig. 1C, primers A and B). V_{k^\text{R}}-intron-RRS rearrangements containing V_{k}\kappa-RRS were most abundant, in part because their smaller size promoted preferential amplification. Amplified V_{k^\text{R}}-intron-RRS rearrangements were gel-purified and cloned, and a total of 52 clones were sequenced across both the V_{k^\text{R}} and the jk-intron-RRS joins (Fig. 2).

These two different recombination joins, present on each PCR product analyzed, provided markers for uniqueness. PCR products that were identical to one another, or that differed by just one nucleotide, were assumed to represent repeated isolates derived from the same initial template (i.e., derived from a single B cell clone). This represents an underestimate because the single base changes could have reflected real differences and because it was possible that some of the apparent repeats were independent events that happened to have identity in the portions of the genes studied, but not in upstream portions of the V_{k} genes. In this sample, at least 37 of the 52 clones represented independent events. Analysis of the V_{k^\text{R}}-junction sequence allowed an assessment of the potential prior functionality of the V_{k^\text{R}}-RS joins just upstream of intron-RS rearrangements. Surprisingly, 15 of the 37 clones (41%) contained V_{k^\text{R}}-RS joins that were in-frame (Fig. 2), and if the apparent repeats were not excluded 23 out of 52 (44%) were in-frame.

To verify the analysis of the PCR-amplified V_{k^\text{R}}-intron-RS rearrangements and to increase the sample size, an independent sampling of V_{k^\text{R}}-intron-RS rearrangements was derived from JCkD/+ splenocytes in the form of B

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**Figure 1.** RS rearrangements inactivate and preserve V_{k^\text{R}} joins. A rearranged, potentially functional \kappa locus (A) can be silenced by two types of RS recombination: V_{k^\text{R}}-RS (B) or V_{k^\text{R}}-intron-RS (C). Type C retains the prior V_{k^\text{R}}-RS rearrangement, and the RS recombination event eliminates the known \alpha acting elements that are critical for efficient rearrangement and expression, thus freezing the locus from further V_{k^\text{R}}-RS recombination. Also shown are the intronic recombination sequence (RS1) (32), the intronic (IE) and 3' kappa (3 E) enhancers (35, 36), and the recombining sequence (RS) (27, 28) element. Probes IIS1 (1) and RS 0.8 (27, 28) are indicated by filled boxes.
cell hybridomas. A total of 133 IgMλ-expressing hybrids were obtained from five separate fusions and their κ locus rearrangements were analyzed. Genomic Southern blot and PCR analysis revealed that at least 74% of the λ+ hybrids (99 out of 133) had inactivated the wild-type κ locus by RS rearrangements (Table 1), a value in accord with previous estimates (31, 34). Two hybridomas apparently had undergone inversionsal Vκ-RS rearrangements, as they showed unique restriction fragments that retained the Cκ locus as revealed by the intron (IVS) probe (data not shown), but scored positive in a Vκ-RS PCR (Fig. 1B). Approximately 25% (26 out of 99) of the hybridomas with RS rearrangements had Jκ-intron-RS joins (Table 1), as detected with primers B and C (Fig. 1C). Genomic Southern blot analysis of (A) productive and (B) nonproductive VκJκ-intron-RS rearrangements from FACS® sorted, IgM λ+ splenic B cells. Vκ gene family and Jκ gene usage were assigned based on homologies to expressed Vκ genes (52) or homology searches of Genbank and the Kabat Ig database (33). Translated Vκ FWR3 and CDR3 sequences are shown for productive rearrangements, whereas FWR3 and CDR3 sequences are shown for nonproductive rearrangements, with the asterisk (*) adjacent to CDR3 in B denoting an out of frame Vκ-Jκ join. The nucleotide sequences of the unarranged Jκ intronic recombining sequence 1 (IR S1) and R element (both of which contain a consensus heptamer sequence adjacent to the Δ symbol) are shown above the sequences of the IR S1-R S joins present in each PCR clone. The R S join sequence for clone 17 was not determined. Underlined nucleotides could be donated by either the IR S1 or the R S sequence, and N region addition (bold) and P-encoded nucleotides are shown between the joins. R repeats denote the number of times a particular sequence was observed.

**Table 1.** κ Locus Rearrangement Status of IgM λ+ Hybridomas

<table>
<thead>
<tr>
<th>Hybridoma</th>
<th>κ Locus Genotype</th>
<th>Productive/Nonproductive</th>
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</thead>
<tbody>
<tr>
<td>Fusion 1</td>
<td>n</td>
<td>37 12 16 9/9* 5/4</td>
</tr>
<tr>
<td>Fusion 2</td>
<td>n</td>
<td>44 10 30 4/4 2/2</td>
</tr>
<tr>
<td>Fusion 3</td>
<td>n</td>
<td>12 1 6 5/4 4/0</td>
</tr>
<tr>
<td>Fusion 4</td>
<td>n</td>
<td>25 9 11 5/2 1/1</td>
</tr>
<tr>
<td>Fusion 5</td>
<td>n</td>
<td>15 2 10 3/1 0/1</td>
</tr>
<tr>
<td>Totals</td>
<td>n</td>
<td>133 34 73 26/20 12/8</td>
</tr>
</tbody>
</table>

**Figure 2.** Sequence analysis of (A) productive and (B) nonproductive VκJκ-intron-RS rearrangements from FACS® sorted, IgM λ+ splenic B cells. Vκ gene family and Jκ gene usage were assigned based on homologies to expressed Vκ genes (52) or homology searches of Genbank and the Kabat Ig database. Translated Vκ FWR3 and CDR3 sequences are shown for productive rearrangements, whereas FWR3 and CDR3 sequences are shown for nonproductive rearrangements, with the asterisk (*) adjacent to CDR3 in B denoting an out of frame Vκ-Jκ join. The nucleotide sequences of the unarranged Jκ intronic recombining sequence 1 (IR S1) and R element (both of which contain a consensus heptamer sequence adjacent to the Δ symbol) are shown above the sequences of the IR S1-R S joins present in each PCR clone. The R S join sequence for clone 17 was not determined. Underlined nucleotides could be donated by either the IR S1 or the R S sequence, and N region addition (bold) and P-encoded nucleotides are shown between the joins. R repeats denote the number of times a particular sequence was observed.
direct PCR-derived clones, whereas 6 hybridomas expressed distinct \( V_k \) genes that were members of families observed in the PCR clone sample and 1 hybridoma expressed a \( V_k32 \) gene, a \( V_k \) family not seen in the PCR clone samples (Figs. 2 and 3 and data not shown).

**Intron/RS Joins.** The sequences of the \( J_k \)-intron-RS joins in both the PCR clones (Fig. 2) and hybridomas (Fig. 3B) were quite varied and were dominated by deletions at both sides of the joins, as up to nine nucleotides were missing from either the \( J_k \)-intron or RS heptamer-flanking sequences. There did appear to be a bias for a particular join (e.g., clone 4, Fig. 2A), which was observed to be associated with 13 independent \( V_kJ_k \) rearrangements. Two of the intron-RS joins contained \( P \) nucleotides and one contained N-region addition nucleotides, consistent with findings described previously (7, 43).

**Rebuilding IgMk Antibodies for Analysis of H/L Pairing and Antigen Specificity.** To determine if the high frequency of in-frame \( V_kJ_k \) rearrangements silenced by intron-RS recombination was due to the inability of \( H \) chains to pair with their \( L \) chains, the \( V(D)J \) and \( V_kJ_k \) rearrangements from hybridomas 2H11 and 15E11 were cloned into \( C_m \) and \( C_k \) expression vectors, respectively. These \( H \) and \( L \) chain constructs were cotransfected into SP2/0 myeloma cells to generate transfectoma clones. Analysis of transfectoma supernatants by IgMk sandwich ELISA revealed that the in-frame \( L \) chains were able to pair with their hybridoma \( H \) chains (Fig. 4), suggesting that ongoing \( R \) S rearrangement was not due to the inability of \( H / L \) chain pairing. The specificity of the \( \mu_k \) transfectoma antibodies remains unknown, however. Attempts in flow cytometry assays to detect recombinant antibody binding to the surfaces of bone marrow cells were unsuccessful (data not shown).

**Discussion**

In this report we examined the DNA sequences of \( V_kJ_k \) joins located upstream of intronic-RS rearrangements in normal, non-Ig Tg B cells to determine the extent to which

![Figure 3. Sequence analysis of \( V_kJ_k \) intron-RS rearrangements from IgMk hybridomas. (A) Sequences of the \( V_kJ_k \) rearrangements. The first digit in the hybridoma name indicates the fusion experiment number. Myeloma fusion partners were either NSO-bcl2 (fusion 1) or SP2/0 (fusions 2-5). \( V_k \) gene family and \( J_k \) gene usage were assigned as described in Fig. 2. \( P \) and \( NP \) denote productive and nonproductive \( V_kJ_k \) rearrangements, respectively. Translated amino acid sequences of \( V_k \) FWR, CDR, and \( J_k \) sequences to the conserved phenylalanine (F) residue are shown for productive rearrangements, and \( V_k \) FWR and CDR sequences, with * denoting an out of frame \( V_kJ_k \) join and # denoting an in-frame stop codon, are shown for nonproductive rearrangements. These sequence data are available from EMBL/Genbank/DDBJ under accession numbers AF087023–AF087034 and AF087460–AF087467. (B) Sequences of the R S rearrangements (as described in Fig. 2). (Published October 5, 1998)

![Figure 4. “Repair” of intron-RS recombination-silenced \( V_kJ_k \) genes by restoration of \( C_k \) exon and surrounding elements reveals that silenced \( L \) chains can pair with their original \( \mu \) chain partner. The graph shows representative results from a \( \mu_k \) ELISA comparing several IgMk transfectoma antibodies (Tfc) to their IgMk parental hybridoma antibodies (Hyb). Antibodies in supernatants were captured on plastic using adsorbed anti-\( \lambda \) chain conjugates. Bars indicate the SD determined from antibodies assayed in triplicate. The concentrations of the hybridoma antibodies were at least 10-fold higher than those of the transfectoma antibodies based on comparison to a TEPC 183 (\( \mu, \kappa \)) standard curve.]

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which RS-mediated recombination silences functionally rearranged κ genes. Nearly half of all VKJκ joins inactivated by RS recombination were in-frame (27 out of 57). This high frequency is clearly incompatible with a strict feedback suppression model of L chain allelic exclusion, which predicts no in-frame VKJκ joins upstream of the RS rearrangements. More strikingly, this high frequency is also significantly higher than 33%, the percentage of in-frame joins expected from random VKJκ rearrangement, indicating that productive VKJκ rearrangements actively induce intron-RS rearrangements. The data also demonstrate a physiological role for the RS element in normal B cell development—the inactivation of functionally rearranged κ genes.

To understand why we conclude that the RS rearrangements were actively induced by functional κ L chains, consider the extreme hypothetical cases of mice in which all κ gene rearrangements result in either autoreactive B cell receptors or nonproductive κ chains (Table 2). If VKJκ and RS rearrangements proceed randomly, albeit with different relative frequencies, then in either case VKJκ joins located upstream of intronic-RS rearrangements should be in-frame at a maximum frequency of one out of three. To significantly exceed this frequency, in-frame VKJκ joins must stimulate the relative rate of (intronic) RS rearrangements. This argument applies to our data because the observed frequency of in-frame joins, 47.4%, is significantly higher than one out of three (p < 0.04, single sample test of a proportion based on a normal approximation). Since it is exceedingly unlikely that the stimulus for increased in-frame rearrangements is mediated by anything other than κ protein, and because κ chains can probably only be perceived by the signaling machinery of B cells through their association with H chains, we conclude that functional κ chains actively stimulate the rate of RS rearrangement based on B cell receptor antigenic specificity. These data also predict that in mice in which the Cκ exon is inactivated, but surrounding ds-acting elements are left intact, VKJκ rearrangement should be extensive, whereas RS rearrangement should be reduced. This is in fact the experimental observation (44).

The statistical argument also excludes the possibility that a high frequency of rearrangeable VKκ pseudogenes, L chains that fail to pair with H chains, or a role for positive selection is responsible for our results. Furthermore, complete sequencing of the coding regions from all the in-frame VKJκ rearrangements derived from λκ+ hybridomas revealed no stop codons or other obvious defects that would have precluded function (Fig. 3 A). It is also unlikely that frequent aberrant H/L chain pairing is responsible for the high frequency of in-frame VKJκ joins in the VKJκ-intron-RS rearrangements, as demonstrated by the ability of H/κL chains from two hybridomas to pair (Fig. 4). Moreover, there are few examples of L chains that fail to pair with H chains and most experiments suggest that virtually all random H/L pairs can associate (40, 45–48). Finally, if a lack of positive selection of surface Ig was responsible for the high frequency of in-frame joins, this would predict that B cells should frequently express two κ chains, a result that has not been observed.

The receptor editing events documented in this study probably do not represent renewed V(D)J recombination in mature B cells, such as has recently been described in the germinatal center (49–51), because the cells analyzed expressed high levels of IgM and λ chain and because they were isolated and, in the case of the hybridomas, stimulated in a manner that should not have induced V(D)J recombination. Another indication that receptor editing in mature B cells is unlikely to explain our results is that the fraction of λκ+ cells in newly formed and mature splenic B cells is nearly identical, suggesting that in unmanipulated mice mature κ+ cells rarely give rise to λκ+ B cells (44). Overall, it would appear from our data that the RS rearrangements that we studied were actually stimulated, rather than inhibited, by productive κ gene rearrangements, probably as the result of immune tolerance-mediated receptor editing in immature B cells. To definitively test the prediction that the κ chains of the cells that we have analyzed generate autotoantibodies in association with the same cell’s heavy chain, it will be necessary to generate mice transgenic for these genes.

<table>
<thead>
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<th>Models</th>
<th>Predicted fraction in-frame</th>
<th>Experimental data</th>
<th>Observed percentage in frame</th>
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<tr>
<td>Perfect feedback regulation with functional κ chain preventing λ rearrangement</td>
<td>0%</td>
<td>27 out of 57 VKJκ-intron-RS loci</td>
<td>47.4% (p &lt; 0.04)</td>
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<tr>
<td>Poor feedback regulation</td>
<td>≤33%</td>
<td></td>
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<tr>
<td>High frequency of VKκ pseudogenes</td>
<td>≤33%</td>
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<tr>
<td>High frequency of H/κ chain mispairing</td>
<td>≤33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any combination of the above</td>
<td>≤33%</td>
<td></td>
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<tr>
<td>Extreme model of editing with random RS rearrangements and all κs autoreactive</td>
<td>33%</td>
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We thank D. Huszar for providing JCκD/JκκD mice; B. Diamond (Albert Einstein College of Medicine, Bronx, N.Y.) for providing the NSO-bcl2 myeloma; S. Sobus for cell sorting; D. Norsworthy for cycle sequence analyses; D. Ikile of the Biostatistics Department for statistical analyses; K. Karjalainen and L. Wysocki for discussions; and M. Hertz, D. Melamed, V. Kouskoff, and other members of the lab for critical reading of the manuscript.

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Address correspondence to David Nemazee, The Scripps Research Institute, Mail drop IM-29, 10550 North Torrey Pines Rd., La Jolla, CA 92037. Phone: 619-784-9528; Fax: 619-784-8805; E-mail: nemazee@scripps.edu

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