Receptor Editing Occurs Frequently during Normal B Cell Development

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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 λ+ cells to determine how frequently in-frame κ genes fail to suppress λ gene rearrangements. To do this, we analyzed recombed VκJκ genes inactivated by subsequent recombing 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 VκJκ 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

The fact that virtually all B cells express a single H and L chain prompted many studies to elucidate the underlying mechanism. One process that clearly contributes to allelic exclusion is the imprecision of V(D)J rearrangement that generates a maximum of one in-frame rearrangement per three attempts (1), but more active feedback processes are also involved. Classic studies showing the ability of a H chain transgene (2, 3) or an L chain transgene (4, and for review see reference 5) to mediate feedback suppression of H and L chain rearrangements, respectively, established important paradigms that have been widely accepted. But in the case of L chain allelic exclusion, this paradigm was weakened by an increasing number of “exceptions”, in which ongoing L chain rearrangement occurred despite expression of functional κ chain (6–9). Studies with autoantibody transgenic (Tg)1 mice suggested that many of the exceptions to the feedback regulation model of L chain allelic exclusion could be explained by postulating self-tolerance-induced receptor editing (10–15). In addition, recent in vitro studies (16–18) and analyses of autoantibody Ig knock-in mice (19, 20) have shown that L chain gene receptor editing can be an important mechanism of B cell tolerance. Despite these findings, it is unclear how frequently receptor editing is used for tolerance induction in normal, non-Ig Tg autoreactive B cells, in part because the extent of autoreactivity in the preselected B cell repertoire is unknown.

The organization of the κ locus, with arrangement of Vκ genes in both sense and antisense transcriptional orientations, the absence of D region gene segments, and the presence of several Jκ gene segments facilitates sequential, nested Vκ-to-Jκ rearrangement attempts (for review see reference 21). In developing B cells, these secondary rearrangements can both rescue receptor expression in cells that fail to assemble in-frame L chains (1, 22) and rescue autoreactive B cells from tolerance elimination by replacing rearranged κ genes with new ones that alter specificity (for review see reference 23). Another way that the organization of the κ locus promotes receptor editing is suggested by the existence of the conserved element known as recombing sequence (RS) in the mouse (or the homologous “κ deleting element” in humans; reference 24). RS is located ~25 kb

1Abbreviations used in this paper: FWR, framework region; Jκ·D/+, 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 κ locus by deletional rearrangements in δs (26–28) (see Fig. 1). In an autoantibody knock-in model system, RS rearrangements can inactivate functional κ genes (20), but the extent of RS-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 κ and λ L chain loci, λ gene rearrangement almost always occurs after κ rearrangement (for review see references 29, 30). Thus, if an appropriate κ gene is not assembled, rearrangement at the λ locus often follows. In λ+ B cells, RS rearrangements usually have deleted the Cκ locus (27, 28, 31) either by recombinating to Vκs 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 κ locus, this latter mode of R S recombination has two important effects: first, unlike nested VκJκ recombinations, it eliminates the Cκ-associated δs-acting enhancer elements that are critical for VκJκ expression and rearrangement (33–36), and second, it retains any VκJκ join that was previously adjacent to Cκ. This physiological knockout of regulatory sequences required for κ gene rearrangement thus “freezes” the locus, allowing an analysis of the VκJκ gene that was assembled adjacent to the Cκ exon just before RS and λ gene rearrangement.

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

Materials and Methods

Mice. Mice homozygous for the targeted deletion of the Jκ-Cκ locus (JκΔD/JκΔD; 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 Medical and Research Center. JκΔD/JκΔD mice were bred with B10.D2nSn/J mice to generate B10.D2nSn/JκΔD+/+ mice (JκΔD+/+), which were used at 6–8 wk of age.

Cell Sorting and Genomic DNA Isolation. Splenic cells from JκΔD/+ 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 IgM+λ+ B cells. Genomic DNA was isolated from cells by overnight 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 Direct PCR Amplified IgR Rearrangements. Genomic DNA from sorted cells was used as a template to amplify VκJκ-intron-RS rearrangements. As shown in Fig. 1, primers A (designated Vκ framework region [FWR] 3; reference 37) and B (RS -101, 5' ACATGGAGTTTTCCCGGAATACTG 3') amplified a product of ~1,450 bps (for Jκx) 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 Vκx-Jκx-intron-RS 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 Jκx-specific (5' CTAACTAGTAAACACTGGTCTTACA 3') and R S-specific (5' AAAGCTTACCATGGGCTCAATCTCGA 3') primers. Both DNA strands from the PCR clones were sequenced over the Vκx-Jκx joins to verify the reading frame.

Production of λ+ H ybridomas. Splenocytes from B10.D2nSn/JκΔD+/+ 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, M O) for 3 d, then fused with N S0-bcl2 (38) myeloma cells for fusion 1 or SP2/0 A 39) myeloma cells for fusions 2 and 5. Hybridoma supernatants were screened by ELISA for secretion of microgram/liter Ig and the lack of κ Ig secretion.

Analysis of Hybridoma Ig R Rearrangements. Hybridoma genomic DNA was digested with EcoR I or BamHI, then fractionated on 0.8% agarose gels, blotted to nylon membrane, and hybridized with the RS 0.8 (27) or IVS probes (Fig. 1 A). Vκx-R S rearrangements were identified by genomic Southern blot analysis using the RS probe and/or by PCR amplification using primer A and primer B to yield a PCR product of ~255 bps. Vκx-Jκx-intron-RS rearrangements were identified by genomic Southern blot analysis using both the RS and IVS probes and/or by PCR amplification using primer C (Jκx intron, 5' CTGACTGCAAGTGTG- CTTTCTTAG 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 Sequence (PE Applied Biosystems). To obtain near-full-length sequences of hybridoma 1-2A7, 1-2E11, 2-2H11, 3-15D6, 3-15C4 and 3-17B10 Vκ genes, a consensus FWR1 oligo (amino acids 1–8) and 5' GTGTGAGTCTGCT-GACCAGCTCTCCA 3') was used with antisense Jκx 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, Vκ 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 H/L Chain Pairing. The H chain V(D)J and the L chain Vκ-Jκ-R S rearrangements from hybridoma 2H11 were genomically cloned as previously described (40), with the modifications that ZAP (Stratagene, La Jolla, CA) was the cloning vector and the RS and IVS probes were used to screen clones for the Vκ-Jκ-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 Vκ-Jκ rearrangement were gel isolated and ligated to pR mSAL, a Cμ expression vector (41) and pSV2-neo-Cκ, a Cκ expression vector (42) respectively. The H chain from hybridoma 15E11 was cloned by PCR amplification using a leader intron oligo (5' GAATCGGAGCACTTTGGAATGACA 3') and an oligo that spanned the Xbal site downstream of Jκx (5' CAG- GCTCCACCAAGCTCCTCCTAGA 3'). The resulting product was digested with EcoO 109I and Xbal and ligated into EcoO 109I and Xbal digested 8 ICμ (40), a Cμ expression vector. The
V_{k\kappa}-RS rearrangement from hybridoma 15E11 was also cloned by PCR using a leader intron oligo (5' TGGAACTCAGGT-TCTACTGGAGACATTG-3') and an oligo which spanned the XbaI site downstream of J_{k\kappa} (5' ACGAATTCTCAGTCAGACCCAGCGTCTACCT 3'). The resulting product was digested with EcoRI and subcloned into a shuttle vector containing V_{k\kappa} leader and promoter elements. An XbaI fragment that contained the promoter elements, leader, and the 15E11 V_{k\kappa} rearrangement was isolated and cloned into the XbaI site in pSV2-neo-C_k (42). The 2H11 and 15E11 H and L chain constructs were cotransfected into SP2/0 myeloma cells and selected for expression of IgM_k as previously described (40).

IgM_k ELISA for Analysis of H/L Pairing. Supernatants from 2H11 and 15E11 H and L chain transfecitoma clones were assayed for IgM_k expression by ELISA. In brief, goat anti-mouse IgM (Southern Biotechnology Associates, Inc., Birmingham, AL) was diluted in PBS, coated onto 96-well Immulon 2HB plates (Dynex Technologies, Inc., Chantilly, VA) 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, 0.04% Tween 20) for 1 h at room temperature. Serial dilution of transfecitoma and parental hybridoma supernatants were added and incubated at room temperature for 2 h. Plates were washed and horseradish peroxidase-conjugated goat anti-mouse k (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_2PO_4/48 mM citrate, pH 4.6) with 0.005% H_2O_2, and OD 410 nm was read using an automated plate reader (Dynatech, Alexandria, VA). Transfectoma and hybridoma antibody concentrations were estimated by comparison to a TEPC 183 (μk) standard curve.

Results

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

A analysis of IgM_λ Cells Reveals Frequent Receptor Editing. Genomic DNA from sorted IgM_λ cells was used as template for a PCR using a panspecific V_{k\kappa} FWR 3 oligonucleotide primer, which recognizes ~80% of V_{k\kappa} genes (37), together with an RS specific primer to amplify V_{k\kappa}-intron-RS rearrangements (Fig. 1 C, primers A and B). V_{k\kappa}-intron-RS rearrangements containing V_{k\kappa} genes rearranged to each of the four functional J_{k\kappa} genes were detected by PCR amplification and Southern blotting (data not shown), but V_{k\kappa}5-intron-RS rearrangements were most abundant, in part because their smaller size promoted preferential amplification. Amplified V_{k\kappa}5-intron-RS rearrangements were gel-purified and cloned, and a total of 52 clones were sequenced across both the V_{k\kappa} and the J_{κα}-intron-RS 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\kappa} genes. In this sample, at least 37 of the 52 clones represented independent events. Analysis of the V_{k\kappa} join sequences allowed an assessment of the potential prior functionality of the V_{k\kappa}5 joins just upstream of intron-RS rearrangements. Surprisingly, 15 of the 37 clones (41%) contained V_{k\kappa}5 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\kappa}-intron-RS rearrangements and to increase the sample size, an independent sampling of V_{k\kappa}-intron-RS rearrangements was derived from JC_{καD}/+ splenocytes in the form of B

Figure 1. RS rearrangements inactivate and preserve V_{k\kappa} joins. A rearranged, potentially functional κ locus (A) can be silenced by two types of RS recombination: V_{k\kappa}-RS (B) or V_{k\kappa}-intron-RS (C). Type C retains the prior V_{k\kappa} join, and the RS recombination event eliminates the known cis-acting elements that are critical for efficient rearrangement and expression, thus freezing the locus from further V_{k\kappa} recombination. Also shown are the intrinsic recombination sequence (RS1) (32), the intronic (IE) and 3' κappa (3 E) enhancers (35, 36), and the recombining sequence (RS) (27, 28) element. Probes IVS (1) and RS 0.8 (27, 28) are indicated by filled boxes.
Table 1. \( \kappa \) Locus Rearrangement Status of IgM \( \lambda \) H hybridomas

<table>
<thead>
<tr>
<th>Locus Rearrangement Status of IgM ( \lambda ) H hybridomas</th>
<th>Productive/Nonproductive</th>
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<tr>
<td>RS (^-) denotes hybridomas that lacked a detectable RS rearrangement. V(k)k-RS and V(\lambda)k-RS are defined in Fig. 1. A asterisk indicates total number of V(k)k-intron-RS loci that were isolated followed by the number that we were able to PCR amplify with the consensus FWR3 oligo.</td>
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<tr>
<th>Fusion 1</th>
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<td>44 10 30</td>
<td>12 1 6</td>
<td>25 9 11</td>
<td>15 2 10</td>
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<td>9 9*</td>
<td>4/4</td>
<td>4/0</td>
<td>5/2</td>
<td>3/1</td>
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<td>5/4</td>
<td>2/2</td>
<td>1/1</td>
<td>0/1</td>
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<td>Totals</td>
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<tr>
<td>133 34 73</td>
<td>26/20</td>
<td>12/8</td>
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Figure 2. Sequence analysis of (A) productive and (B) nonproductive V\(k\)k-intron-RS rearrangements from FACS\(^\text{sort}\) sorted, IgM \( \lambda \) \^ splenic B cells. V\(k\)k gene family and J\(k\)k gene usage were assigned based on homologies to expressed V\(k\)k gene sequences (52) or homology searches of GenBank and the Kabat Ig database. Translated V\(k\)k FWR3 (codons 70-88), CDR3, and J\(k\)k sequences to the conserved phenylalanine (F) are shown for productive rearrangements, whereas FWR3 and CDR3 sequences are shown for nonproductive rearrangements, with the asterisk (\(\ast\)) adjacent to CDR3 in B denoting an out of frame V\(k\)k join. The nucleotide sequences of the unarranged J\(k\)k intronic recombining sequence 1 (IR S1) and R S element (both of which contain a consensus heptamer sequence adjacent to the \(\Delta\) 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 have been donated by either the IRS1 or IRS2 element (both of which contain a consensus heptamer sequence adjacent to the \(\Delta\) symbol) are shown above the sequences of the IRS1-R S joins present in each PCR clone. The RS join sequence for clone 17 was not determined. Underlined nucleotides could have been donated by either the IRS1 or IRS2 element (both of which contain a consensus heptamer sequence adjacent to the \(\Delta\) symbol).
direct PCR–derived clones, whereas 6 hybridomas expressed distinct V\(\kappa\) genes that were members of families observed in the PCR clone sample and 1 hybridoma expressed a V\(\kappa\)32 gene, a V\(\kappa\) family not seen in the PCR clone samples (Figs. 2 and 3 and data not shown).

Intron/RS Joins. The sequences of the J\(\kappa\)-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\(\kappa\)-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\(\kappa\)J\(\kappa\) 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 IgM\(\kappa\) Antibodies for Analysis of H/L Pairing and Antigen Specificity. To determine if the high frequency of in-frame V\(\kappa\)J\(\kappa\) rearrangements silenced by intron-RS recombination was due to the inability of H chains to pair with their L chains, we used the V[D]J and V\(\kappa\)J\(\kappa\) rearrangements from hybridomas 2H11 and 15E11 to clone both C\(\kappa\) and C\(\mu\) 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 IgM\(\kappa\) 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 \(\mu\kappa\) 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\(\kappa\)J\(\kappa\) joins located upstream of intronic-RS rearrangements in normal, non-Ig Tg B cells to determine the extent to
which RS-mediated recombination silences functionally rearranged κ genes. Nearly half of all Vκ/Jκ joints 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 Vκ/Jκ joins upstream of the RS rearrangements. More strikingly, this high frequency is also significantly higher than 33%, the percentage of in-frame joints expected from random Vκ/Jκ rearrangement, indicating that productive Vκ/Jκ 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 Vκ-to-Jκ and RS rearrangements proceed randomly, albeit with different relative frequencies, then in either case Vκ/Jκ 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 Vκ/Jκ joins must stimulate the relative rate of (intronic) RS rearrangement. 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, Vκ/Jκ 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 Vκ 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 Vκ/Jκ rearrangements derived from λ+ hybridomas revealed no stop codons or other obvious defects that would have precluded function (Fig. 3A). It is also unlikely that frequent aberrant H/L chain pairing is responsible for the high frequency of in-frame Vκ/Jκ joins in the Vκ/Jκ-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 germinal 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 λ+ cells in newly formed and mature splenic B cells is nearly identical, suggesting that in unmanipulated mice mature κ+ cells rarely give rise to λ+ 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 autoantibodies in association with the same cell’s heavy chain, it will be necessary to generate mice transgenic for these genes.

Table 2. Analysis of VκJκ Joins in Vκ/Jκ-intron-RS Sequences: Models and Predictions Compared with Experimental Data

<table>
<thead>
<tr>
<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 VκJκ-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>
<td></td>
</tr>
<tr>
<td>High frequency of Vκ pseudogenes</td>
<td>≤33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High frequency of H/κ chain mispairing</td>
<td>≤33%</td>
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<tr>
<td>Any combination of the above</td>
<td>≤33%</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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References


