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Secondary V(D)J Rearrangements and B Cell Receptor-Mediated Down-Regulation of Recombination Activating Gene-2 Expression in a Murine B Cell Line

Jérôme Maës,* Yael Caspi, † François Rougeon, * Joseph Haimovich, 2† and Michele Goodhardt* 

It has recently become clear that recombination of Ig genes is not restricted to B cell precursors but that secondary rearrangements can also occur under certain conditions in phenotypically immature bone marrow and peripheral B cells. However, the nature of these cells and the regulation of secondary V(D)J recombination in response to B cell receptor (BCR) stimulation remain controversial. In the present study, we have analyzed secondary light chain gene rearrangements and recombination activating gene (RAG) expression in the surface IgM\(^+\), IgD\(^-\) murine B cell line, 38C-13, which has previously been found to undergo \(\kappa\) light chain replacement. We find that 38C-13 cells undergo spontaneous secondary V\(\kappa\)-J\(\kappa\) and RS rearrangements in culture, with recombination occurring on both productive and nonproductive alleles. Both 38C-13 cells and the Id-negative variants express the RAG genes, indicating that the presence of RAG does not depend on activation via the 38C-13 BCR. Moreover, BCR cross-linking in 38C-13 cells leads to a rapid and reversible down-regulation of RAG2 mRNA. Therefore, 38C-13 cells resemble peripheral IgM\(^+\), IgD\(^-\) B cells undergoing light chain gene rearrangement and provide a possible in vitro model for studying peripheral V(D)J recombination. The Journal of Immunology, 2000, 165: 703–709.
has previously been shown to undergo light chain gene replacement (22, 23). The 38C-13 cells are surface IgM+, IgD- and produce both \( \kappa \) and surrogate light chains (24). During studies on Id-specific immunotherapy of 38C-13 tumors, it has been observed that Id-negative variant tumors develop in surviving mice (22, 25). The loss of idiotypic specificity was a result of secondary \( \kappa \) gene rearrangements leading to the synthesis of \( \kappa \) chains different from that of the parental cell line or of the lack of light chain production (22, 23, 26). In this study we show that 38C-13 cells undergo spontaneous V-J and RS rearrangement in culture, with recombination occurring on both the productive and the nonproductive alleles. Our results indicate that RS rearrangements are not a last resort but can occur even when the possibility of V-J rearrangement exists. Interestingly, both parental and Id-negative cells express RAG transcripts, showing that the presence of RAG does not depend on activation via the 38C-13 BCR. Furthermore, we find that RAG expression in 38C-13 cells is down-regulated by BCR cross-linking. Our results indicate that 38C-13 cells resemble peripheral IgM+, IgD- B cells undergoing light chain gene rearrangement and provide a possible in vitro model for studying peripheral V(D)J recombination.

Materials and Methods

Cell lines and Abs

The 38C-13 cell line is a murine IgM\( \kappa \)-expressing cell line (21). EH and DB2 are Id-negative variant cell lines of 38C-13 isolated from mice injected with 38C-13 cells and subsequently treated with anti-Id Abs; DB2 produces \( \kappa \) chains that are different from those of 38C-13, whereas EH does not produce \( \kappa \) chains at all (25). All other Id-negative cell lines were isolated from 38C-13 cultures in vitro by limiting dilutions. Absence of Id was determined by ELISA of the culture media directly from the 96 wells of the limiting dilution assay (27).

The 4D2 and D5D10 hybridomas secrete rat anti-38C-13 IgM Id Abs of IgG2b and IgG2a isotypes, respectively (28). The 187.1 hybridoma, secreting rat anti-mouse \( \kappa \) chain Abs, and the 53-6.72 hybridoma, secreting rat anti-mouse CD8 Abs, have been obtained from the American Type Culture Collection (Manassas, VA). Goat anti-mouse IgM has previously been described (29).

Flow cytometric analysis

Expression of \( \mu \)-containing molecules on the cell surface of 38C-13 cells and its Id-negative variants was determined by flow cytometric analysis as previously described (28) with minor modifications. Briefly, cells (0.5–1.0 \( \times \) 10\(^6\)) were incubated first for 30 min at 4°C with 50 \( \mu \)g of aggregated human IgG to block Fc receptors (30). Culture media of 4D2 or 187.1 anti-idiotypic purified goat anti-mouse IgM Abs were then reacted with the cells for 30 min at 4°C before fluorescein-labeled F(ab\(^\prime\))\(_2\) of mouse anti-rat IgM or donkey anti-goat IgG (Jackson ImmunoResearch, West Grove, PA). A FACScan instrument (Becton Dickinson, Mountain View, CA) was used for the flow cytometry.

DNA analysis

Genomic DNA was prepared from \(-10^7\) cells as previously described (31). After restriction cleavage, 25 \( \mu \)g of DNA were fractionated by electrophoresis on 0.7% agarose gel, transferred to Hybond-N membrane (Appligene, Strasbourg, France) in 0.4 M NaOH, and hybridized with radiolabeled probes. The probes used are as follows: 3\'J\( \kappa \) is a 620-bp HindIII-NcoI genomic fragment from the murine Jc-Cx intron (32); RS, a 800-bp Sau3A fragment (33); 3\'J\( \kappa \)e2, a 279-bp fragment obtained by PCR amplification using the forward primer 5\'-CTTGTCTAATGCTCTAAC and reverse primer 5\'-TTGGCAGCAGGCTCCATAC-3'; 3\'J\( \kappa \)e4, a 244-bp fragment generated using the forward PCR primer 5\'-GGTAACTTGTGTGAATTTGTG-3' and reverse primer 5\'-GACTATGACATGCCCCCTCTC-3'; and V\( \kappa \)38C, a 213-bp fragment generated using the forward PCR primer 5\'-AAGCAAGGACCATTAAC-3' and reverse primer 5\'-CATGATTATCATACTGTAGAC-3' (22).

RNA analysis

RNA was prepared from 1–2 \( \times \) 10\(^7\) cells using RNAzol B according to the manufacturer’s instructions (Bioprobe, Richmond, CA). For Northern blot analysis, 20 \( \mu \)g of RNA was fractionated by electrophoresis on 1% agarose–6% formaldehyde gel (31), transferred to Hybond-N membrane (Amersham, Buckinghamshire, U.K.) in 10× SSC, and hybridized with radiolabeled probes. The probes used are a 1.9-kb EcoRV-NcoI RAG2 fragment (34) and a 983-bp G3PDH fragment (Clontech, Palo Alto, CA).

Results

Isolation and characterization of Id-negative variants of the 38C-13 cell line

The parental 38C-13 cell line is a \( \mu \kappa^+\), \( \delta^-\), SLC-expressing murine B cell (21, 24). The DB2 and EH Id-negative variant cell lines have previously been obtained in anti-Id-treated 38C-13 tumor-bearing mice (25). All other cell lines have been obtained from in vitro cultures of 38C-13 cells by limiting dilution in the absence of immunoselection. Because the frequency of spontaneously arising Id-negative variants was only 1–2%, we used a simple and sensitive ELISA assay, which allowed us to screen for the presence of IgM molecules with 38C-13 IgM Id specificity in the culture media of limiting dilution cultures (27). Clones found to be negative for
38C-13 cells undergo secondary Vκ-Jκ rearrangements in culture

It has previously been reported that Id-negative variants of 38C-13 cells isolated after immunoselection with anti-idiotypic Abs had undergone secondary κ gene rearrangements (22, 23, 26). Therefore, we analyzed the rearrangement status of 38C-13 and its spontaneously arising Id-negative variants obtained in vitro.

Southern blot analysis was performed on genomic DNA of 38C-13 and variant cell lines digested with either HindIII or EcoRI and BamHI restriction enzymes. A scheme of the κ locus and the probes used are summarized in Fig. 2A. As previously reported, 38C-13 cells were found to contain two rearranged κ genes (22). The upper 6.4-kb EcoRI-BamHI and 4-kb HindIII rearranged bands detected with the 3′Jκ5 probe (Fig. 2, B and C) also hybridize with both a Vκ38C-specific probe and a 3′Jκ2 probe (data not shown), indicating that they correspond to the productive 38C-13 allele, which has previously been reported to result from a Jκ2 joining (22). We found that the nonproductive allele, corresponding to the lower rearranged band for both digests, contains a V-Jκ5 rearrangement, because it hybridizes to a 3′Jκ5 probe (Fig. 2, B and C) but not to a 3′Jκ4 probe (Fig. 2D and data not shown).

A different restriction pattern was observed for Id-negative variants (Fig. 2, B–D). As expected, the fragment corresponding to the productive allele was lost from all Id-negative variants obtained in vitro, as well as from the EH cell line previously obtained in vivo. New Jκ hybridizing fragments were observed in both κ-negative and κ-positive variants, corresponding to secondary Vκ-Jκ rearrangements on the productive allele. Analysis of the results obtained with the different Jκ probes are summarized in Table I and show that V-Jκ4 rearrangements occur in six of the eight Id-negative variants, whereas only two variants had a V-Jκ5 rearrangement. No Jκ3 rearrangement is observed because this segment is not recombination competent. In conclusion, these results show that 38C-13 cells can spontaneously undergo secondary Vκ-Jκ rearrangements in vitro, giving rise to either functional or nonfunctional κ genes.

38C-13 cells undergo RS rearrangements

The above Southern blot analysis showed that secondary Vκ-Jκ rearrangements occur on the productive 38C-13 allele. However, the secretion of Id-specific IgM molecules were further characterized by FACS analysis with Abs specific for μ, κ, or idiotype determinants. The results for the parental 38C-13 and six representative Id-negative subclones are shown in Fig. 1. Substantial amounts of μ and κ chains are present on the 38C-13 cells as well as on several Id-negative variants. However, as previously reported (24), no κ chains could be detected in certain variants. Nevertheless, μ chains are expressed on the cell surface of these κ-negative cells, albeit at much lower levels than on the parental 38C-13 and κ-positive, Id-negative variants. The μ-containing molecules on the κ-negative variants have previously been shown to consist of μ chains assembled with the A5 and VpreB polypeptide chains (24).

FIGURE 2. Secondary κ gene rearrangements in 38C-13 cells. A, Physical map of the Jκ locus showing probes and restriction enzymes used. Vertical boxes indicate Jκ gene segments, and the filled circle and rectangle represent the κ intronic enhancer (Eix) and constant region (Cκ), respectively. E, EcoRI; H, HindIII; and B, BamHI. B–D, Southern blot analysis of 38C-13 cells and Id-negative variants. Twenty-five micrograms of genomic DNA from 38C-13 cells (Id⁺), Id-negative κ-negative variants (Id⁻ κ⁻), or Id-negative κ-positive variants (Id⁻ κ⁺) were digested with EcoRI and BamHI (B) or HindIII (C and D). Digests were separated through 0.7% agarose gel, transferred to a nylon membrane, and hybridized with the 3′Jκ5 (B and C) or 3′Jκ4 (D) probes. Position of germline fragments (GL) and m.w. markers (sizes in kilobases) are indicated.
in three Id-negative variants (B2, C12, and CYE1), we also observed deletion of the lower band, corresponding to the 38C-13 nonproductive allele (Fig. 2C and Table I). Because the nonproductive allele contains a V-Jk5 rearrangement (Table I), it cannot undergo secondary V-J recombination. However, deletion of the nonproductive allele could be the result of RS rearrangement. RS rearrangements, which were first observed in λ-producing B cells, are V(D)J recombinase-dependent rearrangements that inactivate the κ locus by deletion of either the Cκ exon or the entire Jκ-Cκ region (33). As shown schematically in Fig. 3A, RS rearrangements can occur between a canonical heptamer-nonamer recombination signal sequence situated 3’ of the Cκ region and either 1) a recombination heptamer located in the Jκ-Cκ intron, resulting in the deletion of the Cκ and 3’ region or 2) the recombination signal sequence of a germline Vκ segment situated upstream of the rearranged V gene, leading to the loss of the entire rearranged κ gene. Rearranged RS bands were observed in the three variants that had deleted the nonproductive allele, one κ-positive clone, and two κ-negative clones (Fig. 3B). As expected for RS rearrangements, EcoRI-BamHI fragments of greater than 4 kb were detected with the RS probe, together with the 6-kb germline RS fragment corresponding to the 38C-13 productive allele. These rearrangements resulted in the loss of both the Cκ region and the rearranged Jκ5 segment (Fig. 2C), indicating that they are the product of an RS rearrangement with an upstream germline Vκ segment.

These results indicate that RS rearrangements occur at a relatively high frequency on the 38C-13 nonproductive allele. Therefore, the nonproductive allele is not blocked for further rearrangements in these cells. Moreover, RS recombination was observed in variants with a V-Jk4 on the other allele, which therefore could have undergone a further V-Jk5 rearrangement. This suggests that RS recombination occurs not only when all potential κ gene rearrangements have been exhausted but can take place before a secondary Vκ-Jκ rearrangement.

RAG transcripts are observed in both 38C-13 cells and Id-negative variants

The occurrence of secondary rearrangements in the 38C-13 cell line suggested that the RAG genes may still be expressed in these cells. Northern blot analysis of RAG expression showed that, as expected, RAG1 and RAG2 transcripts are indeed observed in the parental 38C-13 cell line (Fig. 4 and data not shown). Because the specificity of the 38C-13 BCR is unknown, it was possible that, by analogy with receptor editing in bone marrow, the continual expression of RAG genes was due to activation of 38C-13 cells via the interaction of a potential ligand present in the culture conditions with the BCR. However, substantial amounts of RAG2 mRNA were detected in all the Id-negative variants, both in the κ-positive and κ-negative cell lines (Fig. 4). Therefore, the presence of RAG in 38C-13 cells cannot be related to the specificity of 38C-13 BCR because RAG genes are expressed not only in parental 38C-13 cells but also in variants expressing a new BCR or no BCR at all. These results indicate that RAG expression in 38C-13 cells is not induced via BCR engagement.

BCR cross-linking decreases RAG2 expression in 38C-13 cells

The above results show that the IgM+−, IgD−−, 38C-13 cells express RAG and undergo secondary Vκ-Jκ rearrangements in vitro. These properties are also found in IgM+−, IgD−− cells present in

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### Table I. Summary of Vκ-Jκ and RS rearrangements in 38C-13 cells and idotype-negative variants

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Vκ-Jκ Rearrangement</th>
<th>38C-13 Productive</th>
<th>38C-13 Nonproductive</th>
<th>RS Rearrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Id+</td>
<td>Vκ38C-Jk2</td>
<td>VκN-Jk5</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Id− κ−</td>
<td>B2</td>
<td>Jk4</td>
<td>Deleted</td>
<td></td>
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<td></td>
<td>69.28</td>
<td>Jk5</td>
<td>VκN-Jk5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C12</td>
<td>Jk4</td>
<td>Deleted</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EH</td>
<td>Jk4</td>
<td>VκN-Jk5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>137.61</td>
<td>Jk4</td>
<td>VκN-Jk5</td>
<td></td>
</tr>
<tr>
<td>Id− κ+</td>
<td>CYA12</td>
<td>Jk4</td>
<td>VκN-Jk5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYD7</td>
<td>Jk5</td>
<td>VκN-Jk5</td>
<td></td>
</tr>
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<td></td>
<td>CYE1</td>
<td>Jk4</td>
<td>Deleted</td>
<td></td>
</tr>
</tbody>
</table>

*Results for Vκ-Jκ and RS rearrangements of the productive and nonproductive 38C-13 alleles are derived from Southern blots analyzed with the 3’Jκ4, 3’Jκ5, and RS probes as described in Figs. 2 and 3. Vκ38C, Vκ segment of the productive 38C-13 allele; VκN, Vκ segment of the nonproductive 38C-13 allele; −, absent; +, present.*
and G3PDH probes. Results shown are representative of three separate cultures for 48 h (W48) without Ab. Blots were hybridized with the RAG2 lane 1 (Fig. 5, lane 13). These results indicate that, as for germinal center cells, BCR ligation results in down-regulation of RAG2 in 38C-13 cells.

Discussion

In recent years it has become clear that rearrangement of Ig genes is not restricted to early B cell development. Expression of RAG genes and evidence of secondary V-J rearrangement at the k and λ light chain loci have been reported in surface Ig-positive B cells in both the bone marrow and germinal centers (6, 10, 15, 17). However, the origin of these cells and the regulation of (D)J recombination in response to BCR stimulation remain controversial (12, 13). In this study, we have analyzed secondary rearrangements in a B cell line, 38C-13, which has been found to change surface Ig in immunotherapy experiments using anti-Id Abs (22, 23, 25). In parental 38C-13 cells, both k alleles are rearranged: there is a productive Jk2 and a nonproductive Jk5 rearrangement (Ref. 22 and the present study). Id-negative variants were isolated from 38C-13 cultures by limiting dilution and screening for loss of Id. All the 38C-13 variants express μ chains at the surface, but only some express k light chains. This suggests that secondary k gene rearrangements occur during culture, as had previously been observed after treatment with anti-Id Abs (22, 23). Southern blot analysis showed novel k gene rearrangements in all the Id-negative variants analyzed. Eight Id-negative variants were studied in detail, and we found a different restriction pattern for each clone, indicating that each 38C-13 variant had undergone different rearrangements at the productively rearranged k allele. Because these clones had arisen in the absence of any apparent selective pressure, ongoing k gene rearrangement in 38C-13 cells appears to be a spontaneous process rather than one that is induced by BCR ligation with anti-idiotypic Abs.

Secondary k gene rearrangement in 38C-13 cells is not restricted to the productive allele. The nonproductive allele has a V-Jk5 and so cannot undergo further V-J recombination; however, RS rearrangements were observed at the nonproductive allele in three of eight clones analyzed. RS rearrangements were originally thought to be a last resort recombination event leading to deletion of the Ck region before (D)J rearrangements at the λ locus (33). We found that all 38C-13 variants with an RS rearrangement had a V-Jk4 rearrangement on the productive allele. This means that two recombination events had occurred: a Vk38C-Jk2 to V-Jk4 rearrangement on the productive allele and a V-Jk5 to RS rearrangement on the nonproductive allele. We cannot tell whether rearrangement took place first on the productive or the nonproductive allele. However, in either case RS rearrangement occurred in these variants even though secondary V-J rearrangement on the productive allele was still possible. This suggests that RS recombination is quite efficient and does not occur only after all possible V-J rearrangements have been exhausted. Similar conclusions have been reached by Dunda and Corcos (35) and by Retter and Nemazee (36). Our results also show that the productive allele is not preferentially targeted at each round of recombination, indicating that secondary rearrangements in 38C-13 cells do not necessarily proceed on one allele until all possibilities are exhausted before rearrangement can begin on the second k allele. In this our results differ from the generally held view that successive k gene rearrangements occur at the same allele, whereas the other allele remains inaccessible (37). The difference may be related to the fact that previous studies focused on rearrangement in B cell precursors, where the second k allele was in the germline configuration. It has been observed that the germline k allele is methylated (38),

FIGURE 4. RAG2 gene expression in 38C-13 cells and Id-negative variants. Northern blot analysis of total RNA from Id-positive 38C-13 cells (lane 1), Id-negative k-positive variants (lanes 2–4), and Id-negative k-negative variants (lanes 5–9). Twenty micrograms of RNA were run on a 1.1% agarose/6.6% formaldehyde gel, transferred to a nylon membrane, and hybridized with the 32P-labeled RAG2 probe. The G3PDH probe was used as loading control.

FIGURE 5. BCR ligation decreases RAG2 mRNA expression in 38C-13 cells. Northern blot analysis of total RNA from untreated 38C-13 cells (lane 14), 38C-13 cells cultured for 4 h with 10 μg/ml of control (lane 11), or anti-IgM (lane 13) Abs or for indicated times with 10 μg/ml of anti-idiotypic Ab (lanes 1–10 and 12). Lanes 9 and 10, 38C-13 cells were cultured for 48 h with anti-idiotypic Ab, washed, and cultured for another 24 (W24) or 48 h (W48) without Ab. Blots were hybridized with the RAG2 and G3PDH probes. Results shown are representative of three separate experiments.
whereas in 38C-13 cells both the productive and nonproductive \( \kappa \) alleles are demethylated (data not shown) and hence may be equally accessible to recombination acting factors. The analysis of secondary rearrangements in 38C-13 cells further showed that for the productive allele, which originally contained a V-J\(k2\) rearrangement, secondary V-J\(k4\) rearrangements were observed in six of eight Id-negative variants, V-J\(k5\) rearrangements in two of eight variants, and none had RS rearrangements. In contrast, on the nonproductive allele, in three of eight variants the V-J\(k5\) rearrangement is replaced by an RS rearrangement. These results show that, as previously observed (37, 39, 40), ongoing \( \kappa \) gene rearrangement is associated with progressively more 3' elements.

\( \kappa \) genes from the large V\(k4/5\) family have been found to be preferentially utilized in secondary \( \kappa \) gene rearrangements leading to loss of Id specificity in 38C-13 cells (23, 26). Thanks to the recent data of Zachau and coworkers (41), who have systematically cloned and sequenced the murine \( \kappa \) locus, it is now possible to follow the secondary rearrangements occurring in these cells. It appears that the V\(k4\) genes used in the secondary rearrangements lie 3' to the V\(k38C\) gene; however, both the V\(k38C\) gene and most of the V\(k4\) genes are in the opposite orientation with respect to the J\(k\) segments (Fig. 6). Therefore, the V\(k38C\)-J\(k2\) rearrangement on the productive allele occurred by inversion, hence placing the V\(k4\) genes 5' to the rearranged V\(k38C\) gene. Secondary rearrangements using these genes delete the rearranged V\(k38C\) gene, which therefore explains the lack of hybridization observed with the V\(k38C\) probe in the Id-negative variants. Interestingly, there is no strict correlation between distance from the rearranged V\(k38C\) gene and frequency of utilization of the V\(k4\) genes in the secondary rearrangements: V genes situated over 500 kb away from the V\(k38C\) gene are utilized at the same frequency as more proximal genes. Similarly, the results of Zachau and coworkers (42) show that the frequency of expression of a V\(k\) gene in splenic B cells does not depend on its distance from the J\(k\) locus. Nevertheless, in the 38C-13 variants there is a strong bias (9 of 26) toward utilization of the k4 V gene (22, 23, 26), which is situated only 30 kb from the V\(k38C\) gene. It is unclear at present whether the repeated usage of this gene is related to its proximity to the rearranged V gene or to structural properties of this V segment, such as consensus promoter or recombination sequences, leading to more efficient V-J rearrangement.

In line with the findings of secondary \( \kappa \) gene rearrangements in 38C-13 cells, the parental cell line was found to express the RAG1 and RAG2 genes. Continual light chain gene rearrangements and RAG expression have been observed in IgM\(^+\), IgD\(^-\) B cells in both bone marrow (6–8, 11) and germinal centers (14, 18, 19). These cells differ in response to BCR engagement. In bone marrow, BCR cross-linking increases or maintains high levels of RAG (10, 11). This has clearly been demonstrated for autoreactive B cells, consistent with the idea that V(D)J recombination in bone marrow B cells is involved in editing of self-reactive BCR (7–9).

RAG-expressing peripheral B cells are observed in vivo in germinal centers or after in vitro stimulation of splenic B cells with IL-4 and LPS or anti-CD40 Abs (14, 16, 18, 20). In these cells, BCR ligation with Abs or high-affinity ligand causes down-regulation of RAG (18, 20), suggesting that secondary V(D)J rearrangement in peripheral B cells plays a role in receptor diversification. We at first thought that, as in autoreactive bone marrow B cells, constitutive RAG expression and secondary rearrangements in 38C-13 cells might be due to stimulation of the BCR under the culture condition. This was not found to be the case because RAG transcripts were observed not only in the parental cell line but also in the Id-negative variants, showing that RAG expression is not related to the specificity of the 38C-13 BCR. Furthermore, both \( \kappa \)-positive variants, which express a new BCR, and \( \kappa \)-negative variants, which only express the pre-BCR, contain RAG transcripts, indicating that secondary rearrangements in the 38C-13 cell line are not induced via BCR engagement. This was confirmed by treating 38C-13 cells with anti-\( \mu \) or anti-Id Abs. These experiments showed that BCR cross-linking not only did not increase RAG expression but led to rapid and reversible down-regulation of RAG. Therefore, 38C-13 cells resemble RAG-expressing peripheral B cells with respect to BCR-mediated regulation of RAG expression. In addition, these peripheral cells undergoing secondary light chain rearrangements have been found to express markers of immature B cells in that they are IgM\(^+\), IgD\(^-\), B220\(^+\), heat-stable Ag-positive, peanut agglutinin-positive, SLC\(^+\), RAG\(^+\), TdT\(^+\), and GL7\(^+\) and are responsive to IL-7 (19, 43). The 38C-13 cells share all these phenotypic markers, with the exception of GL7 expression (Refs. 22 and 24 and data not shown). Taken together, these results indicate that 38C-13 cells provide a useful in vitro model for the study of secondary V(D)J rearrangements in the periphery.

Continued RAG expression and the extension of light chain gene rearrangement to peripheral B cells, which have been postulated to improve immune responses in germinal centers (20), raise the question of maintenance of allelic exclusion. Unfortunately, we cannot address this issue directly using 38C-13 cells because the nonproductive allele has a V-J\(k5\) rearrangement. However, secondary rearrangements at the A locus have never been observed in this cell line (Ref. 26 and data not shown). Similarly, these cells do not appear to undergo V-gene replacement at the heavy chain locus (22, 26), suggesting that like in B cell precursors, locus accessibility plays a major role in the control of peripheral V(D)J recombination. Continual V(D)J rearrangement in peripheral B cells can also potentially lead to the loss of BCR expression or to the assembly of an autoreactive BCR. How these detrimental consequences of secondary rearrangement in the periphery are prevented

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**FIGURE 6.** Scheme of the utilization of V\(k\) genes in secondary \( \kappa \) gene rearrangements leading to loss of Id specificity in 38C-13 cells. Frequency of utilization of V\(k\) genes was obtained by comparing cDNA sequences from 38C-13 Id-negative variants (22, 23, 26) with murine germline V\(k\) sequences (41). Localization of V\(k4\) genes used in the secondary rearrangements with respect to the V\(k38C\) gene is according to Thiebe et al. (42). The map is not drawn to scale. Arrows indicate transcriptional polarity of the V\(k\) genes.

| Distance from V\(\kappa38C\) (kb) | kh4 | kj4 | kk4 | aj4 | km4 | kn4 | kb4 | an4 | kf4 | 38C | J2 | J3 | J4 | J5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 870 | 750 | 700 | 550 | 525 | 500 | 165 | 70 | 30 | 20 |   |   |   |   |   |   |

| Frequency of utilization | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 3 | 3 | 9 | 1 |

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