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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, ‡† 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 κ light chain replacement. We find that 38C-13 cells undergo spontaneous secondary Vκ-Jκ 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.


Diff erentiation of B lymphocytes progresses through several stages in which genes coding for the Ig heavy and light chains are assembled from germline encoded V, D, and J segments by a series of site-specific recombination events (1, 2). V(D)J recombination is initiated by the recombination activating gene products, RAG1 and RAG2,3 which recognize and cut at recombination signal sequences adjacent to each of the germline coding segments (for review see Ref. 3). The expression of RAG1 and RAG2 is lymphoid-specific and regulated during B and T cell development (4, 5). There are two distinct waves of RAG expression in bone marrow B cell precursors, the first corresponding to Ig heavy chain gene rearrangement in early pro-B cell progenitors. The RAG genes are down-regulated upon expression of a μ heavy chain, which assembles with the surrogate light chain (SLC) proteins A5 and VpreB to form the pre-B cell receptor (pre-BCR) (4). After several rounds of cell division, the RAG genes are re-expressed in pre-B cells where κ and λ light chain gene rearrangements take place. Successful light chain gene assembly permits surface Ig expression and differentiation to the mature B cell stage, a process that involves down-regulation of RAG and suppression of further V(D)J recombination (2, 4). However, the expression of a functional BCR does not always terminate light chain gene rearrangement (6). One well-documented situation in which rearrangement continues is when the Ag receptors on the newly formed immature B cells possess autoreactive specificity (7–9). This secondary V(D)J rearrangement, referred to as receptor editing, occurs in IgM+, IgD− immature B cells in the bone marrow (10, 11). It is presently unclear whether continued light chain gene rearrangement in these cells is a result of direct BCR-mediated up-regulation of RAG or of an arrest in B cell differentiation at the RAG-positive IgM+, IgD− immature B cell stage (10, 12, 13).

Recently, RAG expression and V(D)J recombination have also been observed in germinal centers, which are the site of affinity maturation, where somatic hypermutation of Ig variable region genes occurs (14–18). These secondary rearrangements take place in B cells that have an immature phenotype (12, 13, 19). Whether these cells are newly recruited immature bone marrow B cells that still express RAG or whether RAG is re-expressed in a population of germinal center cells is currently an open question (12–14, 16, 20). Nevertheless, unlike secondary rearrangements occurring in immature bone marrow B cells, where engagement of surface Ig leads to an apparent increase in the level of RAG expression, stimulation of the BCR in peripheral B cells causes a decrease in RAG (18, 20). Furthermore, it has been reported that down-regulation of RAG is only observed upon high-affinity binding of Ag, leading to the suggestion that V(D)J rearrangement contributes to receptor diversification in germinal center cells (20). Therefore, secondary V(D)J recombination appears to be regulated in different ways in germinal center and immature bone marrow B cells via BCR signaling and has been proposed to mediate different physiological functions at these two stages of B cell differentiation: tolerance in bone marrow and improved immune response in germinal center B cells.

The use of cell lines has been very useful in the study of B cell biology. Given the controversy surrounding the nature of the B cells undergoing secondary V(D)J recombination and its regulation, we have analyzed the murine 38C-13 B cell line (21), which

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3 Abbreviations used in this paper: RAG, recombination activating gene; SLC, surrogate light chain; BCR, B cell receptor.
has previously been shown to undergo light chain gene replacement (22, 23). The 38C-13 cells are surface IgM+, IgD− and produce both κ 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 κ gene rearrangements leading to the synthesis of κ 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 IgMκ-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 κ chains that are different from those of 38C-13, whereas EH does not produce κ 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 κ 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 μ-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 × 10⁶) were incubated first for 30 min at 4°C with 50 μg of aggregated human IgG to block Fc receptors (30). Culture media of 4D2 or 187.1 IgM hybridomas or affinity purified goat anti-mouse IgM Abs were then reacted with the cells for 30 min at 4°C before fluorescein-labeled F(ab′)₂ of mouse anti-rat IgG or donkey anti-goat IgG (Jackson ImmunoResearch, West Grove, PA). A FACSort instrument (Becton Dickinson, Mountain View, CA) was used for the flow cytometry.

DNA analysis

Genomic DNA was prepared from −10⁶ cells as previously described (31). After restriction cleavage, 25 μg of DNA were fractionated by electrophoresis on 0.7% agarose gel, transferred to Hybond-N membrane (Amersham, Buckinghamshire, U.K.) in 0.4 M NaOH, and hybridized with radiolabeled probes. The probes used are as follows: 3′ Jκ5 is a 620-bp HindIII-NcoI genomic fragment from the murine Jκ-Cκ intron (32); RS, a 800-bp Sau3A fragment (33); 3′ Jκ2, a 279-bp fragment obtained by PCR amplification using the forward primer 5′-CTTGTGCATCAAGCTTACACCTG3′ and reverse primer 5′-CTTCCAGTCCTGGTCCCCATCAC3′; 3′ Jκ4, a 244-bp fragment generated using the forward PCR primer 5′-GGGTAACTTGTGTGAATTTG-3′ and reverse primer 5′-GACTATTCTCATGTAGAATGG-3′ and reverse primer 5′-GAGTATTCTCATGCTAGAATGG-3′ (22).

RNA analysis

RNA was prepared from 1–2 × 10⁶ cells using RNAzol B according to the manufacturer’s instructions (Bioprobe, Richmond, CA). For Northern blot analysis, 20 μg 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 μκ⁺, δ−, 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...
FIGURE 2. Secondary \( \kappa \) gene rearrangements in 38C-13 cells. A, Physical map of the Jk locus showing probes and restriction enzymes used. Vertical boxes indicate Jk gene segments, and the filled circle and rectangle represent the \( \kappa \) intronic enhancer (Eik) and constant region (Ck), 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 \( \kappa \)-negative variants (Id\(^-\) \( \kappa^-\)), or Id-negative \( \kappa \)-positive variants (Id\(^-\) \( \kappa^+\)) 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' Jk5 (B and C) or 3' Jk4 (D) probes. Position of germline fragments (GL) and m.w. markers (sizes in kilobases) are indicated.

The above Southern blot analysis showed that secondary V\( \kappa \)-Jk rearrangements occur on the productive 38C-13 allele. However, 38C-13 cells undergo secondary V\( \kappa \)-Jk rearrangements in vitro

It has previously been reported that Id-negative variants of 38C-13 cells isolated after immunoselection with anti-idiotypicAbs had undergone secondary \( \kappa \) 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 \( \kappa \) locus and the probes used are summarized in Fig. 2A. As previously reported, 38C-13 cells were found to contain two rearranged \( \kappa \) genes (22). The upper 6.4-kb EcoRI-BamHI and 4-kb HindIII rearranged bands detected with the 3’ Jk5 probe (Fig. 2B and C) also hybridize with both a V\( \kappa \)38C-specific probe and a 3’ Jk2 probe (data not shown), indicating that they correspond to the productive 38C-13 allele, which has previously been reported to result from a Jk2 joining (22). We found that the nonproductive allele, corresponding to the lower rearranged band for both digests, contains a V-Jk5 rearrangement, because it hybridizes to a 3’ Jk5 probe (Fig. 2B and C) but not to a 3’ Jk4 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 Jk hybridizing fragments were observed in both \( \kappa \)-negative and \( \kappa \)-positive variants, corresponding to secondary V\( \kappa \)-Jk rearrangements on the productive allele. Analysis of the results obtained with the different Jk probes are summarized in Table I and show that V-Jk4 rearrangements occur in six of the eight Id-negative variants, whereas only two variants had a V-Jk5 rearrangement. No Jk3 rearrangement is observed because this segment is not recombination competent. In conclusion, these results show that 38C-13 cells can spontaneously undergo secondary V\( \kappa \)-Jk rearrangements in vitro, giving rise to either functional or nonfunctional \( \kappa \) genes.

38C-13 cells undergo RS rearrangements

The above Southern blot analysis showed that secondary V\( \kappa \)-Jk 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 \( \mu \), \( \kappa \), or idiotypic determinants. The results for the parental 38C-13 and six representative Id-negative subclones are shown in Fig. 1. Substantial amounts of \( \mu \) and \( \kappa \) chains are present on the 38C-13 cells as well as on several Id-negative variants. However, as previously reported (24), no \( \kappa \) chains could be detected in certain variants. Nevertheless, \( \mu \) chains are expressed on the cell surface of these \( \kappa \)-negative cells, albeit at much lower levels than on the parental 38C-13 and \( \kappa \)-positive, Id-negative variants. The \( \mu \)-containing molecules on the \( \kappa \)-negative variants have previously been shown to consist of \( \mu \) chains assembled with the A5 and VpreB polypeptide chains (24).
Secondary Rearrangement and RAG Expression in 38C-13 Cells

Summary of Vκ-Jκ and RS Rearrangements in 38C-13 Cells and Idiotype-Negative Variants

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Vκ-Jκ Rearrangement</th>
<th>Vκ-Jκ Rearrangement</th>
<th>RS Rearrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td>38C-13</td>
<td>Vκ38C-Jκ2</td>
<td>VκN-Jκ5</td>
<td>–</td>
</tr>
<tr>
<td>B2</td>
<td>Jκ4</td>
<td>Deleted</td>
<td>+</td>
</tr>
<tr>
<td>69.28</td>
<td>Jκ5</td>
<td>VκN-Jκ5</td>
<td>–</td>
</tr>
<tr>
<td>C12</td>
<td>Jκ4</td>
<td>Deleted</td>
<td>+</td>
</tr>
<tr>
<td>EH</td>
<td>Jκ4</td>
<td>VκN-Jκ5</td>
<td>–</td>
</tr>
<tr>
<td>137.61</td>
<td>Jκ4</td>
<td>VκN-Jκ5</td>
<td>–</td>
</tr>
<tr>
<td>CYA12</td>
<td>Jκ4</td>
<td>VκN-Jκ5</td>
<td>–</td>
</tr>
<tr>
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<td>Jκ5</td>
<td>VκN-Jκ5</td>
<td>–</td>
</tr>
<tr>
<td>CYE1</td>
<td>Jκ4</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′Jκ, 3′Jκ, 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.

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-Jκ5 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-Jκ4 on the other allele, which therefore could have undergone a further V-Jκ5 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 Idiotype-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
and G3PDH probes. Results shown are representative of three separate experiments.

The bone marrow and in germinal centers (10, 15, 17, 18). However, these two cell populations differ in that, unlike the bone marrow cells, BCR cross-linking in the germinal center cells leads to down-regulation of RAG expression (18, 20). Therefore, we cultured 38C-13 cells in the presence of saturating levels (10 μg/ml) of an IgG2a anti-idiotypic mAb (D5D10) or an isotype-matched control monoclonal (53–6.72). Under these conditions, neither the viability nor the proliferation of 38C-13 cells were affected (data not shown). However, treatment of 38C-13 cells with anti-idiotypic but not control Abs resulted in a decrease in RAG2 mRNA levels (Fig. 5). This response was detected within 1 h of Ab treatment, and RAG2 expression remained low for 24–48 h. Removal of the anti-idiotypic Abs restored the expression of RAG2 (Fig. 5, lanes 9 and 10). Similar results were obtained with anti-μ Abs (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 κ 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 V(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 κ alleles are rearranged: there is a productive Jκ2 and a nonproductive Jκ5 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 κ light chains. This suggests that secondary κ gene rearrangements occur during culture, as had previously been observed after treatment with anti-Id Abs (22, 23). Southern blot analysis showed novel κ 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 κ allele. Because these clones had arisen in the absence of any apparent selective pressure, ongoing κ 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 κ gene rearrangement in 38C-13 cells is not restricted to the productive allele. The nonproductive allele has a V-Jκ5 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 Cκ region before V(D)J rearrangements at the λ locus (33). We found that all 38C-13 variants with an RS rearrangement had a V-Jκ4 rearrangement on the productive allele. This means that two recombination events had occurred: a Vκ38C-Jκ2 to V-Jκ4 rearrangement on the productive allele and a V-Jκ5 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 κ allele. In this our results differ from the generally held view that successive κ 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 κ allele was in the germline configuration. It has been observed that the germline κ allele is methylated (38).
whereas in 38C-13 cells both the productive and nonproductive κ alleles are demethylated (data not shown) and hence may be equally accessible to recombination trans acting factors. The analysis of secondary rearrangements in 38C-13 cells further showed that for the productive allele, which originally contained a V-Jxκ2 rearrangement, secondary V-Jxκ rearrangements were observed in six of eight Id-negative variants, V-Jκ5 rearrangements in two of eight variants, and none had RS rearrangements. In contrast, on the nonproductive allele, in three of eight variants the V-Jκ5 rearrangement is replaced by an RS rearrangement. These results show that, as previously observed (37, 39, 40), ongoing κ gene rearrangement is associated with progressively more 3′ elements.

V genes from the large Vκ4/5 family have been found to be preferentially utilized in secondary κ 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 κ locus, it is now possible to follow the secondary rearrangements occurring in these cells. It appears that the Vκ4 genes used in the secondary rearrangements lie 3′ to the Vκ38C gene; however, both the Vκ38C gene and most of the Vκ4 genes are in the opposite orientation with respect to the Jκ segments (Fig. 6). Therefore, the Vκ38C-Jκ2 rearrangement on the productive allele occurred by inversion, hence placing the Vκ4 genes 5′ to the rearranged Vκ38C gene. Secondary rearrangements using these genes delete the rearranged Vκ38C gene, which therefore explains the lack of hybridization observed with the Vκ4 probe in the Id-negative variants. Interestingly, there is no strict correlation between distance from the rearranged Vκ38C gene and frequency of utilization of the Vκ4 genes in the secondary rearrangements: V genes situated over 500 kb away from the Vκ38C 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 Vκ gene in splenic B cells does not depend on its distance from the Jκxκ 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 Vκ38C 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 κ 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 recombination 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 κ-positive variants, which express a new BCR, and κ-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-μ 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+CD43+, 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-Jκ5 rearrangement. However, secondary rearrangements at the κ 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...
is still an open question. However, like 38C-13 cells, RAG-expressing peripheral cells express both a pre-BCR and a BCR (18, 19). Furthermore, peripheral secondary rearrangements are thought to occur in vivo within the selective environment of the germinal center.

Termination of secondary rearrangements by down-regulation of RAG after BCR ligation in germinal center B cells has been likened to positive selection of immature T cells in the thymus (44). Recent evidence for positive selection during B cell development comes from work with CD5+ B cells (45), which also undergo secondary light chain gene recombination in the peritoneal cavity (46). Therefore, regulation of RAG activity in phenotypically immature peripheral B cells via the BCR may play an important role in shaping the B cell repertoire. The 38C-13 cell line, which has the characteristics of these RAG-expressing peripheral B cells and can be induced to down-regulate RAG activity, should help to elucidate this potentially important regulatory pathway.

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References