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Immunology

Plasmacytomas with more than one immunoglobulin κ mRNA: Implications for allelic exclusion

(V$_\kappa$ cDNA sequences/variation in V–J joining/nonproductive recombination/pseudo-V gene/stochastic rearrangement model)

ORA BERNARD, NICHOLAS M. GOUGH†, AND JERRY M. ADAMS

The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia

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ABSTRACT **Although only one allele of an immunoglobulin gene is thought to be expressed as a polypeptide by a given lymphocyte ("allelic exclusion"), three murine plasmacytomas were found to contain more than one κ light chain mRNA species, as evidenced by the sequences of distinct κ cDNA clones. Two different κ cDNA sequences were cloned from BFPC 61 microsomal mRNA, two from MOPC 173, and three from S107. One cDNA sequence from each tumor matches the known secreted polypeptide, while the variant sequences differ in the variable (V) region. Hence fusion of a V$_\kappa$ gene to a joining (J$_\kappa$) gene has occurred independently on separate homologous chromosomes and each allele is transcriptionally competent. The BFPC 61 variant sequence contained a normal V$_\kappa$ sequence linked out of phase to J$_{\kappa 2}$; hence allelic exclusion in this line is accounted for by an error in DNA rearrangement. One S107 variant cDNA has an untranslatable sequence linked to J$_\kappa$–C$_\kappa$ and may derive from a non-V$_\kappa$ or pseudo-V$_\kappa$ gene fused to J$_\kappa$. Another S107 variant cDNA, however, has a proper V$_\kappa$ linked in phase to J$_\kappa$ (albeit missing the first two germline J$_\kappa$ codons) and the MOPC 173 variant sequence also contains a proper V$_\kappa$–J$_\kappa$ join, although it does not encode a tryptophan residue common to all immunoglobulin chains. The presence of two potentially expressible κ mRNAs in both S107 and MOPC 173 suggests that allelic exclusion does not hold in all lymphocytes, or that it sometimes reflects events subsequent to mRNA production, such as inability of certain κ chains to assemble properly with the heavy chain. These observations are compatible with a stochastic model for allelic exclusion in which productive and nonproductive V–J recombination events occur at a certain frequency for each allele.**

In contrast to every other autosomal gene studied, expression of immunoglobulin genes exhibits "allelic exclusion" (1). That is, only one of the two alleles for the heavy chain, and one for the relevant light (L) chain (κ or λ), contributes to the immunoglobulin molecule secreted by a given B lymphocyte or its plasma cell progeny. It is relevant to allelic exclusion that production of an immunoglobulin mRNA requires somatic rearrangement of DNA (see ref. 2 for a review). For the κ locus, one of the 100–300 variable (V$_\kappa$) genes (3) is fused to one of four (or five) joining (J) genes, separated from the constant (C$_\kappa$) region by an intervening sequence (4, 5). Similarly, activation of the heavy (H) chain locus requires steps fusing a V$_H$ gene with one of the D$_H$ (diversity) elements (6, 7) and the D$_H$ element to one of the four J$_H$ genes located near the C$_\mu$ gene (6, 8–10). A simple explanation for allelic exclusion would be that one homologous chromosome is rearranged while the other stays in its embryonic context, and that is the case in some plasmacytomas (11–14) and in a fraction of B cells (15, 16). However, rearrangement is not confined to one allele for many plasmacytomas (12–14, 16–23), B lymphomas (24, 25), and normal B cells (16, 26, 27), and hence other mechanisms must operate. Errors in recombination probably have a significant role, because some plasmacytomas exhibit joining at non-J sites for one allele (19, 23, 24) and at least two have a V and J joined out of translational phase (17, 18, 21), so that only a portion of the immunoglobulin chain can be made.

Allelic exclusion could also result from processes acting subsequent to DNA rearrangement. Selective transcription of one allele has been ruled out as a general mechanism because plasmacytoma MPC 11 is known to produce a κ mRNA fragment from a second allele (19) and MOPC 21 has recently been shown to contain a second full-size κ mRNA (18). The results reported here suggest that κ mRNA production by a second allele may be frequent among plasmacytomas. By analyzing the nucleotide sequences of κ cDNA clones, we have established that each of three plasmacytomas contains more than one full-size κ mRNA species. The sequences obtained demonstrate the importance for allelic exclusion of variation in the V–J recombination point but also reveal that some plasmacytomas contain two properly joined κ mRNAs. Hence properties of the κ polypeptide, such as its ability to combine with the heavy chain into a proper immunoglobulin molecule, must have a significant role in allelic exclusion.

MATERIALS AND METHODS

Construction of the κ cDNA clones has been described (28). We initially examined the κ clone having the longest cDNA insert from each tumor. When partial sequence analysis indicated that it did not correspond to the expressed polypeptide, several other clones were examined for differences in restriction pattern. For sequence analysis, a cloned insert was separated from the pBR322 vector by digestion with restriction endonuclease *Hha* I and electrophoresis on a 5% acrylamide gel. Various restriction fragments (Fig. 1) were then labeled at their 5′ ends by using polynucleotide kinase or their 3′ ends by using DNA polymerase I, recut to produce fragments labeled at a single end, and separated on an 8% acrylamide gel. Sequence analysis by partial chemical degradation was essentially as described (29). The sequences of fragments labeled at 5′ ends were also determined as described by Seif *et al.* (30). Additional data were obtained for clone S107C by inserting the *Pst* I fragment into an M13 phage vector (31) and analyzing the sequence by chain termination (32).

RESULTS

Some Plasmacytomas Synthesize More Than One κ mRNA. On examining κ cDNA clones generated from microsomal poly-

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Abbreviations: L and H, light and heavy chains of immunoglobulins; V and C, variable and constant regions of immunoglobulin chains.

† Present address: European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany.

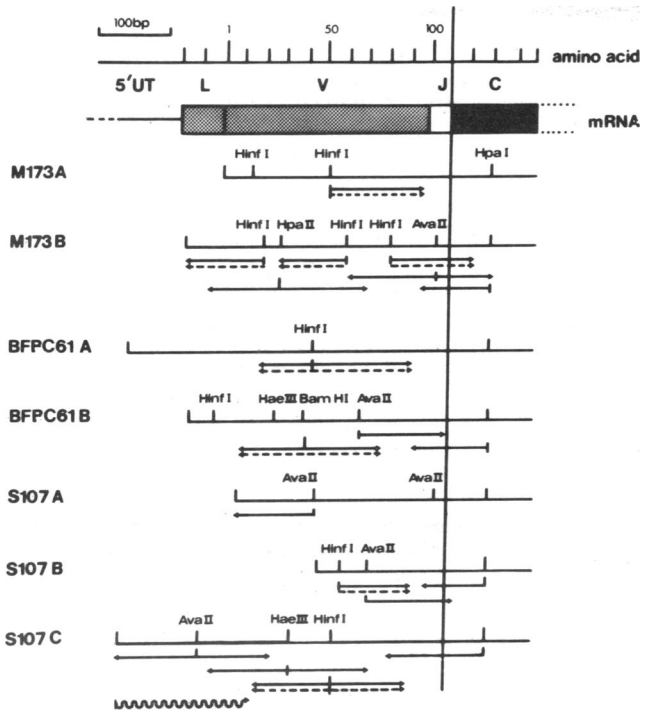

FIG. 1. Restriction maps and sequencing strategy for κ cDNA clones. The *V*, *J*, and 5′ portion of the *C* region of each clone are depicted. bp, Base pairs; 5′ UT, 5′ untranslated region. Solid arrows indicate fragments labeled at 5′ termini, broken arrows those labeled at 3′ termini, and a wavy line, a sequence determined by M13 cloning. Clones M173B, BFPC1B, and S107C were previously designated M173κ14*, B61κ21*, and S107κ4* (3, 28).

adenylylated mRNA of plasmacytomas MOPC 173, BFPC 61, and S107 (28), we found that more than one distinct κ cDNA sequence had been cloned from each plasmacytoma, as indicated by the restriction maps in Fig. 1. Clones from the same tumor exhibited multiple differences in the *V* region but no differences were observed in the C_κ or 3′ untranslated region of any clone. The sequence that proved to correspond to the κ polypeptide known to be expressed by each tumor is designated A. Because the variant sequences B and C were found among the first couple of cDNA clones examined from each line, the corresponding κ mRNAs are probably of comparable abundance. The length of the variant cDNA sequences suggests that those mRNAs are of normal κ mRNA size, and hybridization of a C_κ-bearing fragment to electrophoretically fractionated RNA (33) from S107 and MOPC 173 revealed only full-size κ mRNA.

One BFPC 61 Sequence Has *V* and *J* Out of Phase. The two distinct BFPC 61 cDNA sequences are presented in Fig. 2. We assign clone A as the expressed sequence because the secreted BFPC 61 polypeptide is of the Vκ-21 group or isotype (34) and the A cDNA sequence clearly belongs to that group. Indeed, BFPC 61A is 84% homologous to a Vκ-21 gene from M321 whose sequence has been determined (unpublished results) and 88% homologous to an unexpressed Vκ-21 gene isolated from MPC 11 (19), and it closely matches polypeptides of the Vκ-21 E subgroup (35). BFPC61B also bears an authentic V_κ sequence, as judged by the presence of all nine "invariant" V_κ amino acid residues, such as Cys-23 (36), but its sequence is not closely related to the known V_κ amino acid sequence (36) and hence it probably defines another V_κ isotype. Because the A and B amino acid sequences differ at 26 out of 73 residues, as indicated by the asterisk, the corresponding mRNAs clearly derive from separate alleles. Significantly, the BFPC61B V_κ is joined to the J_κ out of phase by one nucleotide. Hence reading in the V_κ frame will lead to premature termination of translation at the TGA codon starting with the third nucleotide of the C_κ region.

Both MOPC 173 cDNA Sequences Have Proper V_κ–J_κ Joins. The two MOPC 173 cDNA sequences differ at many V_κ positions (Fig. 3). M173A matches the known secreted polypeptide sequence (37) exactly over the codons analyzed. A nonexpressed V_κ genomic sequence (M173B) has been cloned and sequenced recently by Max *et al.* (21). Our M173B cDNA sequence differs from that sequence only at one critical position: the genomic M173B sequence lacks a T residue present near the V_κ–J_κ join in our sequence and hence has V_κ and J_κ out of phase, whereas our cDNA sequence does not. Fig. 4 shows a portion of a sequencing gel demonstrating the sequence TCT-CCT-CCC- (Ser-Pro-Pro) across the V_κ–$J_{\kappa 2}$ join. It is clear that V_κ and J_κ are in phase and the results were as clear-cut for the other strand. Moreover, Fig. 5 shows that our M173B cDNA sequence can be generated by the expected simple crossover between the equivalent germline V_κ gene (21) and $J_{\kappa 2}$, whereas the rearranged M173B genomic sequence requires some extra event. It may, for example, have undergone a one-base deletion at a state subsequent to *V*–*J* joining, either in that M173 tumor line or during molecular cloning. In any case, these results demonstrate that our M173 line contains two distinct κ mRNAs derived from proper V_κ–J_κ joins. It is problematical, however, whether the M173B mRNA would produce a *functional* κ chain, because M173B contains a leucine residue at position 35 rather than the tryptophan found in every κ, λ, and H chain of all species examined, from shark to man (36).

S107 Contains Three Distinct κ RNA Sequences. Fig. 6 displays the three κ cDNA sequences from S107. S107A appears to correspond to the known expressed sequence, because it

FIG. 2. Two BFPC 61 κ cDNA sequences. *V*, *J*, and 5′ C_κ regions are indicated. Positions where the two V_κ amino acid residues differ are indicated with asterisks.

FIG. 3. The two MOPC 173 κ cDNA sequences. Both V_κ sequences were joined to J_2. Differences between the two amino acid sequences are indicated by asterisks. The arginine residue at position 97 in M173A probably reflects a V–J join in which two bases of the codon came from the germline V_κ gene (4, 5). The G_{11} at the 5′ end of MOPC173B was introduced by the cloning method (28).

matches a preliminary amino acid sequence for the very closely related polypeptide TEPC 15 (S. Rudikoff, personal communication). S107B differs at about 37% of amino acids from S107A but appears to be a valid V_κ sequence, because it contains all the invariant V_κ amino acid residues over the region in which the sequence was determined. The S107B V_κ is joined in phase to $J_{\kappa 4}$ but with the first two germline J_κ codons excised and $J_{\kappa 4}$ is properly spliced to C_κ. Thus S107, like MOPC 173, contains two properly joined and potentially translatable κ mRNAs. S107C, on the other hand, is not a true κ mRNA, because the sequence linked to $J_{\kappa 5}$ lacks the invariant V_κ amino acid residues. Moreover, the sequence is not translatable because stop codons occur in all three reading frames. Thus S107C must represent a transcript of a non-V_κ or degenerate (pseudo)-V_κ sequence linked to $J_{\kappa 5}$.

DISCUSSION

Significance of Plasmacytomas with More Than One κ mRNA. Our results establish that three unselected plasmacytomas contain more than one κ mRNA sequence. Because the cDNA clones were derived from microsomal polyadenylyated mRNA (28), it is clear that more than one rearranged κ allele is transcriptionally active in these lines and that the precursor RNAs are processed to mature, full-size κ mRNAs, which are transported to the cytoplasms and bound by ribosomes. κ mRNAs are also made from two alleles in MOPC 21 (18). These findings argue that discrimination at the transcriptional, processing, or transport levels is not the predominant factor in allelic exclusion. We expect that mRNA production by a second allele occurs frequently, but not every rearranged C_κ chromosome may be transcriptionally competent (12). Because the V_κ genes activated on different C_κ alleles are unrelated, homologous chromosomes must rearrange independently.

To account for different amino acids at the V_κ–J_κ join, it has been postulated that the precise site of recombination can vary such that 0, 1, 2, or 3 bases of codon 96 derive from the germline V_κ gene and the rest from the germline J_κ (5, 6). Further variability in the recombination point is demonstrated in our results. First, the BFPC61B sequence has the V_κ and J_κ joined out of phase, as in two other plasmacytomas (17, 18). Thus the κ mRNA species derived from one allele can produce only a κ chain fragment, accounting for allelic exclusion in these lines. Unless there is a special mechanism favoring V_κ and J_κ codons in register, two-thirds of V_κ–J_κ joins will be out of phase. Second, the first two codons of the $J_{\kappa 4}$ germline sequence were bypassed in formation of the S107B V_κ–J_κ allele, thereby markedly shortening the third hypervariable region and presumably altering the antigen-binding site, if indeed this κ chain can associate with a H chain. In any case, it is clear that the V_κ–J_κ joining point can vary over a region of at least six bases.

H chain joining exhibits a similar flexibility, because the first two germline J_H codons are absent from the active V_H–D_H–J_H gene of HPC 76 (8), and one V_H–D_H–J_H allele in pre-B lymphoma ABLS.8 has D_H and J_H out of phase (unpublished results). If joining occurs essentially irrespective of phase for both the V_H–D_H and the D_H–J_H step, V_H and J_H will be in phase in about one-third of V_H–D_H–J_H fusions, because a V_H–D_H join in which, say, one base is lost (-1 phasing) would be compensated by a D_H–J_H join of the -2, $+1$, or $+4$ type. Ability to read

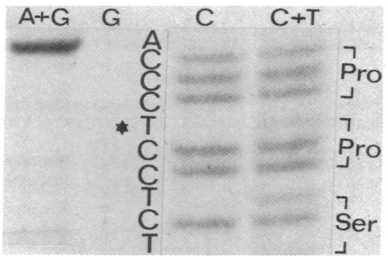

FIG. 4. Portion of a sequencing gel demonstrating the MOPC173B cDNA sequence across the V_κ–J_κ join. The asterisk marks the T residue missing from the genomic MOPC173B sequence studied by Max *et al.* (21).

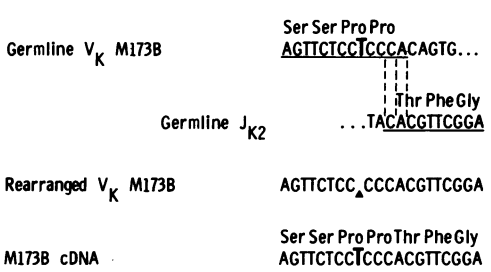

FIG. 5. Relationship of the MOPC173B κ cDNA sequence to the genomic sequences determined by Max *et al.* (21). The T residue present in the cDNA and the germline V_κ sequence (21) is shown in bold, and the corresponding position in the rearranged M173B sequence (21) is marked with an arrowhead. The cDNA sequence can be accounted for by V–J recombination at any one of the three positions indicated.

FIG. 6. The three S107 κ cDNA sequences. S107B is fused to J_4 with two germline J_κ codons deleted, as indicated by the space; S107C uses J_5; the J region of S107A has not been determined. The S107A amino acid sequence shown is a preliminary sequence derived from the virtually equivalent TEPC 15 by S. Rudikoff. One of the stop codons in each reading frame of S107C is underlined.

a given D_H element in each of the three frames in different H chains would markedly increase V_H diversity, and this biological dividend might compensate for the large proportion of inactive heavy chains.

Both S107A and S107B have normal V_κ and J_κ sequences joined in phase (Fig. 6). Similarly, in contrast to the findings of Max *et al.* (21), our MOPC173B as well as the MOPC173A sequence is joined in phase and properly spliced (Fig. 5). These results suggest that allelic exclusion may not hold in all lymphocytes. To test whether S107 and MOPC 173 produce two κ chains, J. Goding (personal communication) has used two-dimensional gel electrophoresis to resolve labeled polypeptides synthesised by the tumors and isolated by binding to *Staphylococcus aureus*, which has a high affinity for certain immunoglobulins (38). S107 and MOPC 173 contained distinct minor chains of ≈25,000 daltons in addition to the major L chain species, whereas BFPC 61 contained only one significant L chain component. Peptide mapping suggests that each species is a κ chain. Thus both S107 and MOPC 173 appear to contain small amounts of a second κ chain, presumably originating from the S107B and MOPC173B mRNAs. Because free L chains are degraded in some tumors, poor assembly of these κ chains with the H chain may explain the low yield. The foreshortened J_κ region in S107B could interfere with chain assembly, as could the absence of the universal tryptophan residue from MOPC173B. These results suggest that allelic exclusion may be a quantitative matter in some lymphocytes, perhaps reflecting the inability of certain L and H chains to assemble into a proper immunoglobulin.

S107C has stop codons in all three reading frames. Kwan *et al.* (40) have recently reported the genomic S107A V_H–J_H sequence. S107C has the same J_H region and the same V_H codons from 90 to 95, but diverges completely upstream from that. We infer that S107C RNA derives from a duplicated S107A V_H–J_H chromosome. One chromosome may have undergone a second rearrangement, such as a deletion extending upstream from codon 90 in V_H S107A. Alternatively, mutation may have led to aberrant RNA splicing with sequences upstream of V_H S107A. While the 5′ segment of S107C lacks the recognizable features of a V_κ region, that segment does label genomic restriction fragment of the sizes labeled by certain valid V_κ probes (unpublished data). This may indicate that the 5′ part of S107C derives from a V_κ-associated sequence.

Models for Allelic Exclusion. Deterministic models, in which activation of one allele directly blocks activation of the other, do not appear tenable. The possibility that only one C_κ allele can undergo rearrangement (15) is clearly ruled out for plasmacytomas, and it appears that about one in three normal κ-expressing B cells has also undergone two C_κ rearrangements (16). Similarly, the present study and other results (18–20, 39) leave no doubt that transcription can occur on more than one rearranged L chain allele. The results fit better with stochastic models (2, 12), in which recombination events occur with a certain frequency for each allele. For example, if we adopt the symbolism (12) of κ^0 as the germline state, κ^+ as a productive rearrangement and κ^- as a nonproductive rearrangement, it is clear that allelic exclusion will occur in the majority of lymphocytes if (*i*) the frequency of κ^+ alleles in a B cell population is much less than κ^0 (an ineffecient recombination machinery) or (*ii*) the frequency of κ^+ is much less than that of κ^- (an error-prone machinery). Because κ-expressing B cells include cells of the $\kappa^+\kappa^0$ and the $\kappa^+\kappa^-$ genotypes (16), both these models may operate for the κ locus, or the $\kappa^+\kappa^0$ cells may be a consequence of a special feedback mechanism (see below). The error-prone model may hold for the H chain locus, because plasmacytomas (7, 13, 16, 23, 24) and B lymphomas (24) rarely exhibit an unrearranged J_H locus and no more than 15% of normal B cells contain a germline J_H allele (16, 26).

As indicated in Table 1, one significant consequence of any strict stochastic model is that not all B lymphocytes will exhibit allelic exclusion. Clearly the ratio of "singles" (here $\mu^+\mu^-$) to "doubles" ($\mu^+\mu^+$) rises as the frequency of productive rearrangement falls. As a consequence, a large fraction of B lymphocytes will be nonfunctional ($\mu^-\mu^-$). The ratio of single to double expressors in the mouse may be more than 100:1 (41, 42). This would correspond to only 2% productive rearrangement, and 96% of lymphocytes would be nonfunctional; even a 10:1 ratio would leave 69% inactive (Table 1). A strict stochastic model applied to both the *H* and *L* loci would be particularly costly in cells, because the frequencies become multiplied. For example, 100:1 discrimination at both the *H* and κ loci would leave only 0.2% of lymphocytes active.

The problem of cell loss is minimized for the L chain loci if we modify the stochastic model by adopting the hypothesis of Alt *et al.* (20) that production of a complete immunoglobulin molecule (H + L chain) shifts the cell from a phase in which κ rearrangement occurs very frequently to one in which it occurs rarely. If a μ-expressing pre-B cell undergoes a $\kappa^0\kappa^0$ to $\kappa^0\kappa^+$ transition and a *functional* κ chain is made, no further rearrangement would occur, whereas an initial $\kappa^0\kappa^-$ could be-

Table 1. Levels of cells exhibiting allelic exclusion predicted for different proportions of productive rearrangement on a strict stochastic model*

Productive events per allele, %†	% doubles‡ ($\mu^+\mu^+$)	% null cells§ ($\mu^-\mu^-$)	Singles/ doubles¶
30	9	49	4.7
17	2.8	69	10
10	1	81	18
3	0.09	94	65
2	0.04	96	100
1	0.01	98	200
0.3	0.0009	99.4	665

* Stochastic models (2, 12) assume that each allele rearranges independently, becoming either productive (here μ^+, for the H chain locus) or nonproductive (μ^-). Hence, for any arbitrarily chosen proportion of productive events in the lymphocyte population, one can calculate the percent of "doubles"—i.e., cells having both alleles productively rearranged ($\mu^+\mu^+$)—the percent of inactive cells—i.e., those with neither allele functional ($\mu^-\mu^-$)—and the ratio of single to double expressors ($\mu^+\mu^-$ to $\mu^+\mu^+$).

† Arbitrarily selected frequencies of productive rearrangement for the lymphocyte population.

‡ If p is the proportion of productive rearrangement per allele, then the proportion of doubles in the lymphocyte population is p^2.

§ If the proportion of nonproductive events is $1 - p$, the proportion of cells with two nonproductive events = $(1 - p)^2$.

¶ Ratio singles ($\mu^+\mu^-$) to doubles ($\mu^+\mu^+$) = proportion singles/proportion doubles = $2p(1 - p)/p^2 = 2(1 - p)/p$.

come either $\kappa^+\kappa^-$ or $\kappa^-\kappa^-$. This feedback scheme permits a higher frequency of productive events than the strict stochastic model. For instance, if the frequencies of κ^+ and κ^- events were the same, $\kappa^0\kappa^0$ precursors would give equal numbers of $\kappa^+\kappa^0$ and $\kappa^-\kappa^0$ cells and about half the latter would become $\kappa^-\kappa^+$ and the rest $\kappa^-\kappa^-$; hence only $\frac{1}{2} \times \frac{1}{2}$ or $\frac{1}{4}$ of the lymphocytes would be $\kappa^-\kappa^-$ and some of these might go on to express λ chains (20, 25, 39).

As do our conclusions on S107 and MOPC 173, this modified stochastic scheme puts emphasis on the ability of a L chain to form a proper immunoglobulin molecule (20), and it would provide allelic exclusion at a L chain locus with minimal cell loss. For the H chain locus, the strict stochastic model may well hold. However, the high loss of lymphocytes predicted in Table 1 could be avoided if the production of a functional μ polypeptide itself led to differentiation changes that depressed further V_H–D_H–J_H fusion events and perhaps activated κ rearrangement. There is evidence for some programming in rearrangement, μ preceding κ (43) and κ precedings λ (20, 25). Thus production of each functional immunoglobulin chain may influence both the recombination process and B lymphocyte maturation.

Note Added in Proof. That S107 expresses two κ chains has also been found by Kwan *et al.* (44).

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