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Restricted association of *V* and *J–C* gene segments for mouse λ light chains

(immunoglobulin/recombination/amino acid sequence variation/hybridoma/myeloma)

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ABSTRACT **The frequencies of diverse rearrangements of variable (*V*)λ to joining (*J*)λ gene segments were examined by Southern blot hybridization in 30 murine B-cell lines, each producing an immunoglobulin λ light chain of known subtype (λ1, λ2, or λ3). For 11 out of 12 λ1 chains, the rearrangement was *V*λ1 → *J*λ1; for 9 out of 9 λ2 chains, it was *V*λ2 → *J*λ2; and for 8 out of 9 λ3 chains, it was *V*λ1 → *J*λ3. Similar results were obtained by considering the partial or complete sequences at the amino acid or cDNA level of 44 other λ chains (24 previously described): for 43 of these chains the rearranged *V–J* gene segments were evidently *V*λ1–*J*λ1 for 28 λ1 chains, *V*λ2–*J*λ2 for 10 λ2 chains, and *V*λ1–*J*λ3 for 5 λ3 chains. Of the combined total of 74 chains there were 3 with unusual *V*λ rearrangements, all involving the *V*λ2 gene segment: for 2 of these unusual chains, the encoding segments were *V*λ2–*J*λ1–*C*λ1 and for one they were *V*λ2–*J*λ3–*C*λ3. Thus, the results for all 74 λ chains show that, in contrast to the apparently unrestricted *V*κ→ *J*κ rearrangements for κ chains, for each of the 3 murine λ-chain subtypes *V–J* recombination is severely restricted: the *V*λ gene segment expressed in λ1 and λ3 chains was nearly always *V*λ1 (95% and 93%, respectively), whereas in λ2 chains it was without exception *V*λ2 (19 out of 19 chains). Therefore *V*λ–*J*λ combinatorial variation is not a significant source of amino acid sequence diversity of λ chains of inbred mice. If the order of the λ gene segments is 5′ *V*λ2-*J*λ2*C*λ2*J*λ4*C*λ4-*V*λ1-*J*λ3*C*λ3*J*λ1*C*λ1 3′, as suggested previously and by the present findings, it appears that (*i*) when a *V*λ gene segment rearranges in a developing B cell it ordinarily recombines with a *J*λ gene segment in the nearest downstream (3′) cluster of *J*λ*C*λ segments, and (*ii*) *V*λ rearrangement to the upstream (5′) cluster is very rare and possibly may not take place at all.**

The commitment of a developing B lymphocyte to produce an immunoglobulin (Ig) light (L) chain depends on a preliminary step in which a variable region (*V*) gene segment recombines with a joining region (*J*) gene segment on the same chromosome (1–5). For mouse L chains of the κ type there are several hundred *V*κ genes (6–8), five *J*κ gene segments (four are functional) and one *C*κ (constant region κ chain) gene segment (4–6), and it seems that any *V*κ segment can recombine with any of the functional *J*κ segments. For mouse L chains of the λ type, the corresponding gene segments (*V*λ, *J*λ, *C*λ) are organized differently. In the inbred strains, there are two *V*λ, four *J*λ, and four *C*λ gene segments (9, 10). Each of the *C*λ gene segments is paired with a unique *J*λ segment about 1 kilobase (kb) on its upstream (5′) side, and the four paired *J*λ–*C*λ gene segments are organized into two clusters (*J*λ2*C*λ2–*J*λ4*C*λ4 and *J*λ3*C*λ3–*J*λ1*C*λ1), probably with one *V*λ gene segment upstream of each cluster (9–12). These gene segments specify the three λ-chain subtypes (13–15). L chains whose amino acid residues from positions 97 or 98 to the COOH terminus (positions 214 or 215) are encoded by the *J*λ1–*C*λ1 or the *J*λ2–*C*λ2 or the *J*λ3–*C*λ3 gene segments are designated, respectively, as λ1, λ2, or λ3 chains (13–15). The fourth pair, *J*λ4–*C*λ4, appears to be a pseudogene (11, 12).

Amino acid sequences of 19 λ chains of the most abundant subtype (λ1) indicate that each of these chains is encoded by a λ gene in which the *V*λ1 and *J*λ1 gene segments are joined (*V*λ1 → *J*λ1) (13, 16, 17). For the other subtypes, only little information is available about their *V* → *J* rearrangements. However, the evidence for 3 λ2 and 2 λ3 chains (14, 18, 19) suggests that for these subtypes there may also be restrictions in the *V*λ → *J*λ recombinations that can occur. The restrictions are interesting for two reasons: (*i*) If consistent, they have the effect of minimizing *V–J* combinatorial variation (4, 5, 20, 21) as a source of amino acid sequence diversity of λ chains. (*ii*) They may contribute to our understanding of the organization and expression of λ genes. To learn more about the consistency and implications of restrictions on *V*λ–*J*λ recombination, we analyzed *V*λ gene rearrangements in 30 B-cell tumors of inbred mice and considered the *V*λ rearrangements responsible for 44 other chains (24 of them previously described). The combined evidence demonstrates marked restrictions of *V*λ–*J*λ rearrangements for λ2 and λ3 chains, as well as for λ1. Three unusual rearrangements were also seen and all of them involved the *V*λ2, not the *V*λ1, gene segment. This difference between *V*λ1 and *V*λ2 is statistically significant; it reinforces the previous suggestion (22) that the linear order of λ-gene segments is probably 5′ *V*2-*J*2*C*2*J*4*C*4-*V*1-*J*3*C*3*J*1*C*1 3′. It thus appears that when a *V*λ gene segment rearranges, it recombines almost exclusively with a *J*λ gene segment in the nearest downstream (3′) cluster; recombination in the upstream (5′) direction is very rare, if it occurs at all.

MATERIALS AND METHODS

λ-Producing B-Cell Clones. The 30 cell lines examined are listed in Table 1. Each secretes a λ chain of a particular subtype. A chain's subtype designation (column 3) is based on the properties of its C domain as determined by one (or more) of three methods: (*i*) amino acid sequence at positions 111 to 214 or 215 (13–15); (*ii*) radioimmunoassays with monoclonal antibodies to the C region of λ1 chains (anti-Cλ1; ref. 23), with purified rabbit antibodies that react with λ2 and λ3 but not with λ1 chains (anti-λ2/3) and other purified rabbit antibodies that react with λ1 and λ3 but not with λ2 chains (anti-λ1/3) (unpublished observations); and (*iii*) electropho-

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Abbreviations: V, variable; J, joining; C, constant; L, light; H, heavy; kb, kilobase(s); bp, base pair(s); NP, 3-nitro-4-hydroxyphenylacetyl; DNP, 2,4,-dinitrophenyl; 1× NaCl/Cit, 0.15 M NaCl/0.015 M Na citrate.

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Table 2. Size (in kb) of *Eco*RI fragments resulting from each of the eight possible $V\lambda \rightarrow J\lambda$ recombinations in BALB/c mice

$J\lambda$ gene segment	$V\lambda$ gene segment	
	$V\lambda 1$	$V\lambda 2$
$J\lambda 1$	7.4	9.0
$J\lambda 2$	4.9	6.5
$J\lambda 3$	2.8	4.4
$J\lambda 4$	4.1	5.8

The unrearranged $V\lambda 1$ and $V\lambda 2$ gene segments are 3.5 and 4.8 kb, respectively. To distinguish between bands of 4.9 and 4.8 kb, representing, respectively, $V\lambda 1 \rightarrow J\lambda 2$ rearrangement and unrearranged $V\lambda 2$, a $C\lambda 2$-specific probe was used. Data are based on refs. 2, 9, and 26.

cally possible recombinations between the two $V\lambda$ and the four $J\lambda$ gene segments can be predicted (Table 2). With the aid of the $V\lambda 1 J\lambda 1$ and the $C\lambda 2$ probes, each of these fragments can be identified and distinguished from the unrearranged $V\lambda$ gene segments. Illustrative results with *Eco*RI digests from cell lines that produce each of the subtypes are shown in Figs. 1 and 2, and all the results are summarized in Table 1.

Of the 12 lines that produced λ1 chains, 11 had only a $V1 \rightarrow J1$ rearrangement and one, hybridoma P5-43.6, had both $V1 \rightarrow J1$ and $V2 \rightarrow J1$ rearrangements. This hybridoma produced an anti-3-nitro-4-hydroxy-phenylacetyl (NP) monoclonal antibody with an unusual λ1 chain: it reacted with antibodies to the C region of λ1 chains (23) and with antibodies to the V region of λ2 chains, but not with antibodies to the C region of λ2 or λ3 chains (unpublished observations). The CNBr fragments of this unusual light chain differed from those of conventional λ1, λ2, or λ3 chains, but agreed with those expected for a chain encoded by $V\lambda 2$–$J\lambda 1$–$C\lambda 1$ gene segments (ref. 15; unpublished observations). It appears, therefore, that in P5-43.6 cells it is the $V2 \rightarrow J1$ rearrangement, not $V1 \rightarrow J1$, that is responsible for the secreted λ chain.

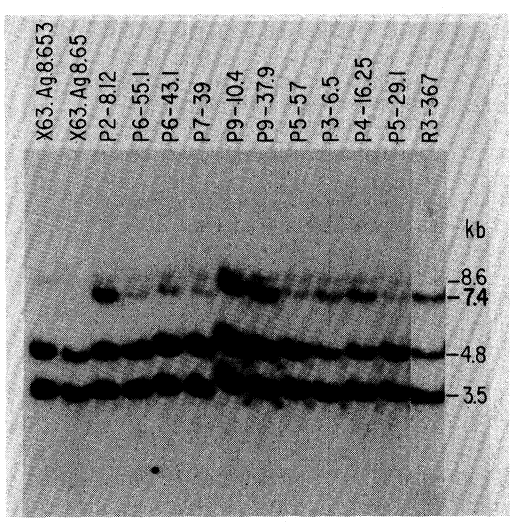

FIG. 1. Southern blot analysis of *Eco*RI-digested DNA from λ1-producing hybridomas, using a $(VJ)\lambda 1$ probe. Ten micrograms of each DNA sample was electrophoresed and analyzed by the Southern blotting technique (25), with 10^7 cpm of probe under conditions described (9). The first two lanes (X63.Ag8.653 and X63.Ag8.65) represent DNA from the fusing myeloma cells used to prepare the hybridomas examined here. Numbers at right mark fragment size in kb. Bands at 8.6, 4.8, and 3.5 kb correspond to unrearranged $J\lambda 1$–$C\lambda 1$, $V\lambda 2$, and $V\lambda 1$ gene segments, respectively. Fragments with $V\lambda$ gene segment rearrangements are in bold face (7.4 kb). *Hin*dIII digests of λ phage DNA were used as molecular weight markers.

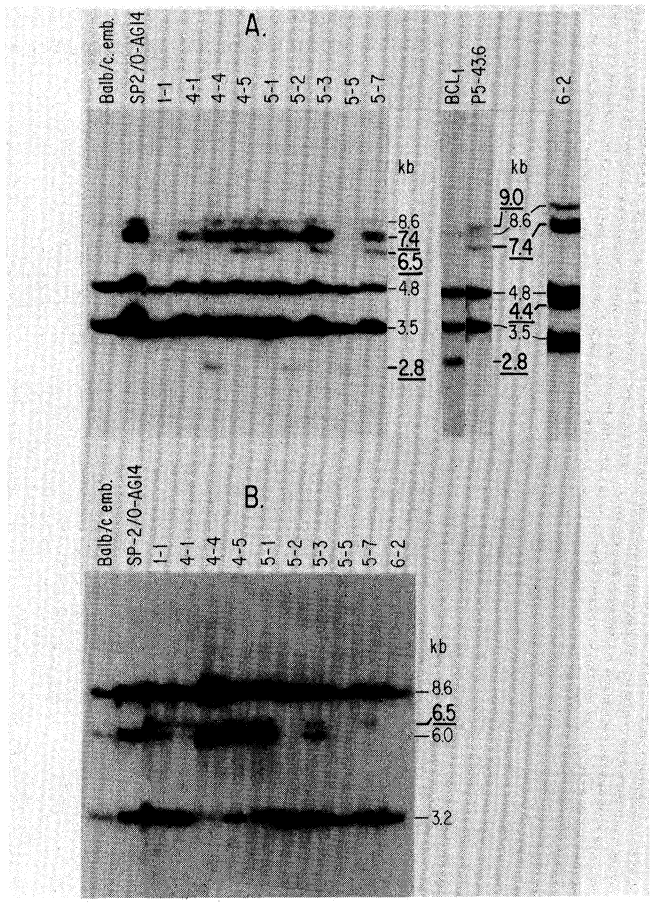

FIG. 2. Southern blot analysis of *Eco*RI-digested DNA from λ2 and λ3-producing B-cell clones with a $(VJ)\lambda 1$ probe (*A*) and a $C\lambda 2$ probe (*B*). Fragment sizes are indicated in kb. $V\lambda \rightarrow J\lambda$ rearrangements are underlined and indicated in bold face. Except for BCL_1 (a B-cell lymphoma) and P5-43.6 (an anti-NP monoclonal antibody), the numbered samples correspond to hybridomas that are designated with the prefix RZ in Table 2. Lanes designated SP2/0-Ag 14 represent DNA from the fusing cell used to prepare these hybridomas. Note the presence in *A* of 9.0- and 4.4-kb bands representing, respectively, $V2 \rightarrow J1$ and $V2 \rightarrow J3$ rearrangements (in P5-43.6 and RZ 6-2). Note also the absence in *B* of a 4.9-kb band, indicative of a $V1 \rightarrow J2$ rearrangement. The 6.0-kb band in *B* represents incomplete digestion with *Eco*RI (9).

Each of the nine λ2-secreting cell lines has a $V2 \rightarrow J2$ rearrangement. However, seven of the nine were hybridomas and they also had a nonproductive $V1 \rightarrow J1$ rearrangement, which was present in, and evidently derived from, the fusing Sp 2/0 myeloma cells that were used to prepare these hybridomas (Fig. 2). Of the nine λ3-secreting cell lines, all but one had the expected $V1 \rightarrow J3$ rearrangement (and the hybridomas that had been prepared by fusion with Sp 2/0 myeloma cells also had, as expected, a $V1 \rightarrow J1$ rearrangement). The one exceptional λ3-producing line is hybridoma 6-2 (Table 1, no. 30). Hy 6-2 produces an unusual λ chain which, on the basis of partial amino acid sequence analysis of a mixture of CNBr fragments, was interpreted as having been encoded by $V\lambda 2$–$J\lambda 3$–$C\lambda 3$ gene segments (22). The validity of this interpretation is borne out by the demonstration that this cell has a $V2 \rightarrow J3$ rearrangement (Fig. 2).

Thus, of the eight theoretically possible $V\lambda$–$J\lambda$ recombinations, only three were not observed: neither $V\lambda 1$ nor $V\lambda 2$ recombined with $J\lambda 4$, and no recombinations between $V\lambda 1$ and $J\lambda 2$ were seen. The $J\lambda 4$–$C\lambda 4$ gene pair appears to be a pseudogene and the absence of any rearrangement to $J\lambda 4$ may be due to a 2-bp deletion in the heptamer recognition

Table 1. Summary of $V\lambda \rightarrow J\lambda$ gene segment rearrangements in mouse B-cell tumors and hybridomas that produce λ-containing immunoglobulins

No.	Tumors and hybridomas	λ-chain subtype	*Eco*RI fragment (in kb) with rearranged $V\lambda$ genes				
			9.0 (*V2J1*)	7.4 (*V1J1*)	6.5 (*V2J2*)	4.4 (*V2J3*)	2.8 (*V1J3*)
1	P2-8.12	λ1		+			
2	P6-55.1	λ1		+			
3	P6-43.1	λ1		+			
4	P7-39	λ1		+			
5	P0-10.4	λ1		+			
6	P9-37.9	λ1		+			
7	P5-57	λ1		+			
8	P3-6.5	λ1		+			
9	P4-16,25	λ1		+			
10	P5-29.1	λ1		+			
11	R3-367	λ1		+			
12	P5-43.6	λ1 (*V2J1C1*)	+	+			
13	RZ 1-1	λ2		(+)	+		
14	RZ 4-1	λ2		(+)	+		
15	RZ 4-5	λ2		(+)	+		
16	RZ 5-1	λ2		(+)	+		
17	RZ 5-3	λ2		(+)	+		
18	RZ 5-7	λ2		(+)	+		
19	ZD 461	λ2		(+)	+		
20	1 BE 79	λ2			+		
21	TEPC-952	λ2			+		
22	RZ 4-4	λ3		(+)			+
23	RZ 5-2	λ3		(+)			+
24	RZ 5-4	λ3		(+)			+
25	RZ 5-5	λ3		(+)			+
26	RZ 5-8	λ3		(+)			+
27	P5-41.6	λ3		+			+
28	BCL_1	λ3					+
29	ABPC-72*	λ3					+
30	RZ 6-2	λ3 (*V2J3C3*)		(+)		+	

The λ-chain subtypes (λ1, λ2, λ3) are defined by their respective C regions (amino acid positions 111–214 or 215; see refs. 13–15). Numbers 1–12 and 27 are anti-NP hybridomas made by fusing myeloma X63 Ag8.653 or X63 Ag8.65 with spleen cells of immune B6 mice (unpublished results). Numbers 13–18, 22–26, and 30 are anti-DNP hybridomas made by fusing myeloma SP2/0-Ag14 with spleen cells of DNP-Ficoll-immunized BALB/c mice (unpublished results). Number 20 is an anti-4-azophthalate hybridoma made with X63 Ag8-653 as the fusing partner (gift of R. Bankert, Roswell Park Institute, Buffalo, NY). Numbers 21 and 29 are myeloma tumors from M. Potter and Litton Bionetics (Silver Spring, MD). Number 19 is a BALB/c anti-DNP hybridoma made with SP2/0-Ag14 as fusing partner (unpublished observations). Number 28 is a B-cell lymphoma generously provided by S. Strober (Stanford Medical School). (+) refers to $V1 \rightarrow J1$ rearrangements in hybridomas that could have been contributed entirely by the myeloma fusing partner (SP2/0-Ag14).

*ABPC-72 also contains a 3.9-kb fragment that hybridized with the *VJ*(λ1) probe and a 4.9-kb fragment that hybridized with a *C*λ1 probe. Both fragments might have arisen from a *V2–J1* rearrangement (9.0-kb fragment expected; see Table 2) in a cell that had a mutation in the *J*λ1–*C*λ1 intron, leading to an unusual susceptibility to *Eco*RI cleavage and the observed 3.9- and 4.9-kb fragments. The possibility that the 3.9-kb fragment represents a $V\lambda1 \rightarrow J\lambda4$ rearrangement was ruled out by its failure to cross-hybridize with a *C*λ1 probe. However, the possibility that this fragment is due to a rearrangement of the *V*λ1 gene segment to the *J2*-like sequence in the *J*λ2–*C*λ2 intron (12) has not been ruled out.

retic properties of its CNBr fragments (15) in comparison with those of prototypic λ chains. A chain with properties that match those of the λ chains from myeloma MOPC-104E (13), MOPC-315 (14), or CBPC-49 (15) is designated as λ1, λ2, or λ3, respectively.

DNA Preparation and Restriction Enzyme Digestion. High molecular weight DNA was extracted from kidney and solid tumors grown as subcutaneous implants in syngeneic BALB/cAnN mice as described (1). DNA was digested to completion with *Eco*RI (Bethesda Research Laboratory, Gaithersburg, MD).

Hybridization Probes. Hybridization probes included ^{32}P-labeled nick-translated copies of encoding sequences of *V*λ1–*J*λ1 and *C*λ2. The *V*λ1–*J*λ1 probe consisted of a 330-base-pair (bp) *Hha* I/*Hae* III fragment of H2020 DNA in the B1 plasmid (9), while the *C*λ2 probe consisted of an ≈350-bp *Bgl* I fragment of the pλII-1 plasmid of MOPC-315 cDNA (gift of A. Bothwell) containing ≈80% of the *C*λ2 encoding sequence (24).

Gel Blotting of DNA Fragments and Hybridization. Ten micrograms of *Eco*RI-digested DNA was electrophoresed through a 0.8% agarose gel, transferred to nitrocellulose paper (25), and hybridized with 10^7 cpm of ^{32}P-labeled probe at 42°C in 2× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate)/50% formamide (9). Filters were washed 3 times in 2× NaCl/Cit/0.1% $NaDodSO_4$ at 42°C–46°C for 15 min each, and then 2 times with 0.1× NaCl/Cit/0.1% $NaDodSO_4$ at 50°C for 15 min each.

RESULTS AND DISCUSSION

From the restriction enzyme maps of λ genes (2, 9, 26) the sizes of the *Eco*RI fragments for each of the eight theoreti-

sequence upstream of *J*4 (11, 12). In all, the *V*λ1 gene segment was rearranged in 22 cell lines; *V*λ2 was rearranged in 11 cell lines. A secreted λ chain (or λ-containing Ig) was associated with 19 of the 22 rearranged *V*λ1 segments and with each of the 11 rearranged *V*λ2 segments (i.e., 3 *V*λ1 rearrangements were not productive). Every rearranged *V*λ1 gene segment was combined with one of the *J*λ gene segments of the *J3C3J1C1* cluster (in 14 cell lines to *J*λ1 and in 8 cell lines to *J*λ3). Of the rearranged *V*λ2 gene segments, 9 were to the *J*λ2 gene segment and 2 were to a *J*λ gene segment of the other cluster (1 to *J*λ3 and 1 to *J*λ1).

Do the *V*λ1 and *V*λ2 gene segments differ significantly in the frequency with which they undergo rare ("anomalous") rearrangements—i.e., *V*1 → *J*2, and *V*2 → *J*3 or *V*2 → *J*1? For the λ chains produced by the 30 cell lines analyzed here there were no anomalous *V*λ1 rearrangements (0/19) and there were 2 anomalous *V*λ2 rearrangements (2/11). With this small sample, the difference between the productively rearranged *V*λ1 and *V*λ2 segments (0/19 vs. 2/11) is statistically not significant ($P = 0.086$). If nonproductive rearrangements are included, the frequency of anomalous rearrangements of *V*λ1 is 0/22 and for *V*λ2 the frequency remains 2/11 (i.e., there were no nonproductive *V*λ2 rearrangements), and the *V*λ1–*V*λ2 difference is still not statistically significant ($P = 0.116$). However, the difference is significant when the sample is enlarged to include additional chains. The expansion is justified because in this study we have found that unusual λ chains, such as hybridomas 6-2 and P5-43.6 (Table 1; Figs. 1 and 2), can be reliably identified by polyacrylamide gel electrophoresis of CNBr fragments (15) and by serological analysis with a panel of antibodies (unpublished observations), as well as by amino acid sequence analyses and Southern blots of restriction enzyme fragmented DNA (Fig. 2). Therefore, for an evaluation of differences in the frequency of anomalous rearrangements of the 2 *V*λ gene segments we include information on 44 additional λ chains: 24 of these chains have been previously described and 20 others are from a new series of anti-2,4,-dinitrophenyl (DNP) monoclonal antibodies recently prepared in this laboratory for analysis of *V*-region mutations (unpublished data). Of the latter 20, 1 is evidently encoded by *V*λ2–*J*λ1–*C*λ1 gene segments, since it reacts, like P5-43.6, with a monoclonal antibody to the C region of λ1 chains and with antibodies to the V region of λ2 chains, but not with antibodies to the C regions of λ2 and λ3 chains. The other 19 in this series have conventional λ chains, including 9 λ1 (*V*λ1–*J*λ1–*C*λ1), 7 λ2 (*V*λ2–*J*λ2–*C*λ2), and 3 λ3 (*V*λ1–*J*λ3–*C*λ3).

The encoding gene segments for the 24 previously described λ chains are evident from partial or complete sequences at the amino acid (13, 14, 16–19) or cDNA (ref. 24; unpublished observations) level. All of the *V*λ–*C*λ associations are conventional in this set of chains, which includes nineteen λ1 (*V*λ1–*J*λ1–*C*λ1), three λ2 (*V*λ2–*J*λ2–*C*λ2), and two λ3 (*V*λ1–*J*λ3–*C*λ3). Combining the results for these additional 44 chains with those for the chains from the 30 cell lines shown in Figs. 1 and 2, we have evidence altogether for 52 *V*λ1 and 22 *V*λ2 rearrangements. All of the *V*λ1 rearrangements and 3 of the *V*λ2 rearrangements were to the *J3C3J1C1* cluster; the remaining 19 *V*λ2 rearrangements were to *J*λ2, in the other cluster. For this enlarged sample the difference in frequency of anomalous rearrangements of *V*λ1 (0/52) and *V*λ2 (3/22) is statistically significant ($P = 0.02$). If, however, the rearrangements are viewed in respect to the 3 *J*λ segments, the statistical significance of the *V*λ1–*V*λ2 difference becomes uncertain, because while there were no anomalous rearrangements to *J*λ2, there were only 2 to *J*λ1 and 1 to *J*λ3. In any event, the present findings mean, in terms of the λ subtypes, that the encoding *V*λ gene segment is *V*λ2 for 19/19 λ2 chains, and is *V*λ1 for 39/41 λ1 chains and for 13/14 λ3 chains; for 5% of λ1 and 7% of λ3 chains, the rearranged *V* segment is *V*λ2.

The difference in frequency of anomalous rearrangements of *V*λ1 and *V*λ2 is probably related to the linear order of the λ gene segments. These segments are all located on chromosome 16 (27), but their order is unknown and the two *J*λ*C*λ clusters have not been physically linked to each other or to the *V*λ segments. However, indirect evidence suggests that one *V*λ gene segment is upstream of each *J*–*C* cluster (9, 10), and it has been proposed, on the basis of the unusual combination of amino acid sequences in the L chain of hybridoma 6-2 ($L^{6\text{-}2}$) (22) that the order is

5′ *V2-J2C2J4C4-V1-J3C3J1C1* 3′.

The demonstration here of the appropriate rearrangement (V2 → J3) in the cells that make this chain (Hy 6-2; Fig. 2), and the identification of two unusual λ1 chains that resemble $L^{6\text{-}2}$ in having rare *V*λ2 recombinations, strengthen the argument for the proposed order. If we assume this order is correct, it would appear that when a *V*λ gene segment rearranges in a developing B cell it ordinarily recombines with a *J*λ gene segment in the nearest downstream cluster, but that in 10–15% of the *V*λ2 rearrangements the recombination takes place to the distant cluster. (These estimates assume that there is no selection for or against unusual *V*–*J* combinations, such as *V2–J1–C1* or *V2–J3–C3*.) Rearrangement to the upstream cluster must be much rarer, and possibly may not take place at all.

Combinatorial variation arising from the recombination of *V*κ with diverse *J*κ gene segments or V_H with diverse D_H and J_H gene segments is an important source of amino acid sequence variation in κ and heavy (H) chains (4, 5, 20, 21). With λ chains this source of variation is limited by the marked restrictions on *V*λ gene segment rearrangements, and this may mean that, in comparison with, say, a *V*κ gene segment, any particular *V*λ gene segment is likely to encode a minimally diversified set of chains and thereby participate in a very limited set of antibody molecules, spanning a narrow range of idiotypes and antigen-binding specificities. The λ2 chains are especially restricted as all of them seem to be encoded by a single *V* and a single *J* (as well as a single *C*) gene segment. Nonetheless, λ chains are abundant in the antibodies made against some antigenic groups—e.g., against NP (28–30), dextran (31–32), and DNP (unpublished observations).

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