Brief Definitive Report

A NEW VARIABLE REGION IN MOUSE IMMUNOGLOBULIN λ LIGHT CHAINS

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The light chains of mouse Igs have been classified into two isotypes, κ and λ. In contrast to the κ chains, the λ chains were shown to display restricted heterogeneity. Two *Vλ* gene segments and four *Jλ-Cλ* gene pairs have been described (1). The three λ subtypes (λ1, λ2, and λ3) are respectively encoded by the combination of *Vλ1-Jλ1-Cλ1*, *Vλ2-Jλ2-Cλ2*, and *Vλ1-Jλ3-Cλ3* gene segments. The *Jλ4-Cλ4* gene pair was never found to be functional (2, 3). In the mouse, study of the λ-bearing Igs is made difficult by the fact that they represent only 5% of total Igs (4). Three strategies were described to increase their level in sera. The first method consisted in immunizing mice with antigens that are known to elicit λ^+ immune responses (5). The second method consisted in producing κ-immunosuppressed mice by repeated injections of anti-κ antibodies (6). We have previously described a third method, which is to inject mice with anti-λ antibodies coupled to LPS (7, 8). In this paper we describe BALB/c hybridomas obtained after injection with anti-λ2 antibodies coupled to LPS and demonstrate the existence of an unexpected new λ light chain.

Materials and Methods

Radioimmunoassays. In this study, five inhibition assays were used to characterize the isotype of the murine Ig light chain. Each system was carried out as follows: Anti-κ/κ assay was carried out by the inhibition of the fixation of ^{125}I–rabbit anti–κ to 48-9 (γ,κ) murine monoclonal Ig. The anti-κ antibodies were isolated from an anti-MOPC 460 (α,κ) antiserum on an XRPC24 (α,κ) Sepharose-AH column. Anti-λ1/λ1 assay was carried out by the inhibition of the fixation of ^{125}I–rabbit anti–λ1 to TNP 15 (μ,λ1) murine monoclonal Ig. The anti-λ1 antibodies were isolated from an anti-J558 (α,λ1) antiserum previously absorbed on an HO-2.2 (μ,κ) column, on a B1-8 (μ,λ1) column. Anti-λ2/λ2 assay was carried out by the inhibition of the fixation of ^{125}I–rabbit anti–λ2 to TNP 9 (γ,λ2) murine monoclonal Ig. The anti-λ2 antibodies were isolated from an anti-MOPC 315 (α,λ2) antiserum repeatedly absorbed on a J558 (α,λ1) column, on a MG8-13 (γ,λ2) column. Anti-λ2/λ1 assay was carried out by the inhibition of the fixation of ^{125}I–rabbit anti–λ2 to TNP 5 (μ,λ1) murine monoclonal Ig. The anti-λ2 antibodies were isolated from an anti-MOPC 315 (α,λ2) antiserum on J558 (α,λ1) column. Subsequently, the anti-α antibodies were absorbed on a MOPC 460 (α,κ) column. Anti-λ1/λ3 assay was carried out by the inhibition of the fixation of ^{125}I–rabbit anti–λ1 to 1.5F9 (γ,λ3) murine monoclonal Ig. The anti-λ1 antibodies were prepared as described above.

Isoelectric Focusing. Cells (8×10^6) were cultured for 30 min in 1 ml of minimum medium, then 300 μCi of [^{3}H]leucine were added for 3 h, after which the supernatants were harvested. Supernatants (50–100 μl) were incubated with 12 μl of protein A–Sepharose pretreated with 5 μl of rabbit anti-λ1 or anti-λ2 immune serum. Half of the immunoprecipitated material was deposited on a 6% acrylamide gel containing 0.1% SDS. After the migration (20 mA constant current), the band containing bromophenol blue

was cut out (light chains migrate with the front) and deposited on a 4% acrylamide gel with 8 M urea and pH 5–8 ampholines. After migration (18 h at 400 V and 1 h at 1,000 V), the gel was fixed and then incubated with enhancer solution. After water washing and drying, the gel was autoradiographed (1-wk exposure on Kodak X-Omat AR film). For more technical details see reference 9.

Dot Blots. Cells (4×10^6) were washed twice in balanced salt solution (BSS) and incubated at 4°C with 90 μl of wall buffer (0.01 M Tris HCl, pH 8.4; 0.14 M NaCl; 1.5 M $MgCl_2$) plus 10 μl of Triton X-100 (10%). After centrifugation, the supernatant was mixed with 100 μl of 2× proteinase K buffer (0.2 M Tris HCl, pH 7.4, 0.3 M NaCl; 0.025 M EDTA; 2% SDS) plus 100 μg of proteinase K and incubated for 30 min at 37°C. Cytoplasmic RNA was extracted by the chloroform method. 440 μl of 20× SSC, 160 μl of 37% formaldehyde, and 200 μl of water were added to 80 μl of RNA solution. After a 15-min incubation at 60°C, 50 μl of this preparation or successive dilutions were filtered through a nitrocellulose filter. The RNA filters were hybridized with ^{32}P-labeled DNA probes ($5–50 \times 10^5$ cpm/ml) in formamide hybridization buffer (50% formamide, 5× SSC, 50 mM Na_2PO_4, pH 6.5, 0.2% SDS, 5× Denhart's, 10 mg/ml of Salmon DNA). Hybridization was carried out at 42°C for 18 h and washing with 0.1× SSC, 0.1% SDS at 42°C. The Vλ1 and the Cλ1 probes were 0.9-kb and 3.6-kb restriction fragments from an Xba I digest of λ1 DNA clones (10). The Cλ2 probe was a 200-bp restriction fragment from Ava I and Pst I digest of the pλII-I insert (11). The probes were generously given by Dr. T. J. Kindt (National Institutes of Health, Bethesda, MD).

Nucleotide Sequencing. RNA was extracted from solid tumors by the LiCl-urea method (12) and poly(A)$^+$-mRNA was isolated on an oligo(dT)-cellulose column. The RNA fractions coding for heavy and light chains were separated on a sucrose gradient (5–20%). Experimental conditions for nucleotide sequencing by the method of Sanger et al. (13) were described by Rocca-Serra (14). The Cλ primer (dTCAGAGGAAGGTG) was synthetized by the laboratory of Dr. Igolen (Institut Pasteur, Paris).

Results and Discussion

Characterization of λ B Cell Clones. To obtain hybridomas secreting λ-bearing Igs, Sp2.0 cells were fused with spleen cells from a BALB/c mouse pretreated 3 d before with rabbit anti-λ2 antibodies coupled to LPS. We selected λ-secreting hybridomas (B hybridomas) using an anti-λ2 mAb that did not recognize κ or λ1 light chains. As the anti-λ2 antibody also reacted with the λ3 chain (8), we attempted to characterize the λ2 or λ3 subtype of our hybridomas by biochemical methods. Isoelectric focusing of [^3H]leucine biosynthetically labeled light chain was performed (Fig. 1). In addition to the two expected isoelectric spectra for the λ2 and the λ3 chains, a third isoelectric spectrum (group III) could be distinguished among the B hybridomas. Group I and group II corresponded, respectively, to the λ2 and λ3 groups, as determined by SDS-PAGE analysis (not shown). The isoelectric spectrum of group III differed also from that of λ1 chains.

A New Variable Region. To characterize these groups serologically, we used rabbit anti-λ antibodies to either variable or constant regions of λ chains (15). Since the homology of sequence between Vλ1 and Vλ2 or between Cλ2 and Cλ3 regions, respectively, is more extensive (>90%) than the homology between the Cλ1 and Cλ2 regions or between Cλ1 and Cλ3 (2, 10, 16, 17), anti-Cλ antibodies can distinguish between λ1 subtype, on one hand, and λ2 or λ3 subtypes, on the other. Anti-Vλ antibodies, however, will recognize all λ subtypes. Thus, we used four RIAs where the anti-λ1/λ1 and the anti-λ2/λ2 interactions, which are more specific for Cλ regions, and the anti-λ1/λ3 and anti-λ2/λ1 interactions, which are more specific for Vλ regions, were inhibited by different

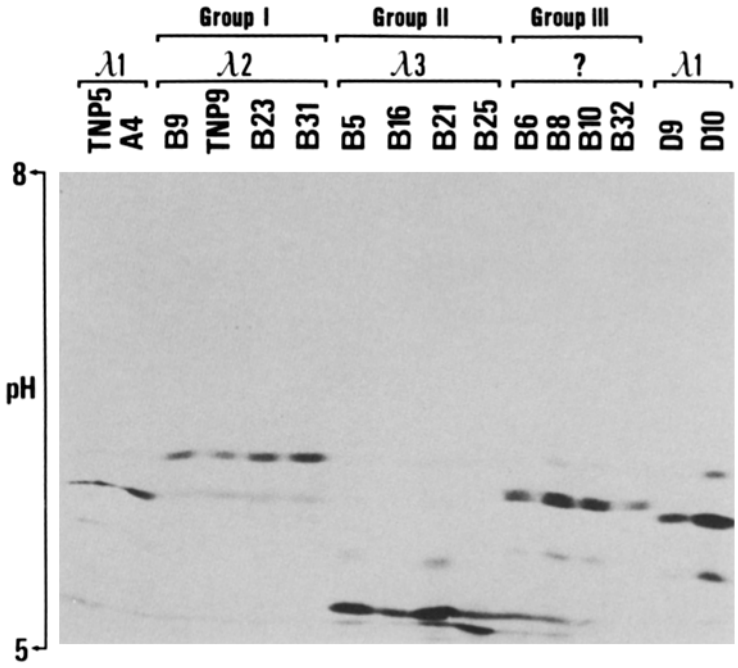

FIGURE 1. Isoelectric focusing of λ light chains. TNP5 (μ,λ1) and TNP9 (γ3,λ2) are two BALB/c anti-TNP hybridomas. Hybridomas B and D derived from a BALB/c mouse treated with rabbit anti-λ2 antibodies coupled to LPS (R anti-λ2-LPS). Hybridomas B were typed λ using an anti-λ2 mAb that reacted with the λ2 and λ3 light chains. Hybridomas D were typed λ1 using an anti-λ1 mAb that did not crossreact with λ2 or λ3.

reagents. As shown in Table I, the hybridomas belonging to group III showed a unique characteristic as compared with those of groups I and II and to λ1 hybridomas. With the exception of the $\lambda 1^+$ cases, all culture supernatants strongly inhibited the anti-λ2/λ2 interaction but not the anti-λ1/λ1 interaction. In addition to this, all supernatants, except for those of group III, inhibited the anti-λ1/λ3 and the anti-λ2/λ1 interactions. These surprising results suggested that the hybridomas of group III used a variable domain different from the two known Vλ regions.

To confirm this hypothesis, cytoplasmic RNA was isolated from each λ Ig–secreting hybridoma and transferred to nitrocellulose filters. Hybridization with Vλ1, Cλ1, and Cλ2 probes was carried out (Fig. 2). RNA of hybridomas belonging to group III hybridized with the Cλ2 probe but not with Cλ1 and Vλ1 probes. On the other hand, the Cλ2 and the Vλ1 probes did hybridize with the RNA of the hybridomas of the λ2 and the λ3 subtypes. These results confirmed that the light chains of the group III hybridomas were encoded by a *V* gene segment that was distinct from the *Vλ1* and *Vλ2* gene segments.

Since the Cλ2 probe hybridized to the RNA of the group III hybridomas, we attempted to sequence the mRNA coding the light chain of the B6 hybridoma belonging to the group III, using a Cλ primer that hybridized with the three *Cλ* genes. The results presented in Fig. 3 demonstrate the existence of a new *V* gene segment. Indeed, the partial sequence of the B6 light chain was significantly different from the known *Vλ1* and *Vλ2* genomic sequences (55% of homology in

TABLE I

Serological Characteristics of Mouse λ Chains

Subtype	Group	Supernatant of hybridoma culture	Percent inhibition of rabbit anti-light chain antibodies/mouse light chain interactions				
			Anti-k/k	Anti-λ1/λ1	Anti-λ2/λ2	Anti-λ2/λ1	Anti-λ1/λ3
λ2	I	B3	12	23	92	78	67
		B9	20	18	92	80	70
		B23	26	21	94	74	70
		B31	15	25	85	55	70
		B15	25	18	91	62	71
λ3	II	B25	10	35	63	67	97
		B21	19	26	68	80	90
		B16	20	32	71	86	96
		B5	23	30	69	61	94
?	III	B8	21	0	73	6	0
		B10	21	0	72	6	10
		B11	27	0	79	14	2
		B22	32	0	71	4	0
		B24	28	3	76	9	3
		B32	19	0	74	5	0
		B6	14	0	75	3	0
λ1	—	D8	12	96	37	77	95
		D9	25	97	26	75	96
		Purified monoclonal Igs:					
k		48-9	76	0	16	10	0
λ1		TNP15	3	88	16	67	88
λ2		M315	30	32	95	81	73
λ3		1.5F9	2	34	81	74	92

Hybridomas B and D derived from BALB/c mouse treated with R anti-λ2-LPS (see legend of Fig. 1). Purified monoclonal Igs 48-9 (γ, κ), TNP 15 (μ, λ1), M315 (α, λ2), and 1.5F9 (γ, λ3) were used as controls in these RIAs. The inhibitor concentration of the purified protein was 5 μg/ml for the anti-λ2/λ2 and anti-λ1/λ3 systems and 625 ng/ml or the other systems.

the sequenced *V* segment). This hybridoma used the *Jλ2* gene segment and probably the *Cλ2* gene segment. However, the sequence between the conserved Cys at position 88 (TGT codon) and the beginning of the *Jλ* gene segment was of unexpected length. Indeed, such a distance has never been described in murine κ or λ light chains (18).

Thus, several lines of evidence demonstrate the existence of a new variable segment used by the light chain of some λ Igs in the mouse. This finding could suggest the existence of other undescribed gene segments since it has already been reported that, in wild mice, the λ genes are more numerous than in the domestic strains (19, 20).

Summary

A series of λ^+ murine hybridomas were derived from a BALB/c mouse after a single injection of anti-λ2 antibodies coupled to LPS. Nine λ B cell clones (five λ2 and four λ3) were expected and seven reacted with antibodies specific for the Cλ2 constant region but showed a particular isoelectric spectrum. Their RNA products did not hybridize with the Vλ probe. The partial DNA sequence of gene segments coding the unexpected light chain of one hybridoma shows that the *V* gene segment has only 55% homology with the *Vλ2* gene segment sequence

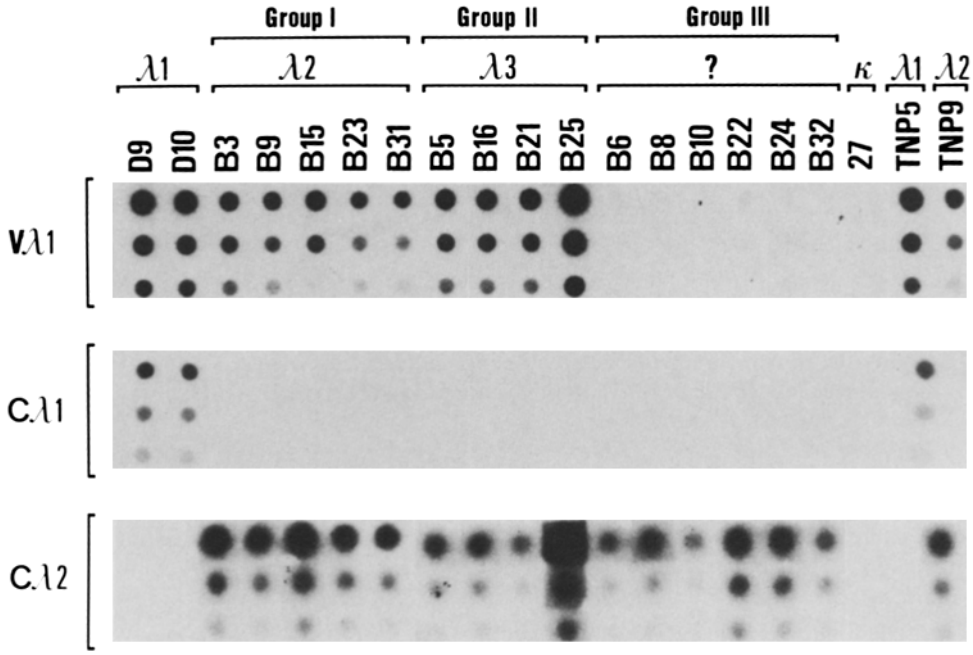

FIGURE 2. Hybridization of λ RNA with the Vλ1, Cλ1 and Cλ2 probes.

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          55              60              65              70
Vλ1   GCTCCAGGTGTTCCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCT
Vλ2   ----------------T---------------------------------
VB6                         tctctgga--xAGCTC---T-CTx-TCGC

                75              80              85
Vλ1   GCCCTCACCATCACAGGGGCACAGACTGAGGATGAGGCAATATATTTCTGT
Vλ2   ---------------------------------T-----G---------
VB6   xA---T-Gcx-TT-xxACATC---Cx---A-----A--x-----CA-----

            90              95
Vλ1   GCTCTATGGTACAGCAACCAT                    TGGGTGTTCGGT    Jλ1
Vλ2   ----------------C----                    -AT--T-----C    Jλ2
VB6   -G-G-GG-TG-Txxx-TTx-GGAACxATTTGTG        -xT--T-----C    JB6
                                               -TTA-T-----C    Jλ3

          100             105                     110
Jλ1   GGAGGAACCAAACTGACTGTCCTA            GGCCAGCCCAAGTCT    Cλ1
Jλ2   --T--------GG-C---------            --T----------C     Cλ2
JB6   --T--------GG-C------c--            --T-a--xxa----x    CB6
Jλ3   A-T--------GG-C---------            --T----------C     Cλ3
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FIGURE 3. Partial DNA sequence of the new *V* gene segment compared with known genomic DNA sequences of *Vλ1* and *Vλ2* gene segments. (*X*) Undetermined nucleotide; (−) Identical nucleotide. Lowercase letters, nucleotide determined once. Amino acid numbering is according to Kabat et al. (21).[1]

and that *Jλ2* and probably *Cλ2* gene segments are used. Taken together, these results demonstrate the existence of a new λ light chain.

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[1] These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00628.

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