A NEW VARIABLE REGION IN MOUSE IMMUNOGLOBULIN
\( \lambda \) LIGHT CHAINS

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The light chains of mouse Ig's have been classified into two isotypes, \( \kappa \) and \( \lambda \). In contrast to the \( \kappa \) chains, the \( \lambda \) chains were shown to display restricted heterogeneity. Two \( V_\lambda \) gene segments and four \( J_\lambda-\Lambda \) gene pairs have been described (1). The three \( \lambda \) subtypes (\( \lambda_1, \lambda_2, \) and \( \lambda_3 \)) are respectively encoded by the combination of \( V_\lambda_1-J_\lambda_1-\Lambda_1, V_\lambda_2-J_\lambda_2-\Lambda_2, \) and \( V_\lambda_1-J_\lambda_3-\Lambda_3 \) gene segments. The \( J_\lambda_4-\Lambda_4 \) gene pair was never found to be functional (2, 3). In the mouse, study of the \( \lambda \)-bearing Ig's is made difficult by the fact that they represent only 5% of total Ig's (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 \( \lambda^+ \) immune responses (5). The second method consisted in producing \( \kappa \)-immunosuppressed mice by repeated injections of anti-\( \kappa \) antibodies (6). We have previously described a third method, which is to inject mice with anti-\( \lambda \) antibodies coupled to LPS (7, 8). In this paper we describe BALB/c hybridomas obtained after injection with anti-\( \lambda_2 \) antibodies coupled to LPS and demonstrate the existence of an unexpected new \( \lambda \) 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-\( \kappa/\lambda \) assay was carried out by the inhibition of the fixation of \( ^{125}\text{I}-\)rabbit anti-\( \kappa \) to 48-9 (\( \gamma, \kappa \)) murine monoclonal Ig. The anti-\( \kappa \) antibodies were isolated from an anti-MOPC 460 (\( \alpha, \kappa \)) antiserum on an \( \text{XRPC24} (\alpha, \kappa) \) Sepharose-AH column. Anti-\( \lambda_1/\lambda_1 \) assay was carried out by the inhibition of the fixation of \( ^{125}\text{I}-\)rabbit anti-\( \lambda_1 \) to TNP 15 (\( \mu, \lambda_1 \)) murine monoclonal Ig. The anti-\( \lambda_1 \) antibodies were isolated from an anti-J558 (\( \alpha, \lambda_1 \)) antiserum previously absorbed on an \( \text{HO}-2.2 (\mu, \kappa) \) column, on a BI-8 (\( \mu, \lambda_1 \)) column. Anti-\( \lambda_2/\lambda_2 \) assay was carried out by the inhibition of the fixation of \( ^{125}\text{I}-\)rabbit anti-\( \lambda_2 \) to TNP 9 (\( \gamma, \lambda_2 \)) murine monoclonal Ig. The anti-\( \lambda_2 \) antibodies were isolated from an anti-J558 (\( \alpha, \lambda_2 \)) antiserum previously absorbed on an HO-2.2 (\( \mu, \kappa) \) column, on a BI-8 (\( \mu, \lambda_1 \)) column. Anti-\( \lambda_2/\lambda_1 \) assay was carried out by the inhibition of the fixation of \( ^{125}\text{I}-\)rabbit anti-\( \lambda_2 \) to TNP 5 (\( \mu, \lambda_1 \)) murine monoclonal Ig. The anti-\( \lambda_2 \) antibodies were isolated from an anti-MOPC 315 (\( \alpha, \lambda_2 \)) antiserum on J558 (\( \alpha, \lambda_1 \)) column. Subsequently, the anti-\( \alpha \) antibodies were absorbed on a MOPC 460 (\( \alpha, \kappa \)) column. Anti-\( \lambda_1/\lambda_3 \) assay was carried out by the inhibition of the fixation of \( ^{125}\text{I}-\)rabbit anti-\( \lambda_1 \) to 1.5F9 (\( \gamma, \lambda_3 \)) murine monoclonal Ig. The anti-\( \lambda_3 \) antibodies were prepared as described above.

**Isoelectric Focusing.** Cells \( (8 \times 10^6) \) were cultured for 30 min in 1 ml of minimum medium, then 300 \( \mu \)Ci of \( [\text{H}] \)leucine were added for 3 h, after which the supernatants were harvested. Supernatants (50–100 \( \mu \)l) were incubated with 12 \( \mu \)l of protein A–Sepharose pretreated with 5 \( \mu \)l of rabbit anti-\( \lambda_1 \) or anti-\( \lambda_2 \) 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 × 10⁶) 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 MgCl₂) 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 ³²P-labeled DNA probes (5–50 × 10⁶ cpm/ml) in formamide hybridization buffer (50% formamide, 5× SSC, 50 mM Na₂PO₄, pH 6.5, 0.2% SDS, 5X 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 VX1 and the CA1 probes were 0.9-kb and 3.6-kb restriction fragments from an XbaI digest of X1 DNA clones (10). The CA2 probe was a 200-bp restriction fragment from AvaI and PstI digest of the pXII-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 Cα primer (dTTCAGAGGAAGGTG) was synthesized 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 Vα1 and Vα2 or between Cα2 and Cα3 regions, respectively, is more extensive (>90%) than the homology between the Cα1 and Cα2 regions or between Cα1 and Cα3 (2, 10, 16, 17), anti-Cα 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 Cα 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 \( \lambda \) light chains. TNP5 (\( \mu,\lambda_1 \)) and TNP9 (\( \gamma_3,\lambda_2 \)) are two BALB/c anti-TNP hybridomas. Hybridomas B and D derived from a BALB/c mouse treated with rabbit anti-\( \lambda_2 \) antibodies coupled to LPS (R anti-\( \lambda_2 \)-LPS). Hybridomas B were typed \( \lambda \) using an anti-\( \lambda_2 \) mAb that reacted with the \( \lambda_2 \) and \( \lambda_3 \) light chains. Hybridomas D were typed \( \lambda_1 \) using an anti-\( \lambda_1 \) mAb that did not crossreact with \( \lambda_2 \) or \( \lambda_3 \).

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 \( \lambda_1 \) hybridomas. With the exception of the \( \lambda_1^+ \) cases, all culture supernatants strongly inhibited the anti-\( \lambda_2/\lambda_2 \) interaction but not the anti-\( \lambda_1/\lambda_1 \) interaction. In addition to this, all supernatants, except for those of group III, inhibited the anti-\( \lambda_1/\lambda_3 \) and the anti-\( \lambda_2/\lambda_1 \) interactions. These surprising results suggested that the hybridomas of group III used a variable domain different from the two known \( V\lambda \) regions.

To confirm this hypothesis, cytoplasmic RNA was isolated from each \( \lambda \) Ig-secreting hybridoma and transferred to nitrocellulose filters. Hybridization with \( V\lambda_1, C\lambda_1, \) and \( C\lambda_2 \) probes was carried out (Fig. 2). RNA of hybridomas belonging to group III hybridized with the \( C\lambda_2 \) probe but not with \( C\lambda_1 \) and \( V\lambda_1 \) probes. On the other hand, the \( C\lambda_2 \) and the \( V\lambda_1 \) probes did hybridize with the RNA of the hybridomas of the \( \lambda_2 \) and the \( \lambda_3 \) 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 \( V\lambda_1 \) and \( V\lambda_2 \) gene segments.

Since the \( C\lambda_2 \) 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 \( C\lambda \) primer that hybridized with the three \( C\lambda \) 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 \( V\lambda_1 \) and \( V\lambda_2 \) genomic sequences (55% of homology in
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-κ/κ Anti-λ1/λ1 Anti-λ2/λ2 Anti-λ2/κ1 Anti-λ1/κ3                                |
| λ2      | I     | B3                              | 12 23 92 78 67                                                                   |
|         |       | B9                              | 20 18 92 80 70                                                                   |
|         |       | B28                             | 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 | 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), TNP15 (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/κ3 systems and 625 ng/ml or the other systems.

The sequenced V segment). This hybridoma used the j/λ2 gene segment and probably the CA2 gene segment. However, the sequence between the conserved Cys at position 88 (TGT codon) and the beginning of the j/λ 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 CA2 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.
and that \( \text{J}_{\alpha}2 \) and probably \( \text{C}_{\alpha}2 \) gene segments are used. Taken together, these results demonstrate the existence of a new \( \lambda \) light chain.

We thank Drs. C. Henderson and D. Juy for their helpful discussions. We also thank Dr. T. J. Kindt for his generosity. We gratefully acknowledge the excellent secretarial help of Ms. Berson.

\textit{Received for publication 24 February 1987 and in revised form 14 April 1987.}

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\footnote{These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00628.}
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