THE NATURAL ABUNDANCE OF λ2-LIGHT CHAINS IN INBRED MICE

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A small fraction of the light (L) chains of mouse immunoglobulins (Igs) are of the λ-type (1). Of the ~22 λ-chains from mouse myeloma tumors whose amino acid sequences have been partially or fully determined, all but one have the same constant (C) domain sequence (2, 3). The exception, L3-15, is the light chain of the myeloma protein (M315) produced by plasmacytoma MOPC-315. L3-15, called a λ2-chain (4), differs from the other mouse λ-chains, called λ1, at 29 of 110 positions in the C region and, compared to the prototype λ1-chain (L1-14), at 11 positions in the variable (V) region (5).

The mouse in which MOPC-315 arose was of the BALB/c 2 type; it derived from the seventh backcross to purebred BALB/c mice beginning with a (BALB/c × C57Bl/Ka) F1 hybrid (6). Because other λ2-chains have so far not been described in BALB/c myelomas, it has long seemed possible that the L3-15 chain might be an allelic variant (of C57Bl/Ka origin) of the λ1-chains produced in BALB/c. However, with so many scattered C-domain amino acid differences between λ1 and L3-15 a more attractive possibility is that L3-15 represents a rarely expressed L-chain isotype, λ2, rather than a λ-allotype (7).

Accordingly, the object of the present study was to establish whether L3-15-like (λ2) chains exist in Igs from BALB/c and other mouse strains and, if so, at what level. Advantage was taken of the Fv fragment of M315 (VH-15 and VH-15) to obtain separate antibody (Ab) preparations to the C and V regions of L3-15 (anti-C15 and anti-VL-15, respectively). With these Ab preparations and 125I-labeled L3-15, two radioimmunoassays were developed to measure the frequencies, in mice of different ages, strains, and immunization background, of serum Igs whose L chains have the respective antigenic
properties of the C and V regions of L. The V region of L has recently become of particular keen interest because its amino acid sequence (5) corresponds almost exactly to the recently determined deoxynucleotide sequence of the first V gene cloned from embryonic mouse DNA (8).

**Materials and Methods**

**Antigens and Antisera.** Dextran 1355S was generously provided by Allene Jeanes, U.S. Dept. of Agriculture, Peoria, Ill. Keyhole limpet hemocyanin (KLH) was purchased from Schwarz/Mann Div., Bectin, Dickinson & Co., Orangeburg, N. Y. Dinitrophenyl (Dnp) was conjugated to KLH as described (9); the protein had 11 Dnp groups/100,000 daltons of KLH. Goat antisera to M315 were obtained from Walter Gray, Gateway Immuno sera Co., Cahokia, Illinois. Three lots were used (11272, 32474, and 31173), each from a different goat that had been repeatedly injected with mildly reduced and alkylated M315. Each antiserum had been adsorbed with M460-Sepharose (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.).

**Immunizations.** 8-Wk-old mice were immunized with KLH or Dnp11-KLH in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) at a 1:9 ratio (aqueous to oil phase) as described (10). Animals received two 0.5-mg i.p. injections 1 wk apart and were tail-bled 1 wk after each injection.

14-Wk-old BALB/c mice were primed with one subcutaneous injection of 10 µg of dextran 1355S. 1 Wk later, they received the first of 5 i.p. injections at 1-wk intervals of 100 µg of dextran. The animals were tail-bled 1 wk after the fourth, fifth, and sixth injections.

**Iodinations.** Myeloma proteins were labeled with carrier-free 125I (11). Chloramine T was used at a 10-fold molar excess over IgA proteins, but at a 1:1 molar ratio to iodinate L chains. Specific activities were usually 3-6 × 10^6 cpm/µg.

**Preparation of the Fv Fragment.** The proteolytic Fv fragment of M315, consisting of just VH plus VL domains was prepared as described (12). Fv preparations having < 1% intact L chains, were obtained with a yield of ~ 20%. Fv was measured by absorbance at 278 nm (E~278, 15.0; mol wt, 23,000 daltons) (12).

**Myeloma Proteins, Immunoadsorbants, and Antiserum Adsorptions.** The following myeloma proteins of the indicated H-chain class and L-chain type were used: M315 (a, λ2), M460 (α, κ), M104E (α, λ1), HOPC-1 (γ2a, λ1), MPC-11 (γ2b, κ), M21 (γ1, κ), LPC-1 (γ2a, κ), and J606 (γ3, κ). X-2, a hybrid protein with the H chain of M315 but the κ-chain from MPC-11, was derived from the fusion of MOPC-315 and MPC-11 tumor cells and was generously provided as the purified protein by G. Siebert.

M315, M460, and M104E were isolated as described (13-15). J chain was the kind gift of Dr. Marian Kosshold (U. of California, Berkeley), who isolated it from MOPC-315 serum (16). M21, MPC-11, LPC-1, and HOPC-1 were purified by gel filtration on Sephadex G-200 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.) followed by chromatography on QAEE-A50 at pH 7.5 (or pH 9.1 for LPC-1) (17). Based on conductivity measurements, myeloma proteins were eluted from QAEE-A50 at the following molar concentrations of NaCl (indicated in parentheses): HOPC-1 (0.21), M21 (0.27), LPC-1 (0.34), and MPC-11 (0.43).

To remove Abs to α-chains and Abs that cross-reacted with λ1-chains, goat antisera to L was passed successively over (a) M460-Sepharose (M460-S), with 2.1 mg M460/g Sepharose 4B (S), and (b) M104E-Sepharose (M104E-S), with 1.3 mg M104E/g S; 1 g adsorbant was used for 0.4 ml antiseraum. Proteins were conjugated to S as described (18).

**Antibodies to the Constant and Variable Regions of L.** Antibodies to the Constant and Variable Regions of L were separated into anti-CL and anti-VL with a column of Fv-Sepharose (Fv-S). 1.2 ml of anti-M315 was passed (after adsorption with M460-S and M104E-S) through 1 g of Fv-S with 4.6 mg Fv/g S. After an initial incubation for 15 min at 25°C, pass-through fractions with A~278~ > 0.08 were pooled to constitute anti-CL.

After washing the column thoroughly (until A~278~ was < 0.015), 2 column volumes of 0.23 M glycine-HCl, pH 2.5, was added. Fractions with pH below 4 were collected until the absorbancy at 278 nm was no longer detectable (~ 1.5 column volumes). The eluted material, anti-VL, was

2 G. R. Siebert, J. F. Harris, and M. L. Gefter, J. Immunol. In press.
was promptly neutralized with 1/10th vol of 2 M Tris-HCl, pH 8.0, and dialyzed extensively against 0.02 M Tris-saline, pH 8.0. The anti-VL recovered from 1 ml of anti-\(\lambda\) processed in this manner had 0.65 A270 U. To avoid Fv denaturation, the Fv-S was not pretreated with glycine-HCl, and each Fv-S column was used only once.

**Preparation of IgG.** IgG was isolated from normal mouse serum by starch block zone electrophoresis followed by two gel filtrations on Sephadex G-200 (19). Over 90% of the first retarded peak, taken as IgG, migrated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (nonreducing conditions) as a band of \(\approx 150,000\) daltons. To deplete some IgG preparations of \(\lambda\)-containing molecules, they were adsorbed (usually in lots of 12 mg) over anti-\(\lambda\)-S, prepared by coupling S with the Ig fraction obtained by ammonium sulfate precipitation (40% saturation) from goat antiserum to L315 (previously adsorbed with M460-S). As a control, a portion of the same mouse IgG preparation was adsorbed with S coupled with a similar Ig fraction (40% ammonium sulfate precipitated) from normal goat serum (NGS-S).

**Preparation of L Chains.** L315 was isolated from purified M315 as described (20). L chains were also obtained from normal serum IgG preparations (see above), some of which were previously adsorbed with anti-\(\lambda\)-S or normal goat serum (NGS)-S. The unbound IgG from both adsorbants (i.e., pass-through fractions) were treated in the same way: at 20 mg/ml they were reduced with 25 mM dithiothreitol (DTT) in 6 M urea-0.2 M Tris-HCl, pH 8.2, carboxymethylated with 57.5 mM iodoacetic acid, and dialyzed for 3 h against 6 M urea-1 M acetic acid before being chromatographed in that solvent on a Sephadex G-100 column. L-chain fractions, selected to minimize contamination with H chains, were dialyzed against 50 mM acetic acid, lyophilized, and rechromatographed on a Sephadex G-100 column under the same conditions.

To prepare soluble L chains for carboxypeptidase A (CPA) digestion, the lyophilized, carboxymethylated (CM)-L chains were taken up in 6 M urea-0.33 M NH4HCO3 and dialyzed against 0.2-0.3 M NH4HCO3, pH 8.0. L chains were measured by absorbance at 278 nm (\(E_{1\text{cm}}^{278} = 11.0\)) (20).

**CPA Digestion of L Chains.** Soluble CM-L chains in 0.2-0.3 M NH4HCO3 (typically at 4 mg/ml) were digested with Worthington bovine pancreatic CPA (diisopropyl phosphofluoridate-treated) at an enzyme:L chain ratio (wt/wt) of \(\approx 1:1,000\). Digestion was stopped after 8 h at 37°C by lyophilization. The enzyme alone and CM-L chains alone served as controls. The lyophilized digests were dissolved and trichloroacetic acid (TCA) was added to 10%. The TCA-soluble material was lyophilized and analyzed for released amino acids on a Durram D 500 analyzer (Durram Dioxex, Sonneyvale, Calif.). Preliminary controls showed that the recovery of free amino acids in the TCA step was 89-92%.

**Radioimmune-Inhibition Assay to Measure IgS with \(\lambda\)-Chains.** To measure the amount of Ig with \(\lambda\)-L chains, various sera or purified Ig fractions were tested for ability to inhibit the reaction between anti-CL\(\text{L}^\lambda\) or anti-VL\(\text{L}^\lambda\) and \(^{125}\text{I-L315}\) in a double Ab radioimmunoassay (21). For standardization, an inhibition curve was obtained with purified M315, centrifuged at 100,000 g for 1 h to eliminate aggregated protein. Maximally, between 75 and 80% of \(^{125}\text{I-L315}\) was bound by anti-CL\(\text{L}^\lambda\) and 50% by anti-VL\(\text{L}^\lambda\). The background, using NGS instead of Abs to L\(\text{L}^\lambda\), was 1-3% and the assay could detect the equivalent of 80 ng M315/ml. The standard deviation among replicate samples was \(\pm 0.8\)% of the added \(^{125}\text{I-L315}\). The frequency (percent) of IgG with \(\lambda\)-L chains was calculated as 100 times the \(\mu\)-g-Eq of M315/\(\mu\)-g of IgG, where \(\mu\)-g IgG was based on sample absorbancy at 278 nm (\(E_{1\text{cm}}^{278} = 14.0\)) and the \(\mu\)-g-Eq M315 was determined by comparing inhibition by the sample with inhibition by protein M315.

**Results**

*The Radioimmune Inhibition Assay for Measuring \(\lambda\)-Associated Ig.* To search for \(\lambda\)-containing IgS, it was particularly useful to employ (a) an antiserum that was raised against the intact M315 molecule (or its Fab fragment) and (b) the isolated L315 chain, rather than intact M315, as radiolabeled antigen in the assay. The first precaution increased the probability that the antiserum recognized the \(\lambda\)-L chain when it was part of an intact Ig, and the second assured that the assay was restricted to Abs against \(\lambda\)-determinants, not H (immunoglobulin heavy) chain (or idiotypic) deter-
TABLE I

Specificity of the Radioimmunoassay for Igs with L Chains with the C Domain of L315.*

| Experiment number | Inhibitors               | Inhibition§ |
|-------------------|--------------------------|-------------|
|                   | Substance                | Amount‡     |             |
|                   |                          | ng          | %           |
| 1                 | None (0)                 | 75          | 0           |
|                   | Fv(VL315 + VL315)        | 75          | 84          |
| 2                 | None (0)                 | 8           | 12          |
|                   | L315                     | 450         | 0           |
| 3                 | None (0)                 | 3           | 12          |
|                   | M315 (a, L2)             | 3           | 91          |
|                   | M315 (a, L2)             | 80          | 0           |
|                   | X-2 (315H,κ)             | 3,800       | 0           |
|                   | HOPC-1 (γ1a,λ1)          | 4,000       | 0           |
|                   | 104E (μ, λ1)             | 4,000       | 0           |
|                   | M460 (a, κ)              | 2,500       | 0           |
|                   | MOPC 21 (γ1, κ)          | 2,500       | 0           |
|                   | MPC-11 (γμκ, κ)          | 2,500       | 0           |
|                   | LPC-1 (γμκ, κ)           | 2,500       | 0           |
| 4                 | None (0)                 | 2           | 12          |
|                   | M315                     | 20          | 71          |
|                   | J606 (γ2κ)               | 1,330       | 0           |

* All tubes had anti-C_L315 as antibody and 125I-L315 (15 ng; 33,000 cpm). In the uninhibited controls, the percent of 125I-L315 bound was 23, 23, 19, and 36% for experiments 1, 2, 3, and 4, respectively.

‡ Amount given indicates highest levels tested for negative controls; lesser amounts also gave no inhibition.

§ Zero indicates binding within ± 1.5% of the amount bound by the uninhibited control (represented by zero in parentheses).

The specificities of the Abs used to assay for Igs with C_L315 and V_L315 are shown in Tables I and II. In the assay for C_L315 (Table I), 75 ng of Fv fragment was not inhibitory, whereas the same amount of L315 inhibited 85%. In contrast, the assay for V_L315 was inhibited to about the same extent by Fv and L315 (Table II). Neither of the λ1-proteins tested (HOPC-1 and 104E) inhibited the assay for C_L315 reaction, and 104E did not inhibit the assay for V_L315. Neither assay was inhibited by M460 (α, κ), by purified myelomas of the γ1-, γ2a-, or γ2b-subclasses, or by X-2 (α, κ), a protein that bears the M315 H chain and is produced by a hybrid cell (MOPC-315/MPC-11) (Materials and Methods). The assay for C_L315 was also not inhibited by a myeloma protein of the γ3-class (J606), or by J chain, a potential contaminant of L315 (15).

Agreement between the Assays for C_L315 and V_L315. To determine whether the assays for C_L315 and V_L315 were quantitatively consistent, both were used to measure M315 in various samples. The results of both assays were in close agreement (Table III) whether the samples tested were sera from mice with growing MOPC-315 tumors or
TABLE II
Specificity of the Radioimmunoassay for Igs with L Chains with the V Domain of L315.

| Experiment number | Inhibitor tested | Amount‡ | Inhibition§ |
|-------------------|-----------------|---------|-------------|
| 1                 | None            | (0)     |             |
|                   | Fv              | 75      | 44          |
|                   | L_{315}         | 75      | 50          |
|                   | L_{315}         | 500     | 90          |
|                   | M315 (a\lambda2) | 14      | 16          |
|                   | M315 (a\lambda2) | 82      | 48          |
|                   | M315 (a\lambda2) | 5,450  | 92          |
|                   | M315 (a\lambda2) | 5,450  | 92          |
|                   | 104E (\mu\lambda1) | 5,450  | 92          |
| 2                 | None            | (0)     |             |
|                   | X-2 (315H,a\kappa) | 1,900  | 0           |
|                   | MOPC-21 (\gamma,\kappa) | 2,500  | 0           |
|                   | LPC-1 (\gamma2,\kappa) | 2,500  | 0           |
|                   | MPC-11 (\gamma2,\kappa) | 2,500  | 0           |

* All tubes had anti-V_L^{315} as antibody and ^{125}I-L_{315} (15 ng; 18,000 cpm). In the uninhibited controls the percent of ^{125}I-L_{315} bound was 26 and 19% for experiments 1 and 2.
‡ Amount given indicates highest levels tested for negative controls; lesser amounts also gave no inhibition.
§ Zero indicates binding within ± 1.1% of the amount bound by the uninhibited control.

TABLE III
Agreement Between the Assays for C_L^{315} and V_L^{315}

| Sample tested   | Concentration of V_L^{315} | Concentration of C_L^{315} | Ratio V_L^{315} : C_L^{315} |
|-----------------|---------------------------|---------------------------|-----------------------------|
| MOPC-315 serum  | 3.85 mg/ml                | 3.4 mg/ml                 | 1.13                        |
| MOPC-315 L.K. serum* | 0.15 mg/ml              | 0.13 mg/ml               | 1.15                        |
| M315‡           | 0.53 mg/ml                | 0.58 mg/ml               | 0.91                        |

average: 1.06

* Serum from animals bearing MOPC-315 L.K. tumor, a subline of MOPC-315 that produces low levels of M315.
‡ The M315 used here was isolated independently of the M315 used to construct the standard calibration curves.

Normal Mouse Serum Igs with \lambda2-Chains. To determine whether L chains with the C domain of L_{315} (i.e., \lambda2-chains) occur in normal mice, sera and purified Ig fractions were tested for the ability to inhibit the reaction between anti-C_L^{315} and ^{125}I-L_{315}. The results, summarized in Table IV, showed suprisingly high serum levels of \lambda2. Serum samples from adult BALB/c mice behaved as though they had (on the average) 80 \mu g/ml of Igs with \lambda2-chains. Similar high values were found in serum from C57Bl/6, AL/N, and NZB mice and much lower values (15–30 \mu g/ml) were found in SJL/J,
TABLE IV
Levels in Sera from Different Mouse Strains of Immunoglobulins with Light Chains having the C and V Domains of L\superscript{315}

| Strain | Age | CL\superscript{315} Average | Range | VL\superscript{315} Average | Range |
|--------|-----|-----------------------------|-------|-----------------------------|-------|
| AL/N   | 15  | 140 ± 88 (4)*                | 86-272| 13.8 ± 6.2 (6)*             | 4.6-18.5|
| NZB    | 15  | 97 ± 24 (4)                  | 76-119| 11.2 ± 2.3 (3)              | 8.5-12.6|
| BALB/c | 15  | 85 ± 36 (13)                 | 23-140| 8.5-12.6                    |
| A/J    | 15  | 76 ± 48 (6)                  | 37-154| 76-119                      |
| LP/J   | >20 | 67 (2)                      | 65-68 | 6.2,7.1                     |
| C57Bl/6| 15  | 66 ± 23 (8)                  | 36-92 | 6.2,7.1                     |
| C57Bl/10| 15  | 61 ± 25 (4)                 | 44-98 | 6.2,7.1                     |
| I/LnJ  | >20 | 59 ± 19 (3)                 | 39-79 | 6.2,7.1                     |
| B10.D2 | 15  | 56 ± 10 (3)                 | 48-67 | 6.2,7.1                     |
| MA/MyJ | >20 | 54 ± 17 (3)                 | 36-70 | 6.2,7.1                     |
| BAB/14 | 15  | 55 (pool of 10)             | 65-68 | 6.2,7.1                     |
| LG/J   | >20 | 51 ± 28 (4)                 | 23-88 | 6.2,7.1                     |
| CBA/J  | 15  | 50 ± 17 (3)                 | 33-77 | 6.2,7.1                     |
| SM/J   | >20 | 49 (2)                      | 49-49 | 6.2,7.1                     |
| C57Br  | 15  | 47 (2)                      | 46-48 | 6.2,7.1                     |
| STR/J  | >20 | 46 (2)                      | 36-56 | 6.2,7.1                     |
| SEC1/ReJ| >20 | 49 (2)                      | 46-53 | 6.2,7.1                     |
| 129/SV | >20 | 46 (2)                      | 44-48 | 6.2,7.1                     |
| AKR/J  | 15  | 41 ± 9 (7)                  | 27-55 | 6.2,7.1                     |
| RF/J   | 15  | 38 ± 7 (7)                  | 29-46 | 6.2,7.1                     |
| Au/SSJ | >20 | 35 (2)                      | 22-48 | 6.2,7.1                     |
| BDP/J  | >20 | 36 (3)                      | 17-63 | 6.2,7.1                     |
| C3H/OH | 15  | 31 ± 7 (3)                  | 23-38 | 6.2,7.1                     |
| SWR/J  | >20 | 29 ± 10 (8)                 | 14-43 | 6.2,7.1                     |
| C3H/HeJ| 15  | 28 ± 6 (9)                  | 21-34 | 6.2,7.1                     |
| DBA/2J | >20 | 28 ± 9 (10)                 | 8-39  | 6.2,7.1                     |
| SJL/J  | >20 | 25 ± 13 (5)                 | 11-46 | 6.2,7.1                     |
| SEA/GnJ| >20 | 24 ± 10 (4)                 | 17-39 | 6.2,7.1                     |
| CE/J   | >20 | 23 (pool of 5)              | 3.4   | 6.2,7.1                     |
| C58/J  | >20 | 20 (2)                      | 18.21 | 6.2,7.1                     |
| RIII/2J| >20 | 20 (2)                      | 16.24 | 6.2,7.1                     |
| BSVS   | >20 | 19 (2)                      | 10.27 | 6.2,7.1                     |
| P/J    | >20 | 15 ± 6 (3)                  | 10-21 | 6.2,7.1                     |

* Numbers in parentheses are the number of mice assayed; ± refers to standard deviation.
‡ Mice >20 wk old were retired breeders.

DBA/2, and BSVS strains. Several other strains had intermediate values. A preliminary study showed that in BALB/c mice, the levels of Igs with \(\lambda\)-chains increased with age from \(\approx 30 \mu g/ml\) at 4 wk to \(\approx 80 \mu g/ml\) between 12 and 15 wk.

After starch block electrophoresis, >90% of the inhibitory activity of serum was found in the Ig-enriched fractions, and after gel filtration on Sephadex G-200, inhibitory activity was found only in the void volume and in the IgG-rich fraction (data not shown). The inhibitory activity of purified BALB/c IgG indicated that it had 0.7% (range 0.48–0.96%) \(\lambda\)-containing Igs. This value is highly significant (\(P < 0.001\)), as the inhibitory activity of purified \(\lambda\)- and \(\kappa\)-myeloma proteins was negligible (corresponded to <0.03%).

Two observations strengthened the probability that the serological test identified
\( \lambda_2 \)-light chains in normal Iggs: (a) purified IgG inhibited >90% of the reaction between \(^{125}\text{I}-\text{L315}\) and anti-C\( \lambda_2 \) and (b) the inhibition curves obtained with purified IgG and M315 were essentially indistinguishable (Fig. 1). If the inhibitor in normal IgG differed from L\(^{315}\), the slope of its inhibition curve would probably have differed from that of M315.

**Normal Mouse Serum Iggs with L Chains Having a V Domain like that of L\(^{315}\).** The radioimmune inhibition assay with anti-V\(^{15}\) showed that molecules with the V region of L\(^{315}\) (V\(^{315}\)) are present in BALB/c serum at a concentration, in M315 equivalents, of 10–14 \( \mu \text{g/mL} \) (Table IV). Because this value is only one-sixth that of \( \lambda_2 \)-chains recognized with anti-C\( \lambda_2 \), it appears that the V domains of most \( \lambda_2 \)-chains in normal Iggs differ from the V domain of L\(^{315}\).

**Increase in C\( \lambda_2 \) and V\(^{315}\) upon Immunization with Dnp-KLH.** Because M315, the only \( \lambda_2 \)-myeloma protein known so far, has Dnp-binding activity, we investigated the effect of immunization with Dnp-KLH on serum \( \lambda_2 \)-levels. As can be seen in Table V, serum from BALB/c, C57Bl/6, and AKR mice immunized with Dnp-KLH had 3–21-fold greater \( \lambda_2 \) levels than preimmune sera. Control sera from mice immunized with KLH alone also increased, but the change over preimmune serum was three- to fivefold greater for the Dnp-KLH-immune than the KLH-immune group. The mice were 6–8 wk old at the start of the experiment and time-course studies showed that in conventionally raised mice, \( \lambda_2 \)-concentrations did not level off until 12–15 wk. Thus, some increase in each group was probably due to aging rather than to immunization.

The average levels of V\(^{315}\) (Table V) in serum from BALB/c, C57Bl/6, and AKR mice immunized with Dnp-KLH were also substantially higher than the level in serum from KLH-immunized animals or serum from unimmunized 15-wk-old mice (Table IV). In one experiment, the level of 92 \( \mu \text{g/mL} \) in pooled sera from Dnp-KLH-immunized BALB/c mice was 10 times higher than the level in serum from the control KLH-immunized BALB/c mice (Table V, group 2).

To determine if the results of the \( \lambda_2 \) assay were due to cross-reaction with naturally occurring \( \lambda_1 \)-Igs, BALB/c mice were immunized with dextran 1355S, which is known...
### Table V

**λ2 Levels in Response to Immunization**

| Group | Strain and age* | Immunogen | Preadmin | Immune | Ratio (Immune)/Preadmin | Ratio (DNP-KLH)/KLH | V1 values (Immune) |
|-------|-----------------|-----------|----------|--------|------------------------|---------------------|-------------------|
| 1     | BALB/c¢ (7)     | KLH       | 27.3     | 31.0   | 1.1                    | 3.0                 | ND                |
|       |                 | Dnp-KLH   | (22-43)  | (15-26) |                        |                     |                   |
| 2     | BALB/c¢§ (10)   | KLH       | ND       | 56     |                        | 7.6                 |                   |
|       |                 | Dnp-KLH   | (Pool of 5) |       |                        |                     |                   |
| 3     | C57BL/6j (8)    | KLH       | 53       | 83     | 1.6                    | 4.1                 | ND                |
|       |                 | Dnp-KLH   | (41-70)  | (38-140) |                        |                     |                   |
| 4     | AKR+ (6-7)      | KLH       | 10.6     | 41.8   | 3.9                    | ND                  |                   |
|       |                 | Dnp-KLH   | (7-14)   | (25-55) |                        |                     |                   |
| 5     | BALB/c (14)     | Dextran   | 68       | 49     | 0.7                    |                     |                   |
|       |                 |           | (47-29)  | (46-79) |                        |                     |                   |

ND = not done.

*Age when immunization was begun is in parentheses; 10 mice per group (5 with Dnp-KLH, 5 with KLH).

§ The immunization protocol in groups 1, 3, and 4 employed 0.5 mg of antigen in a 1:9 ratio with complete Freund’s adjuvant as described in Materials and Methods.

¶ Mice were injected with 100 μg in complete Freund’s adjuvant, then 100 μg in incomplete Freund’s adjuvant on day 7, 100 μg in saline on day 14, and bled 1 wk later.

Numbers are averages per group of five mice (with range of values for individual mice in parentheses). For dextran injections (saline, subcutaneously), see Materials and Methods.

to elicit λ1-containing Abs in this strain (22). The ratio of λ1/λ2 in normal BALB/c serum is 3.6/1 (T. Cotner. Unpublished observations). In sera from dextran-immunized BALB/c mice, the λ1/λ2 ratio was 6.6, and there was no increase in the absolute concentration of λ2 (Table V, group 5).

**CPA Digestion of L Chains.** To evaluate the validity of the serological results by an independent method, CPA-digestion was carried out. This approach took advantage of differences in the carboxy-terminal amino acids of κ-, λ1-, and λ2-chains (cysteine, serine, and leucine, respectively). By reacting reduced L chains with iodoacetate as the alkylating agent, a negative charge was placed on the terminal cysteine of κ and the penultimate cysteines in λ1 and λ2. At pH 8, amino acids with negatively charged side chains are released very slowly by CPA (23). Thus, κ chains, which occur naturally in ~10–20-fold excess over λ-chains (1, 8), are not significantly attacked, allowing detection of amino acids released from proteins that comprise a small fraction of the L-chain preparation. Leucine was detected at a level of 0.016–0.018 mol/mol of L chain in the CPA digests of three L-chain preparations, and a few other amino acids were found at about one-half this level. As expected, the serine of λ1 was not obtained at a significant level because carboxy-terminal serine is poorly released by CPA, especially if the penultimate residue is also a slowly released amino acid (23). To determine if all of the leucine released by CPA was due to λ2, L chains were isolated from a BALB/c IgG preparation that had been adsorbed with either anti-λ2-Sepharose or, as a control, with S coupled with NGS-S. The level of leucine released by CPA from the anti-λ2 adsorbed L chains decreased about twofold (to the background level of the other amino acids), whereas in the L chain from Igs that had
be passed over the control S (NGS-S), the leucine released was unchanged (1.7 mol/100 mol L chain); the difference in level of leucine released was thus \( \approx 0.7 \) mol of leucine/100 mol of L chain. This value may be corrected approximately twofold by taking into account the 50% yield of leucine by CPA digestion of L\textsubscript{315} (reference 20 and T. Cotner. Unpublished observations). Hence, the CPA digestion provides independent support for the serological demonstration that \( \lambda_2 \)-chains account for \( \approx 1 \% \) of the L chains in normal serum Igs of BALB/c mice.

**Discussion**

This study shows that in mice of the BALB/c and several other strains \( \approx 1 \% \) of serum Igs have L chains of the \( \lambda_2 \)-type, confirming earlier tentative serological tests with anti-\( \lambda_2 \)-antisera whose specificity was not fully established (5). The same frequency has been found by analyzing the carboxy-terminal peptides cleaved by trypsin from L chains of normal mouse serum Igs (7). The 1-2% value has been further reinforced by the recent finding that at least three, and probably four, \( \lambda_2 \)-containing myeloma proteins have been identified by screening 260 sera from BALB/c mice with myeloma tumors.\textsuperscript{3} Because sera from all of the 35 inbred strains tested had \( \lambda_2 \)-chains, \( \lambda_2 \) clearly represents an L-chain isotype; i.e., \( \lambda_2 \) is not an allelic variant of \( \lambda_1 \).

The strain survey revealed large differences in serum \( \lambda_2 \)-levels, ranging from 15-30 \( \mu \text{g/ml} \) of Igs with \( \lambda_2 \)-chains in some strains to 140 \( \mu \text{g/ml} \) in AL/N mice. The differences are not associated with differences at the H-2 complex, as BALB/c and NZB (both H-2\( ^b \)) had high levels of \( \lambda_2 \) whereas DBA/2 (also H-2\( ^b \)) had low levels (Table IV). Although differences in \( \lambda_2 \) levels might reflect differences in total Ig (which might be IgCH linked, [24]), control by genes in the heavy chain linkage group cannot explain differences between BALB/c and C3H/He (both Ig-1\( ^a \)) or between C57Bl and SJL or C58 (all Ig-1\( ^b \)). The genetic basis for regulating the expression of this \( \lambda \)-chain isotype may be approached through further study of the large differences in \( \lambda_2 \)-levels in various strains.

The several approaches used to establish the frequency of \( \lambda_2 \)-chains in mouse serum Igs were all based on distinctive serological and chemical features of the C domain of L\textsubscript{315}. In contrast, the frequency of L chains with the V domain of L\textsubscript{315} (i.e., V\textsubscript{L}\textsubscript{315}) could only be measured serologically. Nevertheless, the validity of the assay for V\textsubscript{L}\textsubscript{315} is strongly supported by the agreement between this assay and the one for C\textsubscript{L}\textsubscript{315} in measurements of myeloma protein M315, either in serum of tumor-bearing mice or as a purified protein (Table III). By the serological test, L chains with V\textsubscript{L}\textsubscript{315} appeared to be sixfold less abundant in normal serum Igs than L chains with the C region of L\textsubscript{315}. The radioimmunoassays thus divide \( \lambda_2 \)-chains into at least two subsets: those whose V\textsubscript{L} domains resemble (or are identical) with V\textsubscript{L}\textsubscript{315} and those whose V\textsubscript{L} domains differ from V\textsubscript{L}\textsubscript{315}. We assume (but have not demonstrated) that the L chains which react with anti-V\textsubscript{L}\textsubscript{315} are a subset of all \( \lambda_2 \)-chains.

Comparison of the amino acid sequences in L\textsubscript{315} and in \( \lambda_1 \)-chains shows that their V regions are much more alike than their C regions (4), and the V regions of \( \lambda_1 \) chains from myeloma proteins show remarkably little variation (3). From the radioimmunoassay with anti-V\textsubscript{L}\textsubscript{315}, it appears that the V regions of \( \approx 85 \% \) of \( \lambda_2 \)-chains differ

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\textsuperscript{3} T. Cotner et al. Manuscript in preparation.
from $V_{\lambda}^{\beta 15}$. Though this observation could mean greater variation in the V domains of $\lambda 2$-than of $\lambda 1$-chains, two uncertainties must be emphasized: (a) it is not known whether the V domains of $\lambda 1$-chains in normal serum Igs are as restricted in amino acid sequence as the V domain of $\alpha 1$-chains from myeloma proteins; (b) the extent of amino acid sequence variations in the $\lambda 2$-chains that differ from $L_{\lambda}^{315}$ is unknown.

It is also far from certain that all chains with inhibitory activity in the radioimmunoassay for $V_{\lambda}^{\beta 15}$ have precisely the same amino acid sequence as $V_{\lambda}^{\beta 15}$. If they did, this finding would mean, somewhat surprisingly, that the L chains of $\approx 1$ in 1,000 serum Ig molecules in BALB/c and some other mouse strains are identical with $L_{\lambda}^{315}$. It is alternatively possible that the serological assay detected a set of L chains whose V region amino acid sequences are very similar but not completely identical with each other or with $L_{\lambda}^{315}$. Non-identity of these sequences would fit the recent finding by Tonegawa et al. (8), that although the deoxynucleotide sequence of a $V_{\lambda}$ gene, cloned from mouse embryonic DNA, corresponds almost exactly to the amino acid sequence of $V_{\lambda}^{\beta 15}$ (from position 1 to 98) there are differences at four positions, one in the framework and three in the third hypervariable region. The V gene sequenced by Tonegawa et al. (8), probably represents the germ-line gene for the V region of $\lambda 2$-chains, from which the gene for $V_{\lambda}^{\beta 15}$ was probably derived by a somatic mechanism (mutation or recombination). Other variants, more or less similar to $V_{\lambda}^{\beta 15}$, could account for the V regions of the entire set of $\lambda 2$ chains, with one-sixth of the variants so similar to $V_{\lambda}^{\beta 15}$ as to be serologically indistinguishable from it.

Differences in responses to immunization with Dnp-KLH and with KLH suggest that Dnp-proteins are capable of preferentially stimulating $\lambda 2$-producing cells. This observation is in accord with the high affinity of protein M315 for Dnp and trinitrophenyl ligands (25). The preferential production of $\lambda 2$-containing Abs, and increasing refinements in procedures for producing and selecting clones of fused cells (hybridomas) that make particular Ig chains, may eventually make it possible to analyze directly amino acid variations in V regions of $\lambda 2$-light chains from normal (rather than myeloma) Igs. These variations, viewed in relation to the DNA sequence of the embryonic (probably the germ-line) $V_{\lambda 2}$ gene, should help clarify the basis for the generation of immunoglobulin diversity.

Summary

The amino acid sequence of the constant (C) domain of the light chain of the mouse myeloma protein M315 has not been identified so far in any other myeloma protein. In this study, serological analysis with antiserum to the C-domain of this light chain ($L_{\lambda}^{315}$) showed that $\approx 1\%$ of Igs in normal mouse serum have L chains of the $L_{\lambda}^{315}$ type (called $\lambda 2$). Corroborative evidence was obtained by analysis of the carboxyterminal amino acid removed from normal light chains by carboxypeptidase A. A survey of 35 inbred mouse strains showed that all had $\lambda 2$; the serum level of Igs with $\lambda 2$-chains ranged from $\approx 140 \mu g/ml$ in AL/N mice to $\approx 25 \mu g/ml$ in SJL, BSVS, and eight other strains. In accord with the anti-Dnp activity of M315, sera from mice immunized with Dnp-KLH had three- to fivefold more $\lambda 2$ than sera from control mice immunized with KLH.

It was also possible to measure serum immunoglobulin molecules bearing the $\lambda 2$ variable region of M315 ($V_{\lambda}^{\beta 15}$). In BALB/c sera, the concentration of $V_{\lambda}^{\beta 15}$ was about sixfold lower than that measured for $\lambda 2$. Thus, $\lambda 2$-chains are divided into at least two
subsets: those whose V domain is indistinguishable from V\textsubscript{L}\textsuperscript{15} and those whose V\textsubscript{L} differs from V\textsubscript{L}\textsuperscript{15}. A 10-fold increase in V\textsubscript{L}\textsuperscript{15} was obtained by immunizing BALB/c mice with Dnp-KLH. The relationship of the V\textsubscript{L} domains of normal immunoglobulin \lambda2-chains to the embryonic V\textsubscript{A} gene recently sequenced by Tonegawa et al., is discussed.

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