THE NATURAL ABUNDANCE OF λ2-LIGHT CHAINS IN INBRED MICE*

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A small fraction of the light (L)[1] 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, L^{315}, is the light chain of the myeloma protein (M315) produced by plasmacytoma MOPC-315. L^{315}, 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 (L^{104}), 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) F_1 hybrid (6). Because other λ2-chains have so far not been described in BALB/c myelomas, it has long seemed possible that the L^{315} 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 L^{315} a more attractive possibility is that L^{315} 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 L^{315}-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 (V_L^{315} and V_H^{315}) to obtain separate antibody (Ab) preparations to the C and V regions of L^{315} (anti-C_L^{315} and anti-V_L^{315}, respectively). With these Ab preparations and ^{125}I-labeled L^{315}, 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

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[1] *Abbreviations used in this paper*: A_{278} U, absorbancy at 278 nm × sample vol; Ab, antibody; C, constant domain of an immunoglobulin chain; C_L^{315}, constant domain of M315 light chain; CM-L, S-carboxymethylated light chain; CPA, carboxypeptidase A; Dnp, 2,4-dinitrophenyl; DTT, dithiothreitol; H, immunoglobulin heavy chain; Ig, immunoglobulin; KLH, keyhole limpet hemocyanin; L, immunoglobulin light chain; L^{315}, L chain of M315; M315, M460, etc., are the purified myeloma proteins isolated from the serum of mice bearing the respective myeloma tumors (MOPC-315, MOPC-460, etc.); M460-S, Fv-S, etc. are Sepharose immunoadsorbants to which M460, Fv fragment, etc. are covalently attached; NGS, normal goat serum; −S, Sepharose to which different proteins have been covalently attached; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; V, variable region of an immunoglobulin chain or gene; V_L^{315}, variable domain of the L chain of M315; V_H^{315} variable domain of the H chain of M315.

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properties of the C and V regions of L^{315}. The V region of L^{315} has recently become of particularly 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 Immunosera 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 Dnp_{11}-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 ^{125}I (11). Chloramine T was used at a 10-fold molar excess over IgA proteins, but at a 1:1 molar ratio to iodinate L^{315} chains. Specific activities were usually $3–6 \times 10^6$ cpm/μg.

Preparation of the Fv Fragment. The proteolytic Fv fragment of M315, consisting of just V_H plus V_L 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 ($E^{1\%}_{1\,cm}$, 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 (α, λ2), M460 (α, κ), M104E (μ, 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[2] 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 Koshland (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 QAE-A50 at pH 7.5 (or pH 9.1 for LPC-1) (17). Based on conductivity measurements, myeloma proteins were eluted from QAE-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 antiserum to L^{315} 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 1.5 mg M104E/g S; 1 g adsorbant was used for 0.4 ml antiserum. Proteins were conjugated to S as described (18).

Antibodies to the Constant and Variable Regions of L^{315}: Anti-C_L^{315} and Anti-V_L^{315}. Antibodies to M315 were separated into anti-C_L^{315} and anti-V_L^{315} 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-C_L^{315}.

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-V_L^{315},

[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-V_L^{315} recovered from 1 ml of anti-λ2 processed in this manner had 0.65 A_{278} 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 ≅ 150,000 daltons. To deplete some IgG preparations of λ2-containing molecules, they were adsorbed (usually in lots of 12 mg) over anti-λ2-S, prepared by coupling S with the Ig fraction obtained by ammonium sulfate precipitation (40% saturation) from goat antiserum to L^{315} (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. L^{315} 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-λ2-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 NH_4HCO_3 and dialyzed against 0.2–0.3 M NH_4HCO_3, pH 8.0. L chains were measured by absorbance at 278 nm ($E^{1\%}_{1\,cm}$ = 11.0) (20).

CPA Digestion of L Chains. Soluble CM-L chains in 0.2–0.3 M NH_4HCO_3 (typically at 4 mg/ml) were digested with Worthington bovine pancreatic CPA (diisopropyl phosphofluoridate-treated) at an enzyme:L chain ratio (wt/wt) of ≅ 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(Durrum 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 λ2-Chains. To measure the amount of Ig with λ2-L chains, various sera or purified Ig fractions were tested for ability to inhibit the reaction between anti-C_L^{315} or anti-V_L^{315} and ^{125}I-L^{315} 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}I-L^{315} was bound by anti-C_L^{315} and 50% by anti-V_L^{315}. The background, using NGS instead of Abs to L^{315}, was 1–3% and the assay could detect the equivalent of 80 ng M315/ml. The standard deviation among replicate samples was ± 0.8% of the added ^{125}I-L^{315}. The frequency (percent) of IgG with λ2-L chains was calculated as 100 times the μg-Eq of M315/μg of IgG, where μg IgG was based on sample absorbancy at 278 nm ($E^{1\%}_{1\,cm}$ = 14.0) and the μg-Eq M315 was determined by comparing inhibition by the sample with inhibition by protein M315.

Results

The Radioimmune Inhibition Assay for Measuring λ2-Associated Ig. To search for λ2-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 L^{315} chain, rather than intact M315, as radiolabeled antigen in the assay. The first precaution increased the probability that the antiserum recognized the λ2-L chain when it was part of an intact Ig, and the second assured that the assay was restricted to Abs against λ2-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 L^{315}**

Experiment number	Inhibitors		Inhibition§
	Substance	Amount‡	
		ng	%
1	None		(0)
	Fv(V_L^{315} + V_H^{315})	75	0
	L^{315}	75	84
2	None		(0)
	L^{315}	8	12
	J chain	450	0
3	None		(0)
	M315 (α,λ2)	3	12
	M315 (α, λ2)	80	91
	X-2 (315H,κ)	3,800	0
	HOPC-1 (γ_{2a},λ1)	4,000	0
	104E (μ, λ1)	4,000	0
	M460 (α, κ)	2,500	0
	MOPC 21 (γ_1, κ)	2,500	0
	MPC-11 (γ_{2b},κ)	2,500	0
	LPC-1 (γ_{2a}, κ)	2,500	0
4	None		(0)
	M315	2	12
	M315	20	71
	J606 (γ_2,κ)	1,330	0

* All tubes had anti-C_L^{315} as antibody and ^{125}I-L^{315} (15 ng; 33,000 cpm). In the uninhibited controls, the percent of ^{125}I-L^{315} 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).

minants. The value of the second precaution was evident in some early trials where the presence or absence of anti-α-chain Abs did not alter the anti-M315 serum's activity or specificity in the radioimmunoassay for λ2-chains.

The specificities of the Abs used to assay for Igs with C_L^{315} and V_L^{315} are shown in Tables I and II. In the assay for C_L^{315} (Table I), 75 ng of Fv fragment was not inhibitory, whereas the same amount of L^{315} inhibited 85%. In contrast, the assay for V_L^{315} was inhibited to about the same extent by Fv and L^{315} (Table II). Neither of the λ1-proteins tested (HOPC-1 and 104E) inhibited the assay for C_L^{315} reaction, and 104E did not inhibit the assay for V_L^{315}. 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_L^{315} was also not inhibited by a myeloma protein of the γ3-class (J606), or by J chain, a potential contaminant of L^{315} (15).

Agreement between the Assays for C_L^{315} and V_L^{315}. To determine whether the assays for C_L^{315} and V_L^{315} 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 L^{315}**

Experiment number	Inhibitor tested		Inhibition§
	Substance	Amount‡	
		ng	%
1	None		(0)
	Fv	75	44
	L^{315}	75	50
	L^{315}	500	90
	M315 (α,λ2)	14	16
	M315 (α,λ2)	82	48
	M315 (α,λ2)	5,450	92
	M460 (α,κ)	5,450	0
	104E (μ,λ1)	5,450	0
2	None		(0)
	X-2 (315H,κ)	1,900	0
	MOPC-21 (γ_1,κ)	2,500	0
	LPC-1 (γ_{2A},κ)	2,500	0
	MPC-11 (γ_{2B},κ)	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}
	mg/ml	*mg/ml*	
MOPC-315 serum	3.85	3.4	1.13
MOPC-315 L.K. serum*	0.15	0.13	1.15
M315‡	0.53	0.58	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.

purified M315 isolated independently from the M315 preparation used to construct the standard inhibition curve.

Normal Mouse Serum Igs with λ2-Chains. To determine whether L chains with the C domain of L^{315} (i.e., λ2-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 λ2. Serum samples from adult BALB/c mice behaved as though they had (on the average) 80 μg/ml of Igs with λ2-chains. Similar high values were found in serum from C57Bl/6, AL/N, and NZB mice and much lower values (15–30 μg/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^{315}

Strain	Age	C_L^{315}		V_L^{315}	
		Average	Range	Average	Range
	wk	*μg/ml*	*μg/ml*	*μg/ml*	*μg/ml*
AL/N	15	140 ± 88 (4)*	86–272		
NZB	15	97 ± 24 (4)	76–119		
BALB/c	15	85 ± 36 (15)	23–140	13.8 ± 6.2 (6)*	4.6–18.5
A/J	15	76 ± 48 (6)	37–154		
LP/J	>20‡	67 (2)	65–68		
C57Bl/6	15	66 ± 23 (8)	36–92	11.2 ± 2.3 (3)	8.5–12.6
C57Bl/10	15	61 ± 25 (4)	44–98		
I/LnJ	>20	59 ± 19 (3)	39–79		
B10.D2	15	56 ± 10 (3)	48–67		
MA/MyJ	>20	54 ± 17 (3)	36–70		
BAB/14	15	55 (pool of 10)			
LG/J	>20	51 ± 28 (4)	23–88		
CBA/J	15	50 ± 17 (5)	33–77		
SM/J	>20	49 (2)	49,49		
C57Br	15	47 (2)	46,48		
STB/J	>20	46 (2)	36,56		
SEC1/ReJ	>20	49 (2)	46,53		
129/SV	>20	46 (2)	44,48	6.7 (2)	6.2,7.1
AKR/J	15	41 ± 9 (7)	27–55		
RF/J	15	38 ± 7 (7)	29–46		
Au/SSJ	>20	35 (2)	22,48		
BDP/J	>20	36 (3)	17–63		
C3H.OH	15	31 ± 7 (3)	23–38		
SWR/J	>20	29 ± 10 (8)	14–43	2.7 (2)	2.6,2.9
C3H/HeJ	15	28 ± 6 (9)	21–34		
DBA/2J	>20	28 ± 9 (10)	8–39	3.3 ± 0.5 (4)	
SJL/J	>20	25 ± 13 (5)	11–46	3.3 (3)	
SEA/GnJ	>20	24 ± 10 (4)	17–39		
CE/J	>20	23 (pool of 5)		3.4	
C58/J	>20	20 (2)	18,21		
RIII/2J	>20	20 (2)	16,24		
BSVS	>20	19 (2)	10,27		
P/J	>20	15 ± 6 (3)	10–21		

* 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 λ2-chains increased with age from ≅ 30 μg/ml at 4 wk to ≅ 80 μg/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%) λ2-containing Igs. This value is highly significant ($P < 0.001$), as the inhibitory activity of purified λ1- and κ-myeloma proteins was negligible (corresponded to <0.03%).

Two observations strengthened the probability that the serological test identified

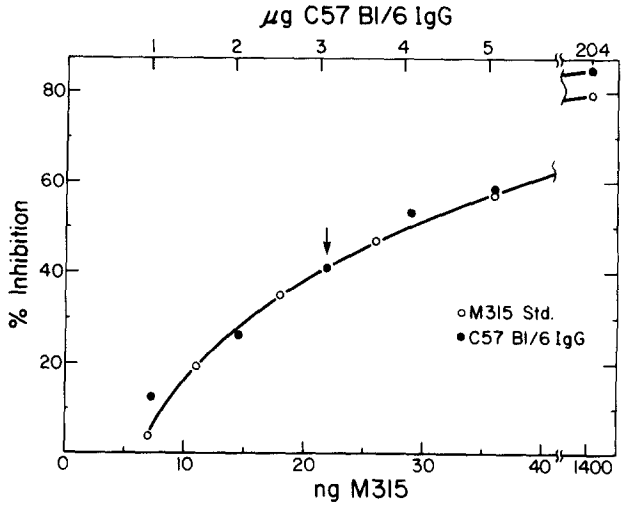

FIG. 1. Comparison of M315 and IgG from normal serum of C57BL/6 mice as inhibitors of anti-C_L^{315} antibodies. Inhibition with the normal serum IgG was fitted to the standard inhibition curve obtained with purified M315 (O) by placing the third of five IgG values (shown by arrow) on the standard curve.

λ2-chains in normal Igs: (*a*) purified IgG inhibited >90% of the reaction between ^{125}I-L^{315} and anti-C_L^{315} 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 Igs with L Chains Having a V Domain like that of L^{315}. The radioimmune inhibition assay with anti-V_L^{315} showed that molecules with the V region of L^{315} (V_L^{315}) are present in BALB/c serum at a concentration, in M315 equivalents, of 10–14 μg/ml (Table IV). Because this value is only one-sixth that of λ2-chains recognized with anti-C_L^{315}, it appears that the V domains of most λ2-chains in normal Igs differ from the V domain of L^{315}.

Increase in C_L^{315} and V_L^{315} upon Immunization with Dnp-KLH. Because M315, the only λ2-myeloma protein known so far, has Dnp-binding activity, we investigated the effect of immunization with Dnp-KLH on serum λ2-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 λ2 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, λ2-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_L^{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 μg/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 λ2 assay were due to cross-reaction with naturally occurring λ1-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	C_L^{315}‖				V_L^{315}‖ (Immune)
			Preimmune	Immune	Ratio (Immune)/(Preimmune)	Ratio (DNP-KLH)/(KLH)	
	wks		*μg/ml*	*μg/ml*			*μg/ml*
1	BALB/c‡ (7)	KLH	27.3	31.0	1.1		ND
			(22–43)	(15–56)		3.0	
		Dnp-KLH	31.0	102.5	3.3		28.4
			(22–34)	(62–145)			(23–34)
2	BALB/c§ (10)	KLH	ND	56			7.6
				(Pool of 5)			(Pool of 5)
		Dnp-KLH	ND	284			92
				(Pool of 5)			(Pool of 5)
3	C57BL/6‡ (8)	KLH	53	83	1.6		ND
			(41–70)	(38–140)		4.1	
		Dnp-KLH	21.7	144	6.6		31.7
			(16–28)	(74–218)			(28–39)
4	AKR‡ (6–7)	KLH	10.6	41.8	3.9		ND
			(7–14)	(25–55)		5.5	
		Dnp-KLH	11.4	245	21.5		49
			(8–14)	(184–400)			
5	BALB/c (14)	Dextran	68	49	0.7		
			(47–89)	(40–79)			

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 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

been 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 ≅ 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^{315} (reference 20 and T. Cotner. Unpublished observations). Hence, the CPA digestion provides independent support for the serological demonstration that λ2-chains account for ≅ 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 ≅ 1% of serum Igs have L chains of the λ2-type, confirming earlier tentative serological tests with anti-λ2-antiserum 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, λ2-containing myeloma proteins have been identified by screening 260 sera from BALB/c mice with myeloma tumors.[3] Because sera from all of the 35 inbred strains tested had λ2-chains, λ2 clearly represents an L-chain isotype; i.e., λ2 is not an allelic variant of λ1.

The strain survey revealed large differences in serum λ2-levels, ranging from 15–30 μg/ml of Igs with λ2-chains in some strains to 140 μg/ml in AL/N mice. The differences are not associated with differences at the H-2 complex, as BALB/c and NZB (both $H\text{-}2^d$) had high levels of λ2 whereas DBA/2 (also $H\text{-}2^d$) had low levels (Table IV). Although differences in λ2 levels might reflect differences in total Ig (which might be IgC_H linked, [24]), control by genes in the heavy chain linkage group cannot explain differences between BALB/c and C3H/He (both $Ig\text{-}1^a$) or between C57Bl and SJL or C58 (all $Ig\text{-}1^b$). The genetic basis for regulating the expression of this λ-chain isotype may be approached through further study of the large differences in λ2-levels in various strains.

The several approaches used to establish the frequency of λ2-chains in mouse serum Igs were all based on distinctive serological and chemical features of the C domain of L^{315}. In contrast, the frequency of L chains with the V domain of L^{315} (i.e., V_L^{315}) could only be measured serologically. Nevertheless, the validity of the assay for V_L^{315} is strongly supported by the agreement between this assay and the one for C_L^{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_L^{315} appeared to be sixfold less abundant in normal serum Igs than L chains with the C region of L^{315}. The radioimmunoassays thus divide λ2-chains into at least two subsets: those whose V_L domains resemble (or are identical) with V_L^{315} and those whose V_L domains differ from V_L^{315}. We assume (but have not demonstrated) that the L chains which react with anti-V_L^{315} are a subset of all λ2-chains.

Comparison of the amino acid sequences in L^{315} and in λ1-chains shows that their V regions are much more alike than their C regions (4), and the V regions of λ1 chains from myeloma proteins show remarkably little variation (3). From the radioimmunoassay with anti-V_L^{315}, it appears that the V regions of ≅ 85% of λ2-chains differ

[3] T. Cotner et al. Manuscript in preparation.

from V_L^{315}. Though this observation could mean greater variation in the V domains of λ2- than of λ1-chains, two uncertainties must be emphasized: (*a*) it is not known whether the V domains of λ1-chains in normal serum Igs are as restricted in amino acid sequence as the V domain of λ1-chains from myeloma proteins; (*b*) the extent of amino acid sequence variations in the λ2-chains that differ from L^{315} is unknown.

It is also far from certain that all chains with inhibitory activity in the radioimmunoassay for V_L^{315} have precisely the same amino acid sequence as V_L^{315}. If they did, this finding would mean, somewhat surprisingly, that the L chains of ≅ 1 in 1,000 serum Ig molecules in BALB/c and some other mouse strains are identical with L^{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^{315}. Nonidentity of these sequences would fit the recent finding by Tonegawa et al. (8), that although the deoxynucleotide sequence of a V_λ gene, cloned from mouse embryonic DNA, corresponds almost exactly to the amino acid sequence of V_L^{315} (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 λ2-chains, from which the gene for V_L^{315} was probably derived by a somatic mechanism (mutation or recombination). Other variants, more or less similar to V_L^{315}, could account for the V regions of the entire set of λ2 chains, with one-sixth of the variants so similar to V_L^{315} 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 λ2-producing cells. This observation is in accord with the high affinity of protein M315 for Dnp and trinitrophenyl ligands (25). The preferential production of λ2-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 λ2-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^{315}) showed that ≅ 1% of Igs in normal mouse serum have L chains of the L^{315} type (called λ2). 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 λ2; the serum level of Igs with λ2-chains ranged from ≅ 140 μg/ml in AL/N mice to ≅ 25 μg/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 λ2 than sera from control mice immunized with KLH.

It was also possible to measure serum immunoglobulin molecules bearing the λ2 variable region of M315 (V_L^{315}). In BALB/c sera, the concentration of V_L^{315} was about sixfold lower than that measured for λ2. Thus, λ2-chains are divided into at least two

subsets: those whose V domain is indistinguishable from V_L^{315} and those whose V_L differs from V_L^{315}. A 10-fold increase in V_L^{315} was obtained by immunizing BALB/c mice with Dnp-KLH. The relationship of the V_L domains of normal immunoglobulin λ2-chains to the embryonic V_λ gene recently sequenced by Tonegawa et al., is discussed.

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