

SEROLOGICAL DISTINCTION OF HEAVY CHAIN VARIABLE REGIONS (V_H SUBGROUPS) OF MOUSE IMMUNOGLOBULINS

I. Common V_H Determinants on the Heavy Chains of Mouse Myeloma Proteins Having Different Binding Sites*

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Extensive sequence homologies in the variable (V)¹ regions of Ig heavy (H) and light (L) chains allow for the division of V regions into subgroups (reviewed in references 1-4). Members of the same subgroup have primary structures more related to each other than to those of any other subgroup. Only the relatively invariant residues are used to classify subgroups; hypervariable residues are ignored.

Evidence for the serological detection of V subgroups was first reported for human kappa chains by Solomon and McLaughlin (5). Serologic typing of κ -chains into three distinct groups correlated with their division into three basic sequences, κ I, κ II, and κ III (6, 7). Evidence for subgroup-specific antigens on human lambda (λ) chains was described shortly thereafter by Tischendorf et al. (8). Recently, Førre et al. (9) reported antisera that distinguish the three major H chain V region (V_H) subgroups in humans. Especially relevant to the latter report is our recent evidence for the serological detection of V_H subgroups in mice (10). This is detailed in the present report.

Materials and Methods

Mouse Plasmacytomas. Most of the myeloma proteins tested in this study derive from NIH BALB/c plasmacytomas. Three tumors came from The Salk Institute, La Jolla, Calif.; J539 was supplied by Dr. M. Cohn and serum from Y5476- and W3082-bearing mice was given to us by Dr. M. Weigert. Myeloma proteins from plasmacytomas not induced in BALB/c mice included: C × BH PC-1 (from a mouse of Bailey's C × BH recombinant inbred line) (11); CBPC 4 and PC 3 [from Ig-

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¹ Abbreviations used in this paper: BSA, bovine serum albumin; C_HI, first constant region domain of H chain; CHO, carbohydrate; 1,6-Gal, β -1,6-D-galactopyranosyl residues; GNAc, N-acetyl-glucosamine; 2,6-Lev, β -2,6-fructosan; LR, linear regression; ManNAc, N-acetyl-mannosamine; PBS, phosphate-buffered saline; V, variable region; V_H, variable region of H chain; V_L, variable region of L chain.

congenic BALB/c mice that carry the allotype genes of C57BL/Ka (C.B mice)]; and 3660 (induced in a NZB mouse by Dr. M. Weigert, Institute for Cancer Research, Philadelphia, Pa.).

BALB/c myeloma proteins of particular interest to this study are those (ABE 48, Y5476, and U10) with specificity for levan, a β -2,6-fructosan (2,6-Lev) (12, 13); and those (T191, S10, JPC-1, X44, X24, J539, and T601) with specificity for β -1,6-D-galactopyranosyl residues (1,6-Gal) (14, 15); two proteins (173 and PC 3) with unknown binding specificity; one protein (M406) with specificity for *N*-acetyl-mannosamine (ManNAc) (16); and one protein (S117) that binds *N*-acetyl-glucosamine (GNAc) (17). Serum from S117-bearing mice was given to us by Dr. R. Riblet, Institute for Cancer Research, Philadelphia, Pa.

Purification of Myeloma Proteins. Chromatographically pure IgG_{2a} proteins (173, U10, and LPC-1) were obtained by precipitating the serum Ig of tumor-bearing mice with 50% (NH₄)₂SO₄ followed by passage of the Ig preparation over a DEAE-cellulose column (at 0.02 M PO₄, pH 7.4). IgG₃ from the J606 tumor was purified by selective precipitation according to the method of Grey et al. (18).

The IgA proteins, ABE 48, and C \times BH PC-1 (gifts of Dr. J. Slack, National Cancer Institute, Bethesda, Md.) and X44 and T601 (gifts from Dr. N. Glaudemans, Lung Cancer Branch, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md.), were specifically purified by elution from 2,6-Lev derivatized to bovine serum albumin-Sepharose (BSA-Sepharose) and from 1,6-Gal-BSA-Sepharose (19, 20). Samples of purified S117 were supplied by both Dr. K. Eichmann (Institute for Immunology and Genetics, German Cancer Research Center, West Germany), and Dr. N. Klinman, Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pa. These were prepared by affinity chromatography on *p*-aminophenyl-GNAc coupled to Sepharose (21).

Preparation of Myeloma H and L Chains. Reduction and alkylation of 173, U10, LPC-1, and J606 and the subsequent separation of the H and L chains was done by the method of Bridges and Little (22), the only difference being that the chains were separated over columns of Sephadex G-150 instead of G-100. Examples of gel filtration profiles of H and L chains from 173 and U10 are shown in Fig. 1. The ratio of the total absorbency of the H/L chain peaks was close to 2.0 and therefore the reduction of H and L chains was virtually complete.

Separated H and L chains were dialyzed against 100-fold and then 10-fold volumes of distilled water. This removed most of the urea and propionic acid and elevated the pH of these preparations to about 5. In this condition, the H and L chains remained soluble except the J606 H chain which precipitated after several days in the cold (4°C). All H and L chain preparations were diluted to the low microgram range in phosphate-buffered saline, pH 7.4 (PBS) immediately before their use as competitors in a radioimmune assay. Lyophilized H and L chains of X44 were supplied by Dr. S. Rudikoff, Laboratory of Cell Biology, National Cancer Institute, Bethesda, Md.

Rabbit Anti-173 Fab Serum. IgG_{2a} Fab fragments of 173 (173 Fab) were obtained by the method of Fahey and Askonas (23). A total of ~10 mg of 173 Fab was injected intramuscularly into a New Zealand rabbit over a period of 9 mo; each injection of 173 Fab (0.5–1.0 mg) was given in complete Freund's adjuvant. After the fifth injection, the rabbit was bled periodically and the antiserum stored at -70°C.

The antiserum was made specific for 173 Fab determinants by absorption with Sepharose beads (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) containing IgG_{2a} from the LPC-plasmacytoma (LPC-1 Sepharose) [see method of Axen et al. (24)]. After repeated absorptions with LPC-1 Sepharose, anti-173 Fab failed to react with a panel of myeloma proteins representative of the major Ig classes, i.e. IgM (MOPC 104E), IgA (MOPC 167), IgG₁ (MOPC 31c), IgG_{2a} (Adj PC5, HOPC-1, and LPC-1), and IgG_{2b} (MOPC 195). The restricted specificity of this absorbed antiserum for determinants on the 173 Ig is referred to as anti-173. As we show later, the IgG_{2a} of another plasmacytoma (U10) shares some determinants with 173. We refer to this cross-reacting specificity as U10-173.

Radioimmune Assay for 173 and U10-173. 173-reactive polystyrene tubes were made as follows (25): 12 \times 75 mm culture tubes (no. 2054; Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) were coated sequentially with 1 ml of a protein solution containing 5 μ g of 173 and 95 μ g of BSA, with 6 ml of 0.5% BSA in PBS and with 1 ml of anti-173 serum (1/200 dilution). To prevent the dissociation of the antibody from the tube surface in subsequent steps, the antigen and antibody were covalently cross-linked by means of 2.5 \times 10⁻⁵ M glutaraldehyde. U10-173-reactive tubes were made in the same manner except U10 was substituted for 173. Competition reactions between

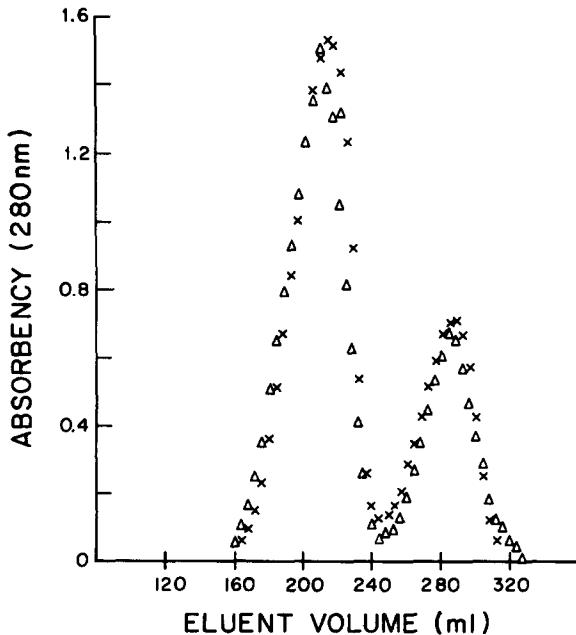


FIG. 1. Gel filtration profiles of separated H and L chains from two IgG_{2a}-producing mouse plasmacytomas, MOPC 173 (Δ) and UPC 10 (\times). The reduced and alkylated proteins were applied to separate Sephadex G-150 columns (2.5×80 cm) that were previously equilibrated with 1 M propionic acid in 4.5 M urea. Eluent fractions having an absorbency greater than 1.2 from the descending limb of the first peak (H chains) were pooled; those fractions having an absorbency greater than 0.5 on the descending limb of the second peak (L chains) were pooled. The ratio of the total absorbency of the H/L chain peaks was close to 2.0.

50 μ g of labeled antigen, ^{125}I -173 or ^{125}I -U10, and varying concentrations of unlabeled antigen were then carried out in these 173- or U10-173-reactive tubes. The reference antigen in the competition reactions was always the same as that used initially to coat the tube surface. As competitors, H and L chains were either added separately or together in different combinations to 173- or U10-173-reactive tubes. For all H and L chain mixtures, the H/L absorbency ratio was held constant at 2/1. Protein concentrations of Ig, H, or L chain solutions were determined from their absorbence (280 nm) using an extinction coefficient (E 1% cm) of 15. Ig preparations were radioiodinated with ^{125}I by the method of Hunter and Greenwood (26) to give a specific activity ranging from 0.4 to 1.0×10^6 cpm/ μ g protein.

Immunoabsorption of Myeloma Proteins. 2,6-Lev-BSA-Sepharose and 1,6-Gal-BSA-Sepharose were given to us by Dr. N. Glaudemans. These immunoabsorbents, together with LPC-1 Sepharose, were used batchwise to absorb solutions of ^{125}I -173, ^{125}I -U10, ^{125}I -X44, and ^{125}I -T601. Each solution was absorbed three to four times with a 5% volume of Sepharose beads and then counted in an Autogamma Spectrometer (Packard Instrument Co., Inc., Chicago, Ill.) to determine how much label was specifically removed.

The maximum quantity of label protein that could be removed by U10-173-reactive tubes was determined as previously described (25). Limiting quantities of the above labeled proteins were added to U10-173-reactive tubes. In this range of antigen addition, the proportion of label bound is independent of the amount added and it corresponds to the fraction of U10-173 antigen in each of the labeled solutions (173, U10, X44, and T601).

Results

Assay for U10-173 Determinants. Rabbit antiserum specific for the IgG_{2a} Fab fragment of 173 (anti-173) was found to react with one of six other IgG_{2a}

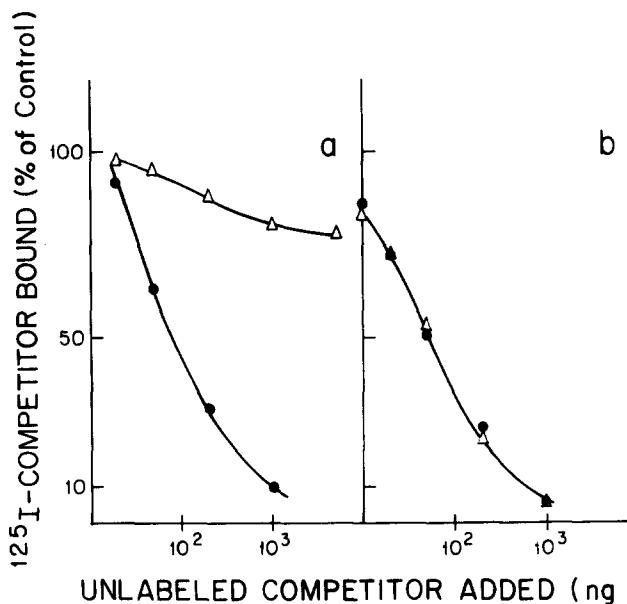


FIG. 2. (a) Antigen competition in 173-reactive tubes between 50 ng of ¹²⁵I-173 and known concentrations of unlabeled 173 (●) and U10 (△); and (b) antigen competition in U10-173-reactive tubes between 50 ng of ¹²⁵I-U10 and known concentrations of unlabeled 173 (●) and U10 (△). In this and subsequent figures, cpm bound is plotted as percent of control (no unlabeled competitor added); in the 173- and U10-173-reactive tubes, approximately 25–50 × 10⁴ cpm were bound to the control tubes. Each point represents the mean of triplicate determinations.

myeloma proteins of BALB/c mice. This cross-reaction occurred with U10, a myeloma protein that specifically binds 2,6-Lev; the specificity of 173 is not yet known. The distinction between 173 determinants shared (U10-173) and not shared (173) is made in Fig. 2a. As shown, the addition of 10³ ng of 173 into 173-reactive tubes reduced the uptake of ¹²⁵I-173 to ~10% of the control value (no unlabeled competitor added), whereas 10³ ng of U10 was not able to reduce the uptake of ¹²⁵I-173 to less than 80% of control. Therefore, U10 could compete for only about 20% of the anti-173 antibodies. To measure these U10-173 determinants exclusively, we coated polystyrene tubes with U10 followed by anti-173 antibodies and carried out antigen competition reactions with a fixed weight of ¹²⁵I-U10 instead of ¹²⁵I-173. The results are seen in Fig. 2b. Here, U10 and 173 were indistinguishable and 10³ ng of either reduced the uptake of ¹²⁵I-U10 to <10% of the control value. Substituting ¹²⁵I-173 for ¹²⁵I-U10 gave the same results as shown in Fig. 2b and therefore U10-173-reactive tubes must recognize the same determinants on both 173 and U10.

Evidence for U10-173 Determinants on 13 of 58 IgA Myeloma Proteins. The possibility that other BALB/c myeloma proteins might carry U10-173 determinants (U10-173⁺) was evaluated as follows: 10⁻⁵ dilutions of each of 102 sera from mice bearing different Ig-producing tumors, together with 50 ng of ¹²⁵I-U10, were added to U10-173-reactive tubes. 10⁻⁵ dilutions of serum from normal mice and from mice bearing the UPC-10 tumor served as negative (U10-173⁻) and positive (U10-173⁺) controls, respectively. Table I shows that sera from 13 of 58

TABLE I
Detection of U10-173 in 13 of 102 Different BALB/c Myeloma Sera*

Ig class of myeloma	No. tested	No. positive
IgM	3	0
IgA	58	13
IgG ₁	10	0
IgG _{2a}	5	0
IgG _{2b}	7	0
Miscellaneous	19	0

* 10^{-5} dilutions of each serum sample, together with 50 ng of ^{125}I -U10, were added to U10-173-reactive tubes. Sera from normal mice and from mice bearing the UPC-10 tumor served as negative and positive controls, respectively.

TABLE II
Comparison of Different U10-173⁺ Myeloma Sera; Evidence for Similar U10-173 Determinants on Myeloma Proteins Having Different Ligand-Binding Specificities

Group	U10-173 ⁺ myeloma sera	Ligand specificity	Comparison of competitive curves; serum dilution needed to reduce ^{125}I -U10 uptake by 50%*		
			LR	Slope	50% values
A	173	Unknown	-0.994	-2.17	1.6×10^{-6}
	PC 3		-0.993	-2.25	2.3×10^{-6}
B	ABE 48	2,6-Lev	-0.941	-1.93	-
	U10		-0.993	-2.32	1.0×10^{-6}
	Y5476		-0.995	-2.33	0.9×10^{-6}
C	T191	1,6-Gal	-0.999	-2.34	7.9×10^{-6}
	S10		-0.998	-2.36	9.9×10^{-6}
	JPC-1		-0.997	-2.32	6.0×10^{-6}
	X44		-0.982	-2.05	19×10^{-6}
	X24		-0.830	-0.936	-
D	S117	GNAc	-0.994	-2.16	1.4×10^{-6}
E	M406	ManNAc	-0.991	-2.61	26×10^{-6}
F	MOEV 48	Unknown	-0.964	-2.40	71×10^{-6}
G	BALB/c serum		-0.994	-2.03	47×10^{-5}

* Relative to control (no unlabeled competitor added), the logit of the percent ^{125}I -U10 bound [$\ln(\% \text{ of control}/100\% \text{ of control})$] was plotted as a function of the dilution of myeloma serum added. This yielded a family of lines for which the coefficients of LR, the slopes, and the 50% values are shown. The two exceptions, ABE 48 and X24, are discussed in the text. Three U10-173⁺ myeloma sera not listed in group C are T601, J539, and CBPC-4.

mice bearing different IgA-producing tumors were U10-173⁺. The remaining sera were U10-173⁻.

Radioimmune Analysis of U10-173⁺ Myeloma Sera. 10 of the 13 IgA myeloma proteins classified as U10-173⁺ in Table I are listed in Table II (the remaining three are indicated in the footnote of Table II). Included in this list

are the two IgG_{2a} proteins of Fig. 2 (173 and U10) plus an additional U10-173⁺ protein (PC 3) from a plasmacytoma that originated in a C.B-17 mouse (27). The grouping of these proteins is according to their ligand-binding specificities.

In Table II, we compare the ability of different myeloma sera to compete with ¹²⁵I-U10 for U10-173-reactive antibody. Serial dilutions of each myeloma serum, together with a fixed quantity of ¹²⁵I-U10 (50 ng), were added to U10-173-reactive tubes. Differences in competitive ability between myeloma sera are expressed as the dilution of serum required to reduce the uptake of ¹²⁵I-U10 by 50%. Except for ABE 48 and X24, all of the myeloma sera of groups A-D reduced the uptake of ¹²⁵I-U10 to <10% of control (no unlabeled competitor added). Logit transformation of these data, i.e., plotting the logit of the percent ¹²⁵I-U10 bound relative to control [$\ln (\% \text{ of control}/100\% \text{ of control})$] as a function of the dilution of myeloma serum added, yielded lines of comparable slope having linear regression (LR) coefficients close to 1. The last two myeloma sera of groups E and F gave logit lines similar to those of groups A-D, but due to the low concentration or low affinity of U10-173⁺ protein in these sera, we could not practically reduce the ¹²⁵I-U10 label below 35% of control. To do so would have required serum dilutions in which background quantities of U10-173 in BALB/c serum are detectable (see group G). Contrasting the above, myeloma sera of ABE 48 and X24 acted as incomplete competitors and were unable to reduce the uptake of ¹²⁵I-U10 below 50% of control. Moreover, logit transformation of these data gave lines having lower LR coefficients and more shallow slopes than any of the other myeloma sera. The same results were obtained with purified ABE 48 and X24 (not shown).

Two conclusions can be made from the results of Table II. First, that ABE 48 and X24 apparently lack some U10-173 determinants. Second, that the remaining myeloma sera contain similar U10-173 determinants; how similar depends on the extent to which differences in reactivity (50% values) reflect differences in U10-173 concentration and/or affinity of antibody for each U10-173⁺ protein.

Radioimmune Analysis of Purified U10-173⁺ Myeloma Proteins. Specific immunoabsorption of purified myeloma proteins representative of groups A-D in Table II was carried out to answer the following: do these proteins have noncross-reacting ligand-binding specificities and do the same molecules carry both the ligand-binding sites and the U10-173 determinants?

In Table III, a comparison between two immunoabsorbents, 2,6-Lev-BSA-Sepharose and 1,6-Gal-BSA-Sepharose, shows that ¹²⁵I-U10 was specifically absorbed by 2,6-Lev-BSA-Sepharose (group B). Conversely, ¹²⁵I-X44 was specifically removed by 1,6-Gal-BSA-Sepharose (group C). The high background in the controls of group C (2,6-Lev-BSA-Sepharose and LPC-1 Sepharose) was presumably due to the presence of the galactosyl residues in Sepharose (see Pharmacia handbook). None of the above immunoabsorbents were able to remove ¹²⁵I-173. Thus, it was clear that the 2,6-Lev- and 1,6-Gal-binding specificities are non-cross-reactive and that 173 recognizes neither of these two ligands. The lower uptake of ¹²⁵I-X44 vs. ¹²⁵I-T601 by U10-173-reactive tubes probably reflected radiolabeling damage of the ¹²⁵I-X44 preparation since it was several weeks older than that of ¹²⁵I-T601. As most of the ¹²⁵I label in groups A-D of Table III was removed by U10-173-reactive tubes and by the appropriate ligand immunoabsorbent as well (groups B and C), the answer to the above question is affirmative.

TABLE III
Immunoabsorption of ^{125}I -Labeled U10-173 $^{+}$ Myeloma Proteins

Group	Labeled myeloma protein	Percent of label removed by immunoabsorbent			
		U10-173-reactive tubes	2,6-Lev-BSA-Sepharose	1,6-Gal-BSA-Sepharose	LPC-1 Sepharose
A	^{125}I -173	87	9	6	12
B	^{125}I -U10	83	87	11	17
C	^{125}I -X44	72	32	70	45
	^{125}I -T601	89	ND*	ND	ND

* ND, not done.

Ligand-Binding Specificities of U10-173 $^{+}$ Myeloma Proteins. At least five different ligand-binding specificities are represented in Table II: unknown specificities (groups A and F); 2,6-Lev (group B); 1,6-Gal (group C); GNAc (group D); and ManNAc (group E). It should be noted that all three of the known 2,6-Lev-binding myeloma proteins of BALB/c were U10-173 $^{+}$, whereas two other myeloma proteins specific for 2,6-Lev, CBPC-2 (from a C.B-20 mouse), and 3660 (from an NZB mouse) were U10-173 $^{-}$. Further, all of the 2,1-Lev-binding myelomas (13) that were tested lacked U10-173 determinants (ABE 4, ABE 47, EPC 109, UPC 61, J606, and W3082). Finally, with respect to 1,6-Gal-binding myeloma proteins, a perfect correlation seemed to hold as all of these proteins were U10-173 $^{+}$ including one that was induced in a C.B-20 mouse (CBPC 4).

Dependence of 173 Determinants on the Combination of H and L Chains. Since U10-173 seemed to represent nonbinding site determinants, it was of interest to study what role H and L chains played in forming the U10-173 structure. For this purpose, it was helpful to evaluate first the contribution of H and L chains to the antigenicity of the 173 structure.

It is clear from Fig. 3 that a mixture of 173 H and L chains was as effective in competing for ^{125}I -173 as was intact 173 Ig (see stippled curve), i.e., both competitive curves reached $\sim 10\%$ of the control value after the total addition of 1,000 ng of unlabeled competitor. When the same weights of 173 H and L chains were added separately or together with LPC-1 L and H chains, respectively, the competition curves were shifted markedly to the right and assumed a different shape than that of the 173H-173L curve. The simplest interpretation for this cooperation between 173H and 173L chains is that the structure of 173 determinants depends on the interaction of both subunits. Thus, H and L chains must recombine in 173-reactive tubes after overnight incubation at 4°C. This is in fact expected since others have already demonstrated that mildly reduced and alkylated H and L chains will recombine noncovalently under conditions of neutral pH (22, 28).

Comparable competitive curves to those of 173H-LPC-1L and LPC-1H-173L resulted when L and H chains from J606 were substituted for LPC-1 (not shown). This did not happen, however, when mixtures of H and L chains between 173 and U10 were added to 173-reactive tubes. As shown in Fig. 3, the combination of 173H and U10L helped restore the 173 structure. This is evident from the shape of the 173H-U10L curve and from its position relative to that of 173H-LPC-1L. The reverse combination of U10H-173L also partly restored the 173 structure (not shown). This means that the H and L chains of U10 and 173 must share

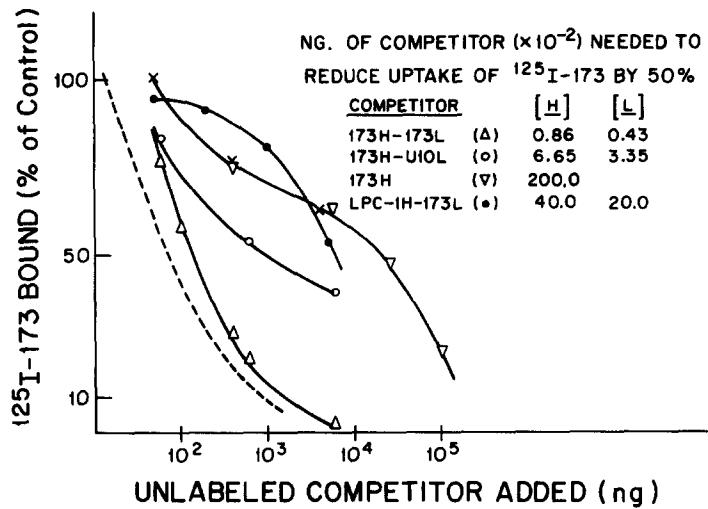


FIG. 3. Contribution of H and L chains to the structure of 173 determinants. 50 ng of ¹²⁵I-173 was added into 173-reactive tubes along with increasing weights of unlabeled H and L chains, added separately or together as follows: 173H + 173L (Δ — Δ); 173H + U10L (\circ — \circ); 173H + LPC-1H (\times — \times) for which only the H chain concentration is plotted; 173H (∇ — ∇); and 173L + 173L (\bullet — \bullet). The ratio of H/L in the subunit mixtures was held constant at 1/1, i.e., the absorbency (280 nm) ratio of H/L was 2/1. The stippled line corresponds to the competitive curve obtained with intact 173 as unlabeled competitor (see Fig. 2). Tabulated on the right-hand side of the figure is the nanograms of competitor needed to reduce the uptake of ¹²⁵I-173 by 50%.

certain homologies in their primary structure. In support of this conclusion is the fact that available N-terminal V_H and light chain V region (V_L) sequences of 173 (29, 30) and U10 (S. Rudikoff, personal communication) are each, respectively, very similar to one another.

From the total weight of H and L chains needed to reduce the uptake of ¹²⁵I-173 to 50% of control, H and L chain recombination was about 60% efficient relative to intact 173 as a competitor (see stippled curve of Fig. 3). This may be an underestimate as recombination of H and L chains appeared to be concentration dependent, i.e., recombination was more complete at total H and L chain concentrations of 1,000 ng than at 200 ng. This criterion for recombination of H and L chains does not, of course, distinguish between HL dimers (or aggregates) and four-chain molecules (H₂L₂).

A comparison of the quantities of H or L chain competitor needed for 50% reduction of ¹²⁵I-173 uptake is tabulated in Fig. 3. For example, approximately 200×10^2 ng of 173H had to be added to 173-reactive tubes in order to displace 50% of the 173 label. This was true whether 173H was added alone or together with unrelated L chain (173H + LPC-1L). But in the presence of 173L, only 0.86×10^2 ng of 173H was needed to displace 50% of the labeled 173. The magnitude of this difference in reactivity can be interpreted to reflect less than 1% contamination of the 173H preparation with unreduced and unseparated HL, H₂L, or H₂L₂. Likewise, the low reactivity of 173L-LPC-1H can be ascribed to contamination of the 173L preparation. An alternate interpretation is that 173H and 173L chains retain enough homology to the intact 173 so as still to bind to anti-173 but with a much reduced affinity.

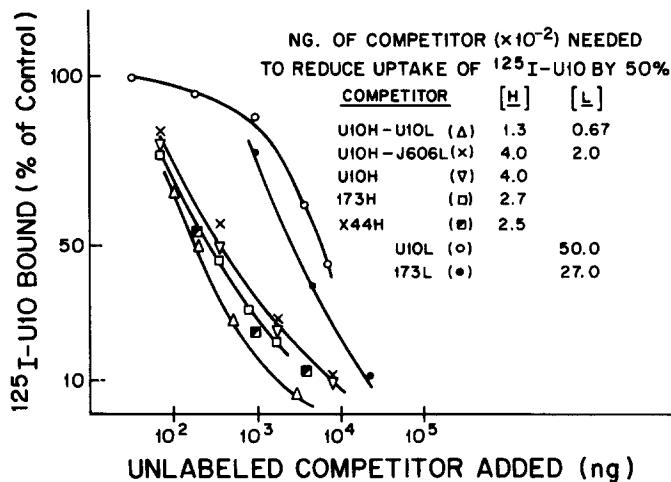


FIG. 4. Contribution of H and L chains to the structure of U10-173 determinants. 50 ng of ^{125}I -U10 was added into U10-173-reactive tubes along with increasing weights of unlabeled H and L chains, added separately or together as follows: U10H + U10L (Δ — Δ); X44H (\blacksquare); 173H (\square — \square); U10H (∇ — ∇); U10H + J606L (x — x) for which only the H chain concentration is plotted; 173L (\bullet — \bullet); and U10L (\circ — \circ). The ratio of H/L in the subunit mixtures was held constant at 1/1, i.e., the absorbency (280 nm) ratio of H/L was 2/1. Tabulated on the right-hand side of the figure is the nanograms of competitor needed to reduce the uptake of ^{125}I -U10 by 50%.

Nondependence of U10-173 Determinants on the Combination of H and L Chains. Unlike 173 determinants which required that both H and L chains be in combination with each other, the U10-173 determinants showed no such requirement. This became evident when various H and L chains were added separately or together into U10-173-reactive tubes in the presence of 50 ng of ^{125}I -U10 (see Fig. 4). Consistent with the low activity of 173L in 173-reactive tubes (Fig. 3), the ability of 173L or U10L to displace ^{125}I -U10 was 38-70 times less efficient than the same weight of U10L mixed with U10H. On the other hand, 173H, U10H, and also X44H were only two to three times less efficient in displacing ^{125}I -U10 than the same weight of U10H mixed with U10L. In the case of 173H, the competitive ability could not be attributed to HL contamination as the same 173H preparation was judged to be virtually pure according to the data of Fig. 3. Therefore, we conclude that U10-173 determinants are on the H chains of 173. Per unit weight of competitor added, 173H, U10H, and X44H gave comparable results: therefore, the H chains of all these proteins must share very similar, if not identical, U10-173 determinants.

It is possible that the configuration of U10-173 determinants might be influenced by the U10L chain. As shown in Fig. 4, the same weight of U10H competed \sim three times more efficiently when added with U10L than with J606L or then when added by itself. A reasonable interpretation of this is that antibodies to U10-173 have greater affinity for U10H when it is combined with U10L than with J606L.

Discussion

We have presented evidence for similar H chain antigens (U10-173) on a number of IgG_{2a} and IgA myeloma proteins known to bind one of four different

carbohydrate (CHO) ligands (2,6-Lev, 1,6-Gal, GNAc, and ManNAc) and on a few myeloma proteins having unknown binding site specificities. What follows in two parts is the basis for concluding that U10-173 is an H chain antigen and the rationale that U10-173 is a V_H framework antigen shared by a small number of related V_H subgroups.

Evidence that U10-173 Determinants are on H Chains Alone. The presence of U10-173 determinants on H chains was ascertained by comparing the ability of isolated H and L chains to compete with labeled antigen in 173-reactive or U10-173-reactive tubes. In such tubes, unlabeled H and L chains could be mixed and recombined after overnight incubation at 4°C. Alternatively, H and L chains could be added separately. Thus, the ability of H and L chains to reduce the uptake of labeled antigen related to whether the antigenic structure in question required only one subunit or the interaction of both subunits. In this way, we found that the addition of increasing weights of 173H and 173L chain mixtures into 173-reactive tubes competed as effectively for ¹²⁵I-173 as did the intact 173 molecule. On the other hand, 173H and 173L chains acted as incomplete competitors when added separately into 173-reactive tubes in the same concentration range as in the mixtures. This result was not changed by interacting 173H chain with unrelated L chains from tumors LPC-1 and J606. However, the antigenic structure of 173 could be partly restored if reciprocal combinations of H and L chains of U10 and 173 were added into 173-reactive tubes. Therefore, it was clear that the structural determinants for 173 required the combination of a specific H and L chain and that both the H and L chains of U10 and 173 shared certain homologies in their primary structure.

The importance of the 173 characterization was that it enabled us to interpret the competitive curves in the U10-173 system. Here, H chain preparations of U10 or 173 gave competitive curves that were displaced by only a factor of 2-3 to the right of the U10H-U10L recombinant curve, whereas the competitive curves generated by L chains of U10 or 173 were shifted to the right of the U10H-U10L curve by a factor of 15-25. This result could not be attributed to 25% or more contamination of the H chain preparations with HL aggregates because the 173H chain preparation behaved in 173-reactive tubes as if it were 99% pure, whereas 173L chains were estimated to have the same order of reactivity or impurity (1-3%) when assayed in either system. Therefore, we concluded that U10-173 determinants are on the H chains alone.

Rationale that U10-173 is a V_H Framework Antigen. That U10-173 determinants are on the V_H was suggested from the results of screening over 100 myeloma proteins in U10-173-reactive tubes. First, U10-173 determinants were found on 13 of 58 IgA proteins and on 3 of 8 IgG_{2a} proteins. It seems unlikely that these determinants would correspond to the first constant region domain (C_HI) of some IgA and IgG_{2a} H chains as then we would have to postulate subclasses of IgA and IgG_{2a} having similar C_HI regions. A second indication for V_H determinants was the restricted association of U10-173 with proteins having one of several anti-CHO specificities. U10-173⁺ proteins included all of the known BALB/c myeloma proteins specific for 2,6-Lev (ABE 48, U10, and Y5476) and all those specific for 1,6-Gal (T191, S10, JPC-1, X44, X24, J539, T601, and CBPC4), plus S117 and M406 which are specific for GNAc and ManNAc residues, respectively. A fourth and different ligand-binding specificity was inferred for 173 and

PC 3 as these two proteins give indistinguishable isoelectric-focusing spectra (unpublished results) and do not bind 2,6-Lev or 1,6-Gal.

The demonstration of very similar, if not identical, U10-173 determinants on the H chains of 173, U10, and X44, each representing a different group of myeloma proteins having noncross-reacting ligand-binding specificities, indicated that we were not dealing with hypervariable amino acid residues that form the binding site. Thus, we presume that U10-173 determinants correspond to framework residues outside the hypervariable regions, and that considerable portions of the V_H framework of proteins in groups A-E of Table II are identical or nearly so. Available structural data support this view. The N-terminal V_H sequences of U10-173⁺ proteins S10, T191, X44 (14), S117 and Y5476 (30), and U10 (S. Rudikoff, personal communication) are identical through the first 27 residues; those of JPC-1 (14), 173 (24) and M406 (16) differ by no more than two replacements through the first 20-27 residues. Further, a comparison between the completed V_H sequence of 173 (29) and the nearly completed V_H sequence of J539 (31) shows only four framework residues that are different. It is to be expected that purified V_H peptides of 173 or J539 (or of U10) would be useful in attempting to localize U10-173 determinants. Such peptides could be tested for their ability to inhibit the uptake of ^{125}I -U10 in U10-173-reactive tubes.

Two different interpretations can be given to the U10-173 data. The first is that U10-173⁺ proteins correspond to at least five different V_H germ-line genes. Here, the rationale is that one or more replacements in the V_H framework defines a distinct V_H germ-line gene. Somatic theories of antibody diversity that presume mutation occurs predominantly in the hypervariable region or is only detectable in these regions due to antigenic selection (32), as well as germ-line theories of antibody diversity (see references 4 and 30), would argue for this interpretation. We know from other data (10) that U10-173 represents only about 1% of the total Ig fraction; therefore, five or more U10-173 V_H genes may account for the expression of 1% of all V_H genes and accordingly there could be as many as 500 V_H genes in the germline. An alternative idea to the above is to consider the V_H frameworks of U10-173⁺ proteins so similar as to represent the product of one V_H germ-line gene. If somatic mutations of V_H genes were not restricted to hypervariable regions and were to occur with unusually high frequency, one could attribute minor differences between V_H frameworks to mutations that do not seriously change the configuration of $V_H V_L$ domains. Accordingly, five or more different anti-CHO specificities might be realized when V_H products of one U10-173 germ-line gene are paired with appropriate V_L gene products.

Summary

16 of more than 100 mouse myeloma proteins, including 3 proteins of the IgG_{2a} class and 13 of the IgA class, were shown to have a similar heavy chain variable region (V_H) antigen(s) (U10-173). The proteins bearing these antigenic determinants (U10-173⁺ proteins) represented at least five different ligand-binding specificities. These findings, taken together with available sequence data for V_H regions of U10-173⁺ proteins, have led us to conclude that U10-173 defines a small number of related V_H subgroups. The ability to detect V_H subgroups in

mice by serological means, as has been done in humans also, promises new and useful kinds of V_H markers for immunologic study.

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