MULTIPLE INDIVIDUAL AND CROSS-SPECIFIC IDIOTYPES ON 13 LEVAN-BINDING MYELOMA PROTEINS OF BALB/c MICE

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Studies of immunoglobulin V-region antigenic determinants (idiotypes) are important in understanding the genetics and evolution of antibodies and the generation of antibody diversity. The finding of homogeneous immunoglobulins (myeloma proteins, Waldenstrom macroglobulins M-components) that bind the same hapten or antigen has added immeasurably to these studies. The best studied systems to date are the human IgM proteins that bind γ-globulin (1–3), and the IgM cold agglutinins (4–6) and the mouse phosphorylcholine (7–10), $\alpha 1 \rightarrow 2$ and $\alpha 1 \rightarrow 6$ dextran (11-16), levan-(11, 14) and galactan-(17) binding myeloma proteins. Generally in these studies two types of antigenic determinants or idiotypes have been found within a functional group, individual antigenic determinants (IdI)[1] or idiotypic determinants specific for the homogeneous immunoglobulin within the group and cross-specific (IdX) or common idiotypic determinants which are shared among some of the functionally related proteins within a group but which are not or very rarely found among unrelated immunoglobulins. Structurally IdI and IdX are both located on the domains of the immunoglobulin molecule formed by the V_L and V_H polypeptides. Some idiotypic determinants are contiguous to or directly involve peptide segments or amino acids in the binding sites and the interaction of hapten with the homogeneous immunoglobulin may block the anti-idiotypic antibody (9, 14, 18). Other V-region idiotypic antigens are genetic in that within the species, they may be derived from polymorphic genes (8, 12, 19–21).

In this study we have examined the idiotypic determinants of the largest group of specific antigen-binding myeloma proteins found thus far in the mouse, namely, the levan-binding proteins. These proteins have been shown to possess two types of binding specificity, one group of 11 proteins binds fructosans such as inulin which are linear polysaccharides with $\beta 2 \rightarrow 1$ linkages and bacterial (Aerobacter) levan; the second group of two proteins binds determinants on bacterial levans which are probably fructosans with $\beta 2 \rightarrow 6$ linkages (11). This latter point has not yet been clearly established since appropriate oligosaccharide inhibitors are not yet available.

[1] *Abbreviations used in this paper:* HA, hemagglutination; HI, hemagglutination inhibition; IdI, individual antigenic determinants; IdX, cross-specific determinants; LBMP, levan-binding myeloma proteins.

The idiotypic sera prepared to levan-binding myeloma proteins (LBMP) were unusual in that they identified a large number of IdX in addition to the usual individual idiotypes. The IdX of LBMP indicated the close relationship of this family of proteins. We were able to show also that some IdI and IdX of LBMP were closely related to the antigen-binding site.

Materials and Methods

Myeloma Proteins. The 13 LBMP used in the study are listed in Table I. The tumors producing these proteins were all of BALB/c origin. The proteins were initially typed with antiallotype antisera

TABLE I
Origin and Characteristics of Plasma Cell Tumors Producing Levan-Binding Myeloma Proteins

Myeloma	Induction method*	Origin	Myeloma protein class	Binding to Levan‡		Ref.
				A. levanicum	Inulin	
J606	M	Salk Inst.	IgG_3	+	+	11, 14, 27, 28
ABPC48	P + V	NIH	IgA	+	−	§
ABPC47	P + V	NIH	IgA	+	+	§
UPC61	M	NIH	IgA	+	+	11
W3082	M	Salk Inst.	IgA	+	+	11, 14, 27, 28
MOPC702	M	NIH	IgA	+	+	§
EPC109	M	NIH	IgA	+	+	§
TEPC803	P	NIH	IgA	+	+	§
ABPC51	P + V	NIH	IgA	+	+	§
ABPC4	P + V	NIH	IgA	+	+	§
UPC10	M	NIH	IgG_{2a}	+	−	11, 14
AMPC1	M	NIH	IgA	+	+	§
ABPC45	P + V	NIH	IgA	+	+	§

In the paper the myeloma proteins will be designated by the first letter followed by the number, e.g. ABPC48 = A48.

* Plasmacytoma (myeloma) was induced by i.p. injection of mineral oil (M), pristane (P) or Pristane and Abelson virus (V).

‡ Agglutination of palmitoyl levan, or palmitoyl inulin-coated SRBC.

§ This paper.

and 11 proteins ABPC48, ABPC47, UPC61, W3082, MOPC702, EPC109, TEPC803, ABPC51, ABPC4, AMPC1, and ABPC45 were of the IgA class and carried C_H determinants $A^{12,13,14}$, one (UPC10) was an IgG_{2a} protein with C_H determinants $G^{1,6,7,8}$ and one (J606) was an IgG3 protein. ABPC4, ABPC47, ABPC48, UPC61, UPC10, TEPC803, EPC109, and AMPC1 were precipitated from ascites or diluted serum at 4°C with 37–50% saturation with ammonium sulfate. The precipitate was resolubilized in phosphate-buffered saline and then dialyzed against physiologic saline. In addition, sera or ascites from 106 different BALB/c plasmacytomas not binding levan were also tested for levan cross-specific idiotypic determinants. J606 and W3082 myeloma proteins were kindly supplied to us by Dr. M. Weigert, Institute for Cancer Research, Philadelphia, Pa.

Anti-Idiotype Antisera. For the most part to successfully prepare anti-idiotype antiserum to BALB/c myeloma proteins, the strain to be immunized has to be of a different IgC_H allotype than the BALBc and also possess the appropriate *H-2*-linked immune response (*Ir*) gene (22, 23). For these reasons strain A/He which lacked $IgC_HA^{12,14}$ of BALBc and possesses the appropriate *Ir-1a* gene which controls the maximal immune response to IgA myeloma protein was selected for immunization with ABPC4, ABPC47, ABPC48, UPC61, TEPC803, EPC109, AMPC1, and W3082 myeloma proteins. Similarly B10.M mice which lacks $IgC_HG^{1, 6, 7, 8}$ of BALB/c and possesses *Ir-1b* were immunized with UPC10 and SJL mice were immunized with J606 myeloma proteins. No allotypic markers have thus far been identified for IgG3 in mice. All mice were immunized according to the method previously described (23). Antisera prepared with IgA myeloma proteins were made specific for idiotype by the addition of 0.025 ml MOPC167 (3 mg/ml) to 0.1 ml of each antiserum to inhibit the allotype. MOPC167 is a BALB/c IgA myeloma which shares the IgC_H allotypes but not idiotype of the IgA antilevan myeloma proteins. Similarly, B10.M anti-UPC10 antisera were made specific for the idiotype by the addition of adj. PC5 (IgG_{2a}) myeloma protein; anti-J606 required no absorption.

Anti-Idiotype Antibody Assay. The hemagglutination (HA) and hemagglutination inhibition (HI) methods previously described using chromic chloride treated SRBC coated with the specific myeloma protein were employed (8). The anti-idiotype specific antisera were first absorbed with an appropriate non-LBMP and the HA titer of the antiserum, for each of the LBMP-SRBC systems was determined. Specific IdI HA systems were then developed by further absorbing the antisera with an appropriate cross reacting LBMP. Cross-specific HA systems were constructed by using an antiserum prepared to one LBMP and SRBC-coupled with a cross-reacting LBMP. The HI method was used to determine if a myeloma protein shared idiotypic specificities with the myeloma protein coupled to the SRBC. The HI titer of a given inhibitor was compared with the HI titer of protein coupled to the SRBC. Inhibitors giving comparable titers were judged to be identical, those giving much less but significant inhibition were arbitrarily judged to be partial determinants. At this time the system is not yet quantitative because we do not have a suitable immunoabsorbant and the distinction between partial and identical is tentative. In most instances approximately 1 mg/ml of the specific myeloma protein inhibitor was used. However, ascites were used as inhibitors for A51, M702, and J606 and the greatest dilution giving complete inhibition was then determined.

Antilevan and Anti-Inulin Activity of Myeloma Proteins. Levan was isolated from culture supernates of *Aerobacter levanicum* ATCC 15552 grown in nutrient broth at 23°C supplemented with 5–10% sucrose. The culture supernates were brought to 80% saturation with ethanol, which precipitated the levan. The levan was solubilized in H_2O and dialyzed extensively. Inulin was obtained commercially from Nutritional Biochemicals Corp., Cleveland, Ohio. The palmitoyl derivative of *Aerobacter levanicum* was prepared according to the method of Tsumita and Ohashi (24); 10 mg of lyophilized levan was dissolved in 1 ml pyridine in a screw cap vial, heated in a water bath, and 1 μl palmitoyl chloride added (Eastman Organic Chemicals, Rochester, N.Y.). The palmitoyl chloride was added quickly to avoid precipitation in the pipette by pyridine. For different polysaccharides, the amount of palmitoyl chloride can be varied to produce optimal derivatives. The mixture was then stirred at room temperature for 48–72 hr, precipitated in 80% saturated isopropyl alcohol, and centrifuged. The precipitate was dissolved in 1 ml water, dialyzed, and coupled to saline-washed SRBC. Occasionally the palmitoyl-levan adhered to the glass vial, and when this occurred the precipitate was dissolved in water. Varying amounts of palmitoyl-levan ranging from 0.05 to 0.2 ml were added to 1 ml 2.5% SRBC. The coupling procedure was carried out at 37°C for 1 hr.

The same procedure was used for inulin except that inulin is relatively insoluble in pyridine. 20 mg inulin was added to 1 ml pyridine, heated, the supernate decanted, and to this 20 μl palmitoyl chloride was added. The oligosaccharide $\beta 2 \rightarrow 1$ fructosan trisaccharide was prepared from Dahlia Inulin by the method of Painter (25). The inulin hydrolysate was evaporated to a syrup that was applied to 3-0 Whatman paper, and the oligosaccharides were separated chromatographically in a butanol:pyridine:water (10:4:3) system for 100 h at room temperature. The oligosaccharides were tentatively identified by their relative migration compared to fructose and sucrose. The trisaccharide was eluted from the band that migrated just behind sucrose. The HI titer of fructosan trisaccharide was determined for each IdI and IdX system. An 0.028 M solution of the trisaccharide was diluted in the microtiter plates and to each dilution the myeloma protein-coated SRBC were added and allowed to stand for 30 min at room temperature. Specific antiserum was then added and the HI titer was read at 3 and 24 h.

Results

Antisera Prepared to One Levan-Binding Myeloma Protein Usually Cross-Reacts with Other Levan-Binding Myeloma Proteins. Idiotypic antisera prepared to 9 (except J606) of the 10 levan-binding myeloma proteins agglutinated both SRBC coated with the immunizing myeloma protein and SRBC coated with other LBMP (Table II). Some of the antisera agglutinated SRBC coated with the other LBMP in roughly equal titers to the homologous protein, e.g., anti-W3082 and anti-E109 antisera.

In some instances much weaker HA titers were observed with the cross-reacting antibodies. This was particularly apparent when one compared the cross-reacting titers with the titer obtained with the homologous proteins. Anti-A48 antiserum is a good example; anti-A48 reacted to A48 protein, with a log 2 titer of 17; to A47 a titer of 14 and E109, U61, W3082, Aml, A4 myeloma proteins titers ranging from 9–12.

TABLE II

Hemagglutination Titers of Cross-Reacting Idiotypic Antibodies for Levan-Binding Myeloma Proteins

Antisera	HA titers (log 2)									
	SRBC coated with myeloma proteins									
	A 4	A 47	A 48	U 61	W 3082	U 10	J 606	E 109	Am 1	T 803
10559 A* anti-A48	12	14	17	10	12	0	0	9	12	10
10548 A anti-A47	0	19	11	0	9	0	0	0	9	0
10544 A anti-A4	17	7	5	13	14	0	0	14	12	13
10570 A anti-U61	16	0	7	19	16	0	0	15	17	15
10789 A anti-W3082	14	0	0	13	17	0	0	12	15	14
10898 A anti-E109	13	0	0	12	14	0	0	14	10	11
10878 A anti-Am1	10	8	8	10	12	0	0	0	17	11
10909 A anti-T803	10	8	8	12	15	0	0	12	12	16
9305 B10.M anti-U10	6	4	5	4	4	13	0	0	0	0
6470 SJL anti-J606	0	0	0	0	0	0	0	0	0	0

10559–10909 absorbed with M167 (IgA) to remove allotype specificities; 9305 absorbed with Adj.PC5 (IgG2a) to remove allotype specificities; 6470 prepared in SJL did not exhibit allotypic specificities.
* A = A/He strain obtained from Delta Uphoff, NIH.

Preparation of Idiotypic Antiserum Specific for Individual Specificities on each Myeloma Protein. Each of the 10 antisera prepared to different LBMP could be made specific for the IdI determinant by absorption with the appropriate cross-reacting myeloma protein (Table III). For example, absorption of anti-A48 antiserum with W3082 myeloma protein removed the cross-reactions with proteins A4, A47, U61, W3082, E109, Aml, and T803 while practically having no effect on the titer to A48 the homologous protein (Tables II, III). Even those antisera showing very strong cross-reactions could be made IdI-specific by absorption with a strong cross-reacting myeloma protein. This is demonstrated by the absorption of anti-U61 with W3082 protein which removed all the strong cross-reactions to A4, W3082, E109, Aml, and T803 and the weaker reaction to A48. In this instance however, the HA titer to U61 coated-SRBC was reduced from 19 to 14 (log 2) after absorption. This was also true of anti-W3082 and anti-

TABLE III

Hemagglutination Titers of Individual Idiotypic Antibodies for Levan-Binding Myeloma Proteins

		HA titers (log 2)									
		SRBC coated with myeloma proteins									
	Absorbed	A 4	A 47	A 48	U 61	W 3082	U 10	J 606	E 109	Am 1	T 803
10559 Anti-A48	W3082	0	0	16	0	0	0	0	0	0	0
10548 Anti-A47	U61	0	19	0	0	0	0	0	0	0	0
10544 Anti-A4	U61	15	0	0	0	0	0	0	0	0	0
10570 Anti-U61	W3082	0	0	0	14	0	0	0	0	0	0
10789 Anti-W3082	U61	0	0	0	0	14	0	0	0	0	0
10898 Anti-E109	T803	0	0	0	0	0	0	0	11	0	0
10878 Anti-Am1	T803	0	0	0	0	0	0	0	0	15	0
10909 Anti-T803	E109	0	0	0	0	0	0	0	0	0	13
9305 Anti-U10	A4	0	0	0	0	0	12	0	0	0	0
6470 Anti-J606	None	0	0	0	0	0	0	11	0	0	0

10559–10909 absorbed with M167 (IgA) to remove allotypic specificities; 9305 absorbed with adj.PC5 (IgG2a) to remove allotypic specificities; 6470 prepared in SJL did not exhibit allotypic specificities.

E109 sera which showed a reduction of titer to the homologous protein after absorption. Anti-J606 antiserum did not require absorption and only identified an IdI on J606.

Identification of Idiotypic IdX among LBMP. As indicated in Table II there were many cross-reactions of the idiotypic sera with other LBMP. In these systems the determinants identified (IdX) were common to the immunizing protein and the "coated protein" but did not involve the IdI determinant. The many cross-reacting sera in Table II suggested common IdX determinants were prevalent among the LBMP. Thus, many of the antisera raised to different LBMP might be identifying the same determinants. This made it possible to construct a variety of HI systems (designated by the letters A to J) (Table IV) in which an antiserum was reacted with a myeloma protein not involved in the immunization. 12 different LBMP were used to inhibit each of the systems described in Table IV and instead of finding the same pattern of inhibition we found a variety of patterns of inhibition. This indicated that there were many cross-specific idiotypic determinants among the LBMP. Based on the pattern of inhibition using 10 IdX systems, 10 corresponding IdX determinants were identified, designated A to J (Table IV). As may be seen some of the inhibitions were judged to be only partial by the low titer of inhibition shown by the LBMP for a given IdX-HA system.

With three antisera, designated 10789, 10898, 10909, more than one IdX system was constructed. The anti-E109 antiserum 10898 identified two different cross-specific determinants D and G. These two specificities were distinguished by the A4 which inhibited G but not the D system and the strong inhibition of A51 in the D system and its relative inability to inhibit the G system. Two different IdX specificities E and F were identified by the anti-T803 antiserum

TABLE IV

Shared Idiotypic Determinants (IdX) on Levan-Binding Myeloma Proteins

Antiserum	Immunizing myeloma protein	SRBC coated with:	IdX system	HI titer (log 2) Myeloma proteins (1 mg/ml) or ascites inhibitors											
				U 10	A 48	A 51	A 4	W 3082	U 61	M 702	E 109	T 803	A 47	Am1	A J606
10789	W3082	A4	A	0	0	6	9	8	7	6	6	8	0	0	0
10789	W3082	T803	B	0	0	0	0	9	8	0	0	8	0	0	0
10789	W3082	Am1	C	0	0	10	5	8	8	>12	6	8	0	8	0
10898	E109	Am1	D	0	0	>12	0	5	7	3	5	4	0	8	2
10909	T803	E109	E	0	0	0	3	10	10	0	11	11	0	2	5
10909	T803	Am1	F	0	0	0	0	8	6	0	0	7	0	8	2
10898	E109	A4	G	0	0	0	10	>12	>12	>12	9	>12	0	6	4
10878	Am1	A4	H	0	0	>12	6	0	5	0	0	0	0	6	0
10559	A48	A47	I	0	7	9	2	6	7	>12	4	6	7	7	2
10548	A47	A48	J	0	4	0	0	6	0	0	0	0	6	6	0

Alternate systems (antiserum to: SRBC coated with): (A) A4:W3082, A4:U61, (B) W3082:U61, T803:W3082, T803:U61; (C) E109:W3082; (D) Am1:T803; (E) U61:W3082.

A51 and M702 preparations were ascites containing at least 1 mg/ml of myeloma protein.

10909. No LBMP was inhibitory in an HI-IdX system that was not directly agglutinated in the corresponding HA system (Table II).

Most of the IdX systems were highly sensitive to inhibition and the myeloma protein inhibitors could inhibit completely at concentrations of 1 μg/ml or less. One system J was relatively insensitive.

Idiotypic Individual Determinants of Anti-Aerobacter and Anti-Inulin Antibodies. The HI system was used to determine if the IdI identified by specifically absorbed antisera described on Table III were shared by any of the 13 LBMP used in this study (Table V). For this purpose each of the specific anti-IdI antisera was reacted to the homologous protein-coated SRBC and then each of the 13 levan proteins were used as inhibitors of this system. No shared IdI was found and each of the 10 LBMP that were used to prepare antisera were shown to have idiotypic individual determinants designated IdI-1 through IdI-10.

Levan IdX and Levan IdI not on 106 Nonlevan-Binding Myeloma Proteins. 106 nonlevan-binding myeloma proteins of various classes were tested for the levan IdI and IdX determinants described in this paper (Table VI). 68 IgA, 2 IgM, 11 IgG1, 9 IgG2a, 13 IgG2b, and 3 IgG3 myeloma proteins were included. Only one myeloma protein (IgA, ABPC28) was found to have a shared determinant (IdI-3) with a LBMP (A47). No other IdI or IdX determinants were found among the 106 nonlevan-binding myeloma proteins.

Inhibition with β2→1 Fructosan Trisaccharide. We attempted to determine the relationship of the IdI and IdX determinants to the fructosan-binding site. To do this, cells coated with LBMP were equilibrated with varying concentrations of fructosan trisaccharide (the highest concentration was 0.028 M) and to these anti-idiotypic antibody was added (Tables VII and VIII). Six IdI systems were not inhibited by 0.028 M trisaccharide: IdI 2, 3, 7, 8, and 9 (Table VII) and IdX I and J and weakly B (Table VII). Inhibition by the highest concentrations of inhibitor was obtained with the IdI-6 and IdX-F systems. Inhibition with lower concentrations of hapten was observed with all of the other IdI and IdX systems indicating that the idiotypic determinants in question were close to if not actually part of the antigen-binding site.

The two IdI systems 2 and 7 (individual specificities of U10 and A48 proteins) and the IdX systems I and J (which identified cross specific determinants on A48 and on several inulin-binding proteins [see Table IV], were not inhibited by β2→1 fructosan trisaccharide. A48 and U10 proteins do not bind β2→1 fructosans and hence it would not be expected that they would be inhibited by the β2→1-linked trisaccharide.

Discussion

In this study we have examined V-region-associated antigenic determinants of 13 LBMP which comprise the largest related group of antigen-binding myeloma proteins thus far described. The LBMP in the BALB/c mouse were divided into two groups: the anti-inulins (11 proteins) and the nonanti-inulin (2 proteins) or the anti-*A. levanicum* levans. The first group is known to bind β2→1-linked fructosans (11) and the second group does not bind β2→1-linked fructosans but is

TABLE V

Individual Antigenic Specificities of Anti-Aerobacter, and Anti-Inulin Antibodies

Antiserum prepared to:	Absorbant	SRBC coated with:	Anti-Aerobacter			Anti-inulin and Aerobacter (myeloma proteins [1 mg/ml] or ascites inhibitors)										IdI determinant
			U 10	A 48	A 51	A 4	W 3082	U 61	M 702	E 109	T 803	A 45	A 47	Am 1	J 606	
U10	A4	U10	9	0	0	0	0	0	0	0	0	0	0	0	0	7
A48	W3082	A48	0	9	0	0	0	0	0	0	0	0	0	0	0	2
A4	U61	A4	0	0	0	9	0	0	0	0	0	0	0	0	0	6
W3082	U61	W3082	0	0	0	0	8	0	0	0	0	0	0	0	0	5
U61	W3082	U61	0	0	0	0	0	7	0	0	0	0	0	0	0	4
E109	T803	E109	0	0	0	0	0	0	0	8	0	0	0	0	0	8
T803	E109	T803	0	0	0	0	0	0	0	0	11	0	0	0	0	10
A47	U61	A47	0	0	0	0	0	0	0	0	0	0	4	0	0	3
Am1	T803	Am1	0	0	0	0	0	0	0	0	0	0	0	10	0	9
J606	None	J606	0	0	0	0	0	0	0	0	0	0	0	0	6	1

A51 and M702 preparations were ascites containing at least 1 mg/ml of myeloma protein.

TABLE VI

Myeloma Proteins that do not Bind Levan Tested for Lev IdI and Lev IdX Determinants

Antiserum	Antigen	IdI determinants	IgA 68	IgM 2	IgG1 11	IgG2a 9	IgG2b 13	IgG3 3
Anti-J606	J606	1	0	0	0	0	0	0
Anti-A48	A48	2	0	0	0	0	0	0
Anti-A47	A47	3	1*	0	0	0	0	0
Anti-U61	U61	4	0	0	0	0	0	0
Anti-W3082	W3082	5	0	0	0	0	0	0
Anti-A4	A4	6	0	0	0	0	0	0
Anti-U10	U10	7	0	0	0	0	0	0
Anti-E109	E109	8	0	0	0	0	0	0
Anti-Aml	Aml	9	0	0	0	0	0	0
Anti-T803	T803	10	0	0	0	0	0	0
		IdX determinants						
Anti-A4	U61	A	0	0	0	0	0	0
Anti-T803	W3082	B	0	0	0	0	0	0
Anti-E109	W3082	C	0	0	0	0	0	0
Anti-E109	Aml	D	0	0	0	0	0	0
Anti-T803	E109	E	0	0	0	0	0	0
Anti-T803	Aml	F	0	0	0	0	0	0
Anti-E109	A4	G	0	0	0	0	0	0
Anti-Aml	A4	H	0	0	0	0	0	0
Anti-A48	A47	I	0	0	0	0	0	0
Anti-A47	A48	J	0	0	0	0	0	0

* Levan IdI-3 found on AbPc 28 IgA myeloma protein.

hypothesized to bind fructosans with $\beta 2 \rightarrow 6$ linkages. The latter specificity is as yet unproven.

The division of the levan-binding myelomas into two groups is further supported by a limited amount of structural data on partial amino acid sequences of the V_{kappa} and V_{HIII} subgroups of five of the proteins. In the mouse there are at least 25 subclasses of kappa and one of lambda based on the amino terminal sequence to Cys23 (16, 26, 27). V_H sequences have recently been divided into four large groups and within each of these there is evidence to suggest still further subdivisions (28). Functionally related proteins with hapten-binding activities of $\alpha 1 \rightarrow 3$ dextran (14, 15) $\alpha 1 \rightarrow 6$ dextran (14), and β6D galactan (17) all fall into the same V_{kappa} and V_{HIII} subclasses based upon partial amino terminal sequences through the Cys 23 peptide for $\beta 1 \rightarrow 6$D galactans and the first Wu-Kabat hypervariable region for the $\alpha 1 \rightarrow 3$ dextrans. The myeloma proteins W3082, J606 (27, 28), A4, and Aml (unpublished data of Vrana and Rudikoff) fall into the same V_{kappa} and V_{HIII} subclasses while the noninulin *Aerobacter* levan-binding proteins (28) W5476 (P. Barstad, personal communication), and U10 (S. Rudikoff, unpublished data) fall into different V_{kappa} and V_{HIII} subclasses. Thus, the antilevans are divided into two different hapten-binding groups comprising different V_L and V_H pairs that make up the binding sites.

A striking finding in the present experiments was the large number of different

TABLE VII

Inhibition of IdI Systems with B2 → 1 Fructosan Trisaccharide (FTS)

Antiserum to myeloma protein		Dilution (reciprocal)	IdI system	FTS giving complete inhibition
				mol
9302	U10	800	7	NI
10559	A48	100,000	2	NI
10878	Am1	50,000	9	NI
10898	E109	500	8	NI
10548	A47	50,000	3	NI
10544	A4	12,000	6	0.0035
10570	U61	12,000	4	0.00087
10789	W3082	50,000	5	0.00175
10909	T803	12,000	10	0.00175
6470	J606	100	1	NI

NI = No inhibition at 0.028 M.

TABLE VIII

Inhibition of IdX Systems with B2 → 1 Fructosan Trisaccharide (FTS)

Antiserum to myeloma protein		SRBC coupled with:	Antiserum dilution	IdX system	FTS giving complete inhibition
					mol
10544	A4	U61	400	A	0.00175
10909	T803	W3082	800	B	0.028
10898	E109	W3082	500	C	0.00087
10898	E109	Am1	100	D	0.00087
10909	T803	E109	400	E	0.00043
10909	T803	Am1	100	F	0.00175
10898	E109	A4	500	G	0.00087
10878	Am1	A4	100	H	0.00043
10559	A48	A47	800	I	NI
10548	A47	A48	100	J	NI

idiotypic determinants among the 11 inulin-binding myeloma proteins Table IX. These proteins as mentioned are probably coded by V_L and V_H structural genes of the same subgroup. Each of the proteins thus far tested in this group was found to have its own specific IdI determinant and further each protein had two to nine cross-specific determinants. These multiple antigenic differences reflected by the presence of IdI determinants and dissimilarities in distribution of IdX determinants appear to be due to a structural variation in different and discontinuous portions of the V_{kappa} and V_H polypeptide chains. Some changes are related to the antigen binding site region as demonstrated by the ability of the fructosan hapten to inhibit the interaction of anti-idiotype antibody from binding the idiotypic determinant, while others not inhibited by hapten were outside the sites. Structural studies on the antigen-binding sites of the MOPC603 (29) and New protein (30) have revealed that the binding site region is a continuous surface formed by five or six loops or bends of beta-pleated sheets. These particular segments of the V_L and V_H regions correspond to the Wu-Kabat hypervariable

TABLE IX

Cross-Specific (IdX) System

Myeloma protein	IdI	Hapten inhibition	IdX determinants									
			A	B	C	D	E	F	G	H	I	J
			+	+	+	+	+	+	+	+	–	–
U10	7		–	–	–	–	–	–	–	–	–	–
A48	2		–	–	–	–	–	–	–	–	+	+
A47	3		–	–	–	–	–	–	–	–	+	+
J606	1		–	–	–	±	+	±	+	–	±	–
U61	4		+	+	+	+	+	+	+	+	+	–
W3082	5		+	+	+	+	+	+	+	–	+	+
T803	10		+	+	+	+	+	+	+	–	+	–
E109	8		+	–	+	+	+	–	+	–	+	+
Am1	9		–	–	+	+	±	+	+	+	+	+
A4	6		+	–	+	–	±	–	+	+	±	–
A51	—		+	–	+	+	–	–	–	+	+	–
M702	—		+	–	+	±	–	–	+	+	+	+

region (31). Variations in different loops are probably responsible for the multiple antigenic differences which would account for the observation that many of the IdX determinants appear to vary independently of each other rather than coordinately. For example, IdX determinants A and D may occur in the same protein while in others only A or D are present and in still other proteins neither of the two determinants was seen.

The most intriguing aspect of the idiotypes of the LBMP aside from their multiplicity was the fact that most of them involved structures near the binding site without apparently disturbing the ability to bind the antigen. Regardless of what the biological mechanism of variation is, it apparently induces sequence changes close to but not directly involved in antigen binding. The structural variations responsible for the antigenic differences might be regarded as permissive changes not affecting the function of the molecules. It should be noted that this includes not only the binding of the hapten but also includes interactions of the myeloma proteins with the whole carrier polysaccharide.

The genetic basis for such diversity among functionally related proteins is not resolved. It could be due to a cluster of closely related structural genes that have duplicated and accumulated mutations but which have not yet diverged by losing their ability to form molecules that bind $\beta 2 \rightarrow 1$ linked fructosans. Alternatively, it could be argued the differences would have resulted from mutations that have developed in somatic cells in single V_{kappa} and V_H structural genes. The two mechanisms, multiple germ line genes and somatic mutations, are not mutually exclusive and it is possible that the multiplicity of idiotypes in the LBMP is due to a combination of both mechanisms.

It is of interest to note that the noninulin-binding protein A48 shared two IdX determinants with proteins in the inulin-binding group. These determinants were not inhibited by hapten. This similarity among proteins of different subclasses may be due to a structural relationship between V_{kappa} or V_H genes.

Possibly in the mouse one of the genes evolved from the other, by duplication. Alternatively, the antigenic similarity may simply be due to chance. Other possibilities are that A48 may be completely different from any of the other proteins by having different V_{kappa} or V_H subunits or may have evolved from an unusual pair of V_L and V_H units one of which is from the type involved in B2→1 fructosan-binding proteins while the other is in the B2→6 fructosan-binding group.

Three other relevant systems of antigen-binding myeloma proteins have been studied antigenically and structurally in the BALB/c mouse: the α1→3 dextrans (12, 13, 15), β1→6D galactans (17), and the T15-S63 group of phosphorylcholine-binding proteins (7). In all three of these systems the specific binding function is governed by specific pairs of V_L and V_H genes. IdI and IdX determinants have been found in the α1→3 dextran system and we have found IdI (17) and IdX (unpublished observations) in the β1→6D galactan system. The T15-S63 system has proven to be unusual, inasmuch as all proteins in this group have the same IdI determinant which also happens to be a genetic antigen (8). If a somatic mutational process were in fact responsible for the variations it would be expected that all V-region genes would be subject to change, which apparently is not the case with the T15-S63 phosphorylcholine-binding proteins. This result suggests that variations are determined at the germ line gene level and that multiplicity of idiotypes reflects multiple genes. Very recently however some evidence has been presented that there are a variety of normal phosphorylcholine-binding proteins in the mouse (32). The selection of a predominant type in both antibody and myeloma populations may be the result of factors that select these clones preferentially.

Summary

13 levan-binding myeloma proteins (LBMP) of BALB/c origin were classified into two groups with different binding specificities; one group of 11 proteins bound β2→1 fructosans, a second group of two proteins bound fructosans probably of β2→6 linkage. Anti-idiotypic sera prepared to 10 of the proteins in the appropriate strains of mice identified numerous idiotypic determinants. Each protein used for immunization had its own unique individual idiotypic specificities (IdI) and in addition most of the proteins carried two–nine cross-specific or shared idiotypes (IdX) that were found only among LBMP, and not found in 106 non-LBMP. Most of the IdX determinants and only four of the IdI determinants of the β2→1 fructosan binding group were located in the antigen-binding site. The multiplicity of antigenic differences in this functionally related group of immunoglobulins reveals an unexpected degree of heterogeneity in V-regions that appears to be unrelated to binding.

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