

IDIOTYPES OF INULIN-BINDING MYELOMA PROTEINS LOCALIZED TO VARIABLE REGION LIGHT AND HEAVY CHAINS: GENETIC SIGNIFICANCE

By R. LIEBERMAN, M. VRANA, W. HUMPHREY, JR., C. C. CHIEN, AND M. POTTER

(From the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, and the Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014)

Idiotypes are antigenic determinants on variable (V)¹ regions of antibodies. Two types of idiotypes have been described. Individual specificities or idiotypes (IdI) unique to a specific antibody (1-5); cross-specific idiotypes (IdX) shared among immunoglobulins that are usually functionally related (4-10).

IdI and IdX determinants have been found on human (1-4, 8, 11), rabbit (12-17), and mouse antibodies (5-7, 9-10, 18-28). Antigenic specificities that determine IdI and IdX have been localized to V region light chain (V_L), V region heavy chain (V_H), or to both chains (2, 8, 29-38). An idiotypic region can be localized to or near a complementary region when anti-idiotypic antibody is blocked by hapten.

Many IdX have been shown to be genetically controlled (20-28). In the mouse genes controlling α 1-3 dextran (24), phosphorylcholine (23), phenylazoarsonate (22), and inulin (28), cross-specific idiotypes were shown to be linked to the allotype (C_H).

In BALB/c mice a series of 11 myeloma proteins that bind inulin, and bacterial levan have been described (10, 28). These proteins all have hapten-binding specificity for a series of β 2,1-linked fructan oligosaccharides. Each of the proteins in this group has individual antigenic (idiotypic) specificities (IdI) as well as one or more shared idiotypes (IdX). IdI and IdX antisera can be prepared in the homologous species, and hence are used in detecting polymorphic differences between strains.

Genetic analysis has shown that the cross-reacting hapten-inhibitable idiotypes on the inulin-binding myeloma proteins (InuBMP) are linked to allotype (28), which suggests that these idiotypes may be useful as markers for genes coding for the V_H region. However, their use as such is limited until the light chains are shown not to contribute to the expression of the idiotypes. If the idiotypic region reflects conformational contribution of both V_L and V_H , then it reflects structures controlled by two different unlinked genes and such genetic studies are precluded unless one uses strains which have similar V_L repertoires for inulin. In the present study, recombinants of different InuBMP were prepared to determine if the idiotypes associated with InuBMP could be localized.

Materials and Methods

Myeloma Proteins. The BALB/c IgA InuBMP used in this study have been previously described and include EPC109 (E109), Am1 (A1), ABPC4 (A4), TEPC803 (T803), W3082, UPC61

¹ *Abbreviations used in this paper:* HA, hemagglutination; HI, hemagglutination inhibition; IdI, individual idiotypes; IdX, cross-specific idiotypic; InuBMP, inulin-binding myeloma proteins; V, variable; V_H , variable region heavy chain; V_L , variable region light chain.

(U61), ABPC47N (A47N), and a new protein TEPC957 (T957) (10). All of these proteins agglutinate both inulin (β 2,1 fructan) and bacterial levan (β 2,1-2,6 fructan) coated SRBC. The InuBMP were purified on affinity columns with inulin-coupled Sepharose 4B (39). Another protein, XRPC24 (X24), used in these studies which binds galactan and not inulin was purified on a bovine serum albumin galactoside column (40).

Anti-Idiotype Antisera. Production of anti-idiotypic antisera has been described (10). A/He mice were immunized with each of the InuBMP and the antisera were made specific for inulin idiotypes by absorption with a BALB/c IgA non-InuBMP. Anti-IdI antisera were made specific by absorbing anti-idiotypic antisera with the cross-reactive InuBMP (10).

Assay to Determine Specificity of Anti-Idiotypic Antibody. Antisera were tested in the hemagglutination (HA) and hemagglutination inhibition (HI) systems for idiotypic antibodies as previously described (10). SRBC coated with a specific InuBMP by the chromic chloride method (10) were tested with anti-idiotypic antiserum and the HA titer was determined for each of the InuBMP. The antiserum was tested with each of the InuBMP SRBC with which it cross-reacted; the InuBMP were then used as inhibitors of this system and the HI titer was determined.

Titration of Inulin and Levan Antibodies. Inulin and levan antibody titers were determined by HA with SRBC coated with *O*-stearoyl inulin or levan (28). Levan was obtained from culture supernates of *Aerobacter laevanicum* ATCC 15552 (28).

Separation and Reconstitution of H and L Chains of Myeloma Proteins. The method for separating L and H chains has been described (41), and consisted of dialyzing the purified protein against 0.15 M Tris-HCl-0.15 M NaCl-2 mM Na₂ EDTA, reduction with 10 mM dithiothreitol, and alkalination with 20 mM iodoacetamide. The chains were separated on a G100-6M urea Sephadex column. Purity of H and L chain preparations was checked by polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate and contamination was found to be <2%. The H and L chain was recombined as described by Manjula et al. (42). Recombinants from three protein pairs were made: E109-U61, A4-T957, and E109-X24 and put on a Sephadex G200 column to isolate the recombinant monomers (H2L2 mol wt 180,000). Monomers of recombinants of E109H + U61L, E109L + U61H, A4H + T957L, A4L + T957H, E109H + X24L, and E109L + X24H were obtained with a yield of 25-90% of the total protein applied to the column. Dr. D. J. Streetkirk of the National Cancer Institute, National Institutes of Health kindly determined by fluorescence titration, that the binding sites of all the monomers were functional except for E109-X24 which did not bind inulin or galactan.

Results

Inulin and Levan Activity of Recombinants of L and H Chains of U61, E109, A4, and T957 InuBMP. Monomers of recombinant molecules of U61L + E109H and U61H + E109L were tested for HA of SRBC coated with inulin and bacterial levan (Table I). Both recombinants agglutinated inulin- and levan-coated SRBC with titers similar to the U61 and E109 7S molecules. No agglutination was obtained with U61 or E109 L and H chains alone.

Similarly, monomers of recombinants of A4L + T957H and A4H + T957L agglutinated inulin- and levan-coated RBC while, A4H or A4L alone showed no agglutination. When recombinants were made between E109 and X24, the recombinant molecules did not agglutinate inulin- and levan-coated SRBC.

Localization of Individual Idiotypes of U61, E109, A4, and T957 to Specific InuBMP Immunoglobulin Chains. Antisera specific for only the InuBMP used in its preparation identify individual antigenic specificities (IdI) (Table II). Monomers of recombinant molecules of L and H chains from four different InuBMP (U61, E109, A4, and T957) were used as inhibitors of anti-IdI sera each reacting with SRBC coated with the homologous protein. Recombinant molecules made with homologous chains (e.g., E109L-E109H) retained their IdI (Figs. 1-4). Dissociated L and H chains did not inhibit IdI antisera. IdI antisera for E109, U61, T957, and A4 identified antigenic determinants which were

TABLE I
Inulin and Levan HA Titers of InuBMP Recombinants and Individual L and H Chains

Myeloma protein (1 mg/ml)	Inulin SRBC	Levan SRBC	Log 2 HA titer	
U61	15	16		
E109	12	10		
U61L + E109H	10	9		
U61H + E109L	10	8		
U61L	0	0		
U61H	0	0		
E109L	0	0		
E109H	0	0		
E109H + X24L	0	0		
E109L + X24H	0	0		
T957	7	10		
A4	6	12		
A4L + T957H	4	10		
A4H + T957L	4	10		
A4L	0	0		
A4H	0	0		

Recombinants are monomeric (H2L2 mol wt 180,000); U61, E109, T957, and A4 are InuBMP; X24 binds galactan but not inulin or levan.

TABLE II
Specificity of Anti-InuIdI Sera

Antiserum	Immuno- gen	Absorbent	Antiserum dilution	Myeloma protein SRBC	InuBMP							
					A4	W3082	T803	U61	E109	A47N	Am1	T957
<i>HI titer (log 2)</i>												
11142	E109	T803	3,000	E109	0	0	0	0	<u>11</u>	0	0	0
10570	U61	T803, W3082	200	U61	0	0	0	<u>7</u>	0	0	0	0
10544	A4	U61, W3082	100	A4	<u>9</u>	0	0	0	0	0	0	0
11442	T957	W3082	3,000	T957	0	0	0	0	0	0	0	<u>11</u>

Underlined numbers indicate titers of InuIdI.

expressed only when the L chains of these four respective proteins were present (Figs. 1-4) since the anti-IdI sera for each of these proteins could only be inhibited by molecules containing the appropriate L chain.

In the case of A4 IdI some A4H was also required for its expression (Fig. 4). The IdI of U61, E109, and T957 are not inhibitable by the hapten fructan trisaccharide while the A4 IdI is associated with the antibody site and is hapten inhibitable.

Localization of Cross-Specific Idiotypes (IdXB System). In addition to IdI, InuBMP share idiotypes (IdX) with other InuBMP. IdX determinants can also be shared by normal inulin antibodies (28). One IdX, IdXB, specificity has been previously described (10, 28) and was found on U61, W3082, and T803 but not on E109, A4, A47N, Am1, and T957 InuBMP. Antisera used to identify InuIdXB are shown in Table III. Recombinant molecules U61L + E109H and U61H + E109L were used to inhibit the anti-InuIdXB system. Specifically anti-

Ig CHAIN LOCATION OF E109 Inu Idi

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor		HI Titer (Log 2)						
			H	L	0	2	4	6	8	10	12
Anti-E109 Abs. W3082	E109	NI	E109	E109	████████████████████						
			E109	U61							
			U61	E109	████████████████████						
			-	E109							
			E109	-							
			E109	X24							
			X24	E109							

FIG. 1. Localization of E109 Idi to E109 L chain; also requires an H chain of an InuBMP to be expressed. Recombinant molecules of L and H chains of E109, U61 (InuBMP), and X24, a noninulin-binding myeloma protein, were tested as inhibitors of specific anti-E109 Idi serum reacted with E109-coated SRBC. E109 Idi is not in the antibody-combining site and is not inhibitable (NI) by fructan trisaccharide. W3082 is an InuBMP.

Ig CHAIN LOCATION OF U61 Inu Idi

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor		HI Titer (Log 2)						
			H	L	0	2	4	6	8	10	12
Anti-U61 Abs. T803+W3082	U61	NI	U61	U61	████████████████████						
			E109	U61	████████████████████						
			U61	E109							
			-	U61							
			U61	-							

FIG. 2. Localization of U61 Idi to U61 L chain; also requires an H chain of an InuBMP to be expressed. Recombinant molecules of L and H chains of E109 and U61 (InuBMP) were tested as inhibitors of specific anti-U61 Idi serum reacted to U61-coated SRBC. U61 Idi is not in the antibody-combining site and is not inhibitable (NI) by fructan trisaccharide. T803 and W3082 are InuBMP.

T803 (serum 10914) absorbed with E109 tested with W3082-coated SRBC was inhibited by U61 but not E109, and was also inhibited by U61L + E109H but not by U61H + E109L or by E109L and H chains alone (Fig. 5). Thus the shared IdXB appears to require the U61 L chain and an InuBMP H chain.

Localization of A4 IdXA. InuIdXA antiserum identifies a determinant shared by many InuBMP including A4, W3082, T803, U61, and E109 but not on

Ig CHAIN LOCATION OF T957 Inu IdI

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor		HI Titer (Log 2)						
			H	L	0	2	4	6	8	10	12
Anti-T957 Abs. W3082+A4	T957	NI	T957	T957	████████████████████						
			A4	A4	█						
			T957	A4	█						
			A4	T957	████████████████████						
			A4	-	█						
			-	A4	█						
			W3082	W3082	█						

FIG. 3. Localization of T957 IdI to T957 L chain; also requires an H chain of an InuBMP to be expressed. Recombinant molecules of L and H chains of T957 and A4 InuBMP were tested as inhibitors of specific anti-T957 serum reacted with T957-coated SRBC. T957 IdI is not in the antibody-combining site and is not inhibitable (NI) by fructan trisaccharide. W3082 is an InuBMP.

Ig CHAIN LOCATION OF A4 Inu IdI

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor		HI Titer (Log 2)						
			H	L	0	2	4	6	8	10	12
Anti-A4 abs. U61 + W3082	A4	0.0035	A4	A4	████████████████████						
			T957	T957	█						
			T957	A4	████████████████████						
			A4	T957	█						
			A4	-	█						
			-	A4	█						
			U61	U61	█						
W3082	W3082	█									

FIG. 4. Localization of A4 IdI primarily to A4 L chain; also requires an H chain of an InuBMP to be expressed. Recombinant molecules of L and H chains of A4 and T957 InuBMP were tested as inhibitors of specific anti-A4 serum reacted with A4-coated SRBC. A4 IdI is associated with the antibody site and is hapten (fructan trisaccharide) inhibitable. W3082 and U61 are InuBMP.

TABLE III
Specificity of Anti-InuIdXB Serum

Antiserum	Immunogen	Antiserum dilution	Myeloma proteins on SRBC	Myeloma proteins (1 mg/ml)							
				U61	E109	A4	W3082	T803	A47N	Am1	T957
				<i>Log₂ HI titer</i>							
10914 Abs E109	T803	4,000	W3082	10	0	0	>8	7	0	0	0
10570 " "	U61	4,000	W3082	8	0	0	>8	>8	0	0	0
10789 " "	W3082	3,000	U61	10	0	0	>8	>8	0	0	0
10909 " "	T803	1,000	T803	9	0	0	>8	>8	0	0	0

InuIdXB is a cross-specific idiotype designated B found on U61, W3082, and T803 myeloma proteins (10).

Ig CHAIN LOCATION OF U61 Inu IdXB

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor		HI Titer (Log 2)						
			H	L	0	2	4	6	8	10	12
Anti-T803 Abs. E109	W3082	0.00087	U61	U61							
			E109	E109							
			W3082	W3082							
			U61	E109							
			E109	U61							
			E109	-							
			U61	-							
			-	E109							
			-	U61							
			X24	E109							
E109	X24										

FIG. 5. Localization of IdXB to U61 L chain; also requires an H chain of an InuBMP to be expressed. IdXB is shared by U61, T803, and W3082 InuBMP and is inhibitable by the hapten, fructan trisaccharide. Recombinant molecules of L and H chains of U61 and E109 were tested as inhibitors of a specific anti-IdXB serum reacted to W3082-coated SRBC.

T957, Am1, or A47N (10, 28). Antisera identifying the InuIdXA system are shown on Table IV. In addition antisera identifying another specificity (not previously described) designated InuIdXA' showed the same pattern of cross-reactions as InuIdXA except that it also cross-reacted with A47N (Table IV). Recombinant molecules A4H + T957L and A4L + T957H were used as inhibitors of the anti-IdXA and anti-IdXA'. Anti-E109 serum absorbed with T957 and tested with A4-coated SRBC was inhibited by A4 but not T957, and was also inhibited by A4H + T957L but not by A4L + T957H nor A4L or A4H chains alone (Fig. 6). The IdXA determinant was associated with the A4H chain but required an InuBMP L chain.

TABLE IV
Specificity of Anti InuIdXA Serum

Antiserum	Immuno- gen	Antiserum dilution	SRBC coated with:	InuIdX identified	A4	T957	W3082	T803	U61	E109	A47N	Am1
11142 Abs T957	E109	5,000	A4	A'	<u>12</u>	<u>0</u>	>8	8	8	>8	10	0
11142 " "	E109	5,000	A47N	A'	<u>9</u>	<u>0</u>	>8	8	8	>8	10	0
11142 " "	E109	5,000	U61	A'	<u>7</u>	<u>0</u>	>8	8	8	>8	10	0
10789 " "	W3082	100	A4	A	<u>8</u>	<u>0</u>	>8	8	8	4	0	0
11171 " "	A47N	400	A4	A'	<u>7</u>	<u>0</u>	>8	8	8	>8	8	0
10544 " "	A4	400	U61	A	<u>9</u>	<u>0</u>	>8	8	8	>8	0	<u>0</u>
10544 " "	A4	1,000	A47N	A	<u>13</u>	<u>0</u>	>8	8	8	>8	0	<u>0</u>

InuIdXA is an inulin cross-specific idio type found on A4, W3082, T803, U61, and E109. A new InuIdXA-like cross idio type (A') is similar to InuIdX except that it is also present on A47N.

Ig CHAIN LOCATION OF A4 Inu IdXA

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor		HI Titer (Log 2)						
			H	L	0	κ	2	4	6	8	10
Anti-E109 Abs. T957	A4	0.0087	A4	A4	████████████████████						
			T957	T957							
			A4	T957	████████████████████						
			T957	A4							
			A4	-							
			-	A4							
			X24	E109							
			E109	X24							

FIG. 6. Localization of IdXA to A4 H chain; also requires an L chain of an InuBMP to be expressed. IdXA is found on A4, W3082, T803, U61, E109, and A47N InuBMP. Recombinant molecules of L and H chains of A4 and T957 were tested as inhibitors of a specific anti-IdXA serum reacted to E109-coated SRBC.

Discussion

The topography of six idiotypes associated with InuBMP were studied. The U61, E109, T957, and A4-IdI and IdXB of U61 were determined by the V_L , but only when the V_L was bound to a V_H domain from another InuBMP. The IdXA on A4 was determined by the V_H , but only when the V_H was bound to the appropriate V_L domain. Apparently a specific V_HV_L pair is needed for the expression of these idiotypes because the dissociated V_L and V_H failed to exhibit the idiotypes. Though five of these idiotypes are determined by the L chain one cannot discern whether a part of the idio type is contributed by the H chain or if the idiotypes are exclusively on the L chain. In regard to the latter possibility the H chain may be needed to stabilize the L chain in its native

conformation such that the L chain idiotype can be expressed. Whatever the role of the H chain is in idiotype expression, it is likely that the V_H from an InuBMP is necessary since the H chain from a galactan-binding myeloma protein (X24) combined with E109L chain didn't result in expression of any E109 idiotypes.

There have been many idiotypes linked to allotype reported in the mouse, including the IdXB idiotype associated with U61L discussed in this paper (22-24, 28). It is often assumed that these idiotypes are markers for genes coding for the V_H region since there is linkage of V_H to C_H (22-28, 43). The contribution of the L chain to expression of these idiotypes must be understood before these markers can be used in genetic studies. For example in previous genetic studies (28) it was presumed that InuIdXB was a V_H marker since it appeared to be associated with the BALB/c C_H allotype. In these studies backcross progeny of BALB/c (IdXB⁺) × C57BL (IdXB⁻)F₁ × C57BL showed that the 50% of the progeny which carried the BALB/c allotype were IdXB⁺ while the 50% lacking the BALB/c allotype were IdXB⁻. However, linkage of IdXB to allotype is difficult to understand since the findings in this paper show that the InuIdXB idiotype is determined by L chain differences questions the former assumption that InuIdXB is a V_H marker, but shows instead that it is a marker for a specific V_H - V_L pair which depends on the structure of V_L for its expression. Thus the backcross data may indicate: (a) that there is no polymorphism of the L chain and the V_L of BALB/c and C57BL are the same but require the appropriate V_H (BALB/c) to be expressed? or (b) that there is indeed polymorphism of the L chains but selection among the L chains of C57BL for the appropriate L chain to pair with the V_H of BALB/c is regulated by the H chain of BALB/c?

Congenetic strain data (28) permits further analysis of idiotype genetics. CB20 presumably has the L chains of BALB/c and the H chain of C57BL and is IdXB⁻, indicating that it has the wrong V_H for the V_H - V_L pair that determines IdXB. BAB₁₄ on the other hand is identical to CB20 except that at the 14th generation when a separate line was established a crossover occurred in the V_H region so that V_L and V_H markers are of the BALB/c type and the C_H is of the C57BL type. This strain is IdXB⁺, showing expression of the appropriate V_L - V_H pairs.

The more provocative finding is in the BC-8 congenic strain which presumably has the C57BL L chain and BALB/c H chain. These mice are IdXB⁺, which suggests: (a) that the L chain of C57BL and BALB/c are the same and either one uses the V_H of BALB/c to express IdXB or (b) that there is regulation of the C57BL L chain by the H chain of BALB/c which selects among the L chains, for the appropriate one, permitting expression of IdXB.

IdXB can be used as both a H and L chain marker as long as proper strain combinations are used. The idiotype is potentially useful as a L chain marker so long as both parental strains have the same or permissive V_H genes. Specifically, the V_H in BALB/c and RIII appear to be very similar because IdXA and IdXG are present in both while the IdXB is lacking in RIII (28).

Conversely, to study the genetic differences of a V_H , it must first be shown that the strains compared have similar V_L . Idiotypes linked to allotypes should

not be considered V_H markers until it is demonstrated that the L chains in the strains involved are not polymorphic.

Summary

Idiotypes of inulin-binding myeloma proteins (InuBMP) were determined primarily by variable region light chains (V_L) or by variable region heavy chains (V_H) but needed both chains to be expressed. Recombinant molecules were used to show that individual idiotypes (IdI) of U61, E109, T957, and A4 InuBMP and cross-specific idiotypes (IdXB) of U61 were primarily determined by V_L while cross-specific idiomorph (IdXA) of A4 was determined mainly by V_H . The assignment of genes controlling idiotypes to V_H based on allotype linkage (e.g., IdXB) is dubious until the role of the L chain in determining that idiomorph is assessed. IdXB has been shown to be a V_L - V_H marker which presumably is controlled by two unlinked genes. However IdXB can be used as a L chain marker in combinations of strains differing in their L chain genes but having the same permissive H chain genes. Conversely IdXB can be used as a H chain marker in strains having the same permissive L chain genes but differing in their H chain genes.

Received for publication 30 June 1977.

References

1. Kunkel, H. G., M. Mannik, and R. C. Williams. 1963. Individual antigenic specificity of isolated antibodies. *Science (Wash. D. C.)*. 140:1218.
2. Grey, H. M., M. Mannik, and H. G. Kunkel. 1965. Individual antigenic specificity of myeloma proteins. Characteristics and localization to subunits. *J. Exp. Med.* 121:561.
3. Kunkel, H. G. 1970. Individual antigenic specificity, cross specificity and diversity of human antibodies. *Fed. Proc.* 29:55.
4. Williams, R. C., H. G. Kunkel, and J. D. Capra. 1968. Antigenic specificities related to cold agglutinin activity of gamma M. globulins. *Science (Wash. D. C.)*. 161:379.
5. Potter, M., and R. Lieberman. 1970. Common individual antigenic determinants in five of eight BALB/c IgA myeloma proteins that bind phosphoryl choline. *J. Exp. Med.* 132:737.
6. Eichmann, K. 1972. Idiotypic identity of antibodies to streptococcal carbohydrate in inbred mice. *Eur. J. Immunol.* 2:301.
7. Carson, D., and M. Weigert. 1973. Immunochemical analysis of the cross reacting idiotypes of mouse myeloma proteins with anti-dextran activity and normal anti-dextran antibody. *Proc. Natl. Acad. Sci. U. S. A.* 70:235.
8. Capra, J. D., and J. M. Kehoe. 1974. Structure of antibodies with shared idiomorph: the complete sequence of the heavy chain variable regions of two immunoglobulin M anti-gamma globulin. *Proc. Natl. Acad. Sci. U. S. A.* 71:4032.
9. Pawlak, L. L., and A. Nisonoff. 1973. Distribution of cross-reactive idiotypic specificity in inbred strains of mice. *J. Exp. Med.* 137:855.
10. Lieberman, R., M. Potter, W. Humphrey, Jr., E. B. Mushinski, and M. Vrana. 1975. Multiple individual cross-specific idiotypes on 13 levan-binding myeloma proteins of BALB/c mice. *J. Exp. Med.* 142:106.
11. Childs, R., and T. Feizi. 1975. Cross idiotypic specificity among heavy chains of microglobulins with blood group I and i specificities. *Nature (Lond.)*. 255:562.

12. Oudin, J., and M. Michel. 1969. Idiotype of rabbit antibodies. I. Comparison of idiotype of antibodies against *Salmonella typhi* with that of antibodies against other bacteria in the same rabbits, or of antibodies against *Salmonella typhi* in various rabbits. *J. Exp. Med.* 130:595.
13. Brown, D. G., and R. M. Krause. 1968. Individual antigenic specificity of antibodies to streptococcal carbohydrates. *J. Exp. Med.* 128:968.
14. Eichmann, K., and T. J. Kindt. 1971. The inheritance of individual antigenic specificities of rabbit antibodies to streptococcal carbohydrates. *J. Exp. Med.* 134:532.
15. Winfield, J. B., J. N. Pincus, and R. G. Mage. 1972. Persistence and characterization of idiotypes in pedigreed rabbits producing antibodies of restricted heterogeneity to pneumococcal polysaccharides. *J. Immunol.* 108:1278.
16. Oudin, J. 1975. Idiotype of antibodies. In *The Antigens*. M. Sela, editor, Academic Press, Inc., New York. 2:278.
17. Klapper, D. G., and T. J. Kindt. 1974. Idiotypic cross reactions among antistreptococcal antibodies in an inbred rabbit population. *Scand. J. Immunol.* 3:483.
18. Brant, B. W., and A. Nisonoff. 1970. Quantitative investigation of idiotypic antibodies. IV. Inhibition by specific haptens of the reaction of antihapten antibody with its idiotypic antibody. *J. Exp. Med.* 132:951.
19. Potter, M., E. B. Mushinski and C. P. J. Gludemans. 1972. Antigen binding IgA myeloma proteins in mice: specificities to antigen β -D 1 \rightarrow 6 linked galactose side chains and a protein in wheat. *J. Immunol.* 108:295.
20. Kuettner, M. G., A. L. Wang, and A. Nisonoff. 1972. Quantitative investigations of idiotypic antibodies. VI. Idiotypic specificity as a potential genetic marker for the variable regions of mouse immunoglobulin polypeptide chains. *J. Exp. Med.* 135:579.
21. Sher, A. and M. Cohn. 1972. Inheritance of an idio type associated with the immune response of inbred mice to phosphorylcholine. *Eur. J. Immunol.* 2:319.
22. Pawlak, L. L., E. B. Mushinski, A. Nisonoff, and M. Potter. 1973. Evidence for the linkage of the IgC_H locus to a gene controlling the idiotypic specificity of anti-*p*-azophenylarsonate antibodies in strain A mice. *J. Exp. Med.* 137:22.
23. Lieberman, R., M. Potter, E. B. Mushinski, W. Humphrey, Jr., and S. Rudikoff. 1974. Genetics of a new IgV_H (T15 Idiotype) marker in the mouse regulating natural antibody to phosphorylcholine. *J. Exp. Med.* 139:983.
24. Blomberg, B., W. R. Geckler, and M. Weigert. 1973. The immune response to dextran in mice. In *Specific Receptors of Antibodies, Antigens and Cells*. Third International Convocation on Immunology, Buffalo, New York. S Karger AG, Basel, Switzerland. 285.
25. Imanishi, T., and O. Mäkelä. 1974. Inheritance of antibody specificity. I. Anti (4-hydroxy-3-nitro-phenyl)-acetyl of mouse primary response. *J. Exp. Med.* 140:1498.
26. Imanishi, T., and O. Mäkelä. 1975. Inheritance of antibody specificity. II. Anti-(4-hydroxy-5-bromo-3-nitrophenyl)-acetyl in the mouse. *J. Exp. Med.* 141:840.
27. Eichmann, K. 1975. Genetic control of antibody specificity in the mouse. *Immunogenetics* 2:491.
28. Lieberman, R., M. Potter, W. Humphrey, Jr., and C. C. Chien. 1976. Idiotypes of inulin-binding antibodies and myeloma proteins controlled by genes linked to the allotype locus of the mouse. *J. Immunol.* 117:2105.
29. Grey, H. M., and M. Mannik. 1965. Specificity of recombinations of H and L chains from human γ G-myeloma proteins. *J. Exp. Med.* 122:619.
30. Roholt, O. A., G. Rodzinski, and D. Pressman. 1965. Preferential recombination of antibody chains to form effective antibody-combining sites. *J. Exp. Med.* 122:785.
31. Roholt, O. A., G. Rodzinski, and D. Pressman. 1966. Recovery of antibody activity from inactive hybrids of H and L chains. *J. Exp. Med.* 125:191.

32. Bridges, S. H., and J. R. Little, Jr. 1971. Recovery of binding activity in reconstituted mouse myeloma proteins. *Biochemistry* 10:2525.
33. Givol, D., P. H. Strausbach, E. Hurwitz, M. Wilchek, J. Haimovich, and H. N. Eisen. 1971. Affinity labeling and cross-binding of heavy and light chain of a myeloma protein with anti 2,4-dinitrophenyl activity. *Biochemistry*. 10:3461.
34. Wells, J. V., H. H. Fudenberg, and D. Givol. 1973. Localization of idiotypic antigenic determinants in the Fv region of murine myeloma protein MOPC 315. *Proc. Natl. Acad. Sci. U. S. A.* 70:1585.
35. Kunkel, H. G., R. J. Winchester, F. C. Joslin, and J. D. Capra. 1973. Similarities in the light chains of idiotypic specificities. *J. Exp. Med.* 139:128.
36. Ghose, A. C., and F. Karush. 1974. Chain interactions and idiotypic specificities of homogeneous rabbit anti-lactose antibodies. *J. Immunol.* 113:162.
37. Huser, J., J. Haimovich, and J. C. Jaton. 1975. Antigen binding and idiotypic properties of reconstituted immunoglobulin G derived from homogeneous rabbit anti-pneumococcal antibodies. *Eur. J. Immunol.* 5:206.
38. Forre, O., T. E. Michaelson, and J. B. Natwig. 1976. Light chains and recombinant IgG of human IgG anti-D. *Scand. J. Immunol.* 5:155.
39. Vrana, M., J. Tomasic, and C. P. J. Glaudemans. 1976. Purification of homogeneous murine immunoglobulins with anti-fructosan specificity. *J. Immunol.* 116:1662.
40. Jolley, M. E., S. J. Rudikoff, M. Potter, and C. P. J. Glaudemans. 1973. Spectral changes on binding of oligosaccharides to murine IgA myeloma immunoglobulin. *Biochemistry*. 12:3039.
41. Rudikoff, S., and M. Potter. 1973. Variable region sequence of the heavy chain from a phosphocholine binding myeloma protein. *Biochemistry*. 13:4033.
42. Manjula, B. N., C. P. J. Glaudemans, E. B. Mushinski, and M. Potter. 1976. Subunit interactions in mouse myeloma proteins with anti-galactan activity. *Proc. Natl. Acad. Sci. U. S. A.* 73:932.
43. Mage, R. G. 1977. Structure and expression of V_H and C_H allotypic determinants. *Prog. Immunol.* In press.