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

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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 idiotype can be localized to or near a complementary region when anti-idiotype antibody is blocked by hapten.

Many IdX have been shown to be genetically controlled (20–28). In the mouse genes controlling α1–3 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 β2,1-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 idiotype 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), Aml (A1), ABPC4 (A4), TEPC803 (T803), W3082, UPC61

[1] *Abbreviations used in this paper:* HA, hemagglutination; HI, hemagglutination inhibition; IdI, individual idiotypes; IdX, cross-specific idiotype; 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 (β2,1 fructan) and bacterial levan (β2,1-2,6 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-idiotype 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-idiotype antisera with the cross-reactive InuBMP (10).

Assay to Determine Specificity of Anti-Idiotype 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-idiotype 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 Na_2 EDTA, reduction with 10 mM dithiothreitol, and alkalation 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
					HI titer (log 2)							
11142	E109	T803	3,000	E109	0	0	0	0	11	0	0	0
10570	U61	T803, W3082	200	U61	0	0	0	7	0	0	0	0
10544	A4	U61, W3082	100	A4	9	0	0	0	0	0	0	0
11442	T957	W3082	3,000	T957	0	0	0	0	0	0	0	11

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 Idl

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor H	Inhibitor L	HI Titer (Log 2) 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 Idl 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 Idl serum reacted with E109-coated SRBC. E109 Idl 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 Idl

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor H	Inhibitor L	HI Titer (Log 2) 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 Idl 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 Idl serum reacted to U61-coated SRBC. U61 Idl 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 Idl

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor H	Inhibitor L	HI Titer (Log 2) 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 Idl 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 Idl 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 Idl

Antisera	SRBC Coated With	Fructan Trisacch. Inhibition 0.028 M	Inhibitor H	Inhibitor L	HI Titer (Log 2) 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 Idl 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 Idl 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
				Log 2 HI titer							
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).

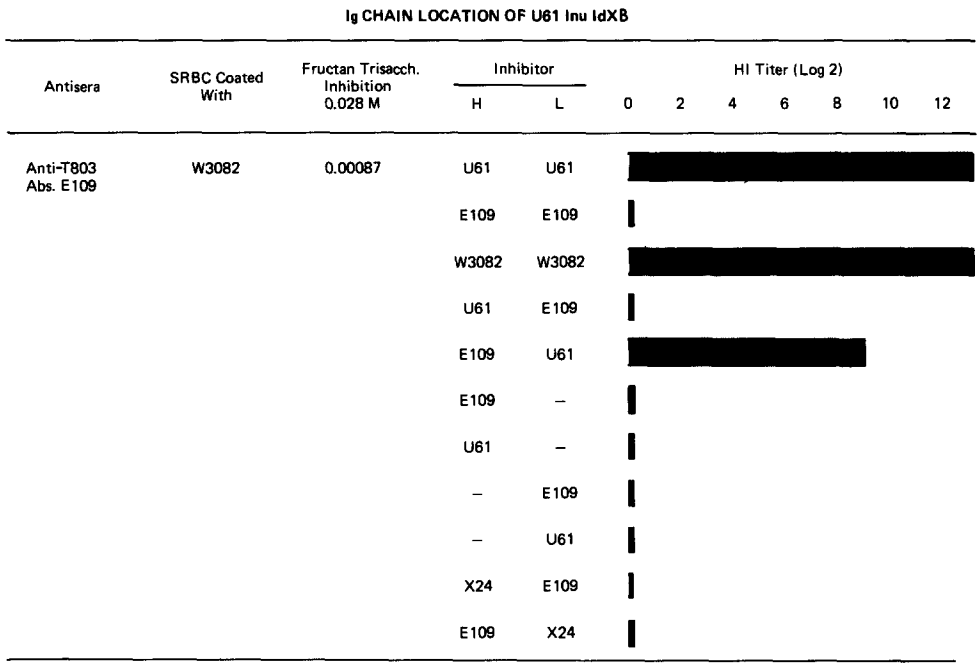

FIG. 5. Localization of IdXB to U61 L chain; also requires an H chain of an InuBMP to be expressed. IdXB is shared by U61, T803, and W3082 InuBMP and is inhibitable by the hapten, fructan trisaccharide. Recombinant molecules of L and H chains of U61 and E109 were tested as inhibitors of a specific anti-IdXB 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'	12	0	>8	8	8	>8	10	0
11142 " "	E109	5,000	A47N	A'	9	0	>8	8	8	>8	10	0
11142 " "	E109	5,000	U61	A'	7	0	>8	8	8	>8	10	0
10789 " "	W3082	100	A4	A	8	0	>8	8	8	4	0	0
11171 " "	A47N	400	A4	A'	7	0	>8	8	8	>8	8	0
10544 " "	A4	400	U61	A	9	0	>8	8	8	>8	0	0
10544 " "	A4	1,000	A47N	A	13	0	>8	8	8	>8	0	0

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

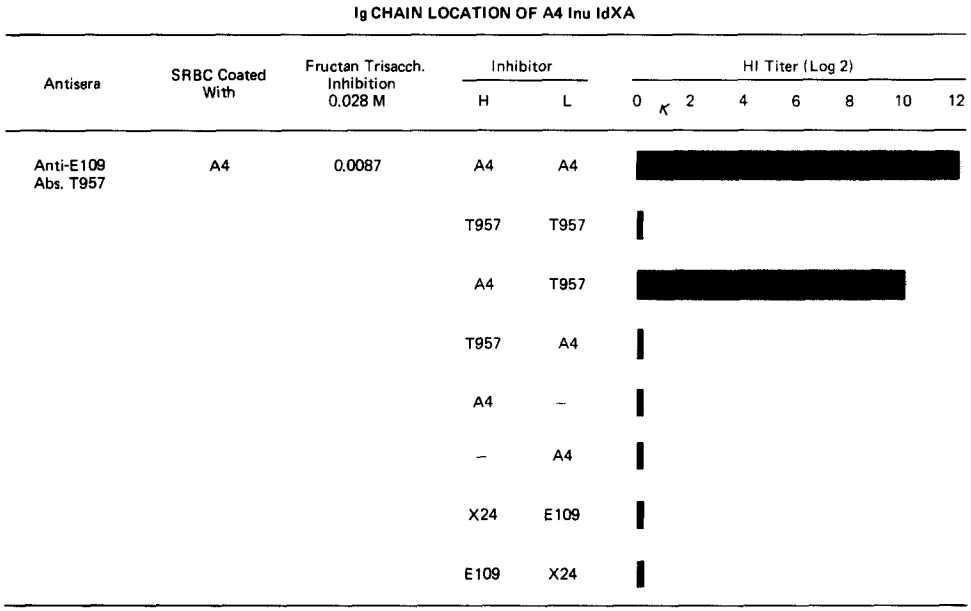

FIG. 6. Localization of IdXA to A4 H chain; also requires an L chain of an InuBMP to be expressed. IdXA is found on A4, W3082, T803, U61, E109, and A47N InuBMP. Recombinant molecules of L and H chains of A4 and T957 were tested as inhibitors of a specific anti-IdXA serum reacted to E109-coated SRBC.

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 idiotype 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 idiotypic 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 ($IdXB^-$)F_1 × 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?

Congenic 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. BAB_{14} 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 idiotype (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 idiotype 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.

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