

Structural Characterization of Mouse Immunoglobulin Allotypic Determinants (Allotopes) Defined by Monoclonal Antibodies

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Abstract. We have generated a new series of monoclonal antibodies recognizing allotypic determinants on mouse IgG₁, IgG_{2a}, and IgG_{2b}. In this communication we describe their reactivity at the molecular level. A number of genetic specificities (as defined by reactivity with sera from inbred strains) were divided into subspecificities (allotopes) by these analyses. With the exception of one allotope located in the hinge region of *Igh-1^b*, all other 23 allotopes examined were preserved upon reduction and alkylation of immunoglobulin antigens. To further analyze the role of immunoglobulin conformation in presenting the allotopes, we assayed their presence on mixed *Igh-1^a/Igh-4^a* heavy chain molecules. The *Igh-1^a* determinants were maintained, but the *Igh-4^a* determinants were lost. Taken together, our results indicate that genetic polymorphisms at the *Igh* loci generate an enormous antigenic complexity, much of which relies on tertiary and quaternary protein structure for expression.

Introduction

As revealed by alloantisera, mouse immunoglobulin heavy chains are highly polymorphic (Herzenberg and Herzenberg 1978, Lieberman 1978). These serologically detected genetic polymorphisms, known as allotypes, are composed of a constellation of individual antigenic determinants or allotopes. The generation of monoclonal allotope-specific antibodies makes it possible to analyze the pre-

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Abbreviations used in this paper: DNP, dinitrophenyl; DTE, dithioerythreitol; Igh, immunoglobulin heavy chain; SDS, sodium dodecyl sulfate.

sentation of these antigenic epitopes at the molecular level. For our initial examinations of the structural basis for the allotypic genetic complexity, we used a small library of monoclonal antibodies to IgG_{2a} allotypes (Oi and Herzenberg 1979). We have since obtained monoclonal antibodies reactive with allotypes present on immunoglobulin heavy chains of the IgG_{2a} (*Igh-1*) IgG_{2b} (*Igh-3*), and IgG₁ (*Igh-4*) (see Green 1979 and the preceding paper by Huang et al. (1983) for immunoglobulin genetic nomenclature). The reactivity of these antibodies with inbred strains of mice is described in the companion paper (Huang et al. 1983). Here, we characterize the topographical relationship of the allotypes on individual immunoglobulin molecules. We also demonstrate that the expression of some of these polymorphic determinants depend on tertiary and quaternary structure.

Materials and Methods

Somatic cell hybridization. The generation of hybridoma antibody producing cell lines has been described (Oi and Herzenberg 1980, Huang et al. 1983). Antibodies IA7 and BG1 were a gift from Drs. M. and G. Bosma (Fox Chase Institute for Cancer Research); rat monoclonal antibodies 2b-180.4 and 2b-168.1 were a gift from Dr. M. Scharff (Albert Einstein College of Medicine). Antibodies are referred to by hybridoma and clone number.

Hybrid cell line 93-1.5 produces antigen-specific Igh-1^a/Igh-4^a mixed molecules and was generated by somatic cell hybridization as follows. Hybridoma 29-B1 synthesizes anti-DNP of the Igh-1^a allotype and hybridoma 27-4.4 synthesizes anti-dansyl antibody of the Igh-4^a allotype. Using standard drug-selection procedures, thymidine kinase deficient and hypoxanthine-guanine phosphoribosyl transferase deficient sublines were isolated from 29-B1 and 27-4.4, respectively. The derivative lines were fused with polyethylene glycol 1500 (BDH Chemicals) and hybrid cells were selected using hypoxanthine-aminopterin-thymidine selection medium (Littlefield 1964). Cells were cloned using the fluorescein-activated cell sorter (Parks et al. 1979) and screened for anti-dansyl activity associated with an Igh-1^a heavy chain by solid-phase radioimmune assay (see below). Such activity would reflect assembly of Igh-4^a anti-dansyl and Igh-1^a anti-DNP heavy chains into a single molecule. Clone 93-1.5 exhibited this reactivity and was subsequently shown to produce both parental heavy chains (see text).

Reduction and alkylation of immunoglobulins. Immunoglobulins at 2 mg/ml in 0.05M Tris-HCl, pH 8.1, 0.15M NaCl containing 10 mM dithioerythritol (DTE) were incubated on ice for 1 h. Reduction was terminated by addition of 100 mM iodoacetamide to alkylate free-sulphydryl groups. In parallel, a portion of the DTE-treated immunoglobulin was incubated with 10 mM dehydroascorbic acid to rapidly reform disulfide bonds. Electrophoresis under non-reducing conditions on sodium dodecyl sulfate (SDS) gels confirmed that reduced and alkylated preparations contained only dissociated molecules, and that reduced and reoxidized immunoglobulins contained intact immunoglobulin molecules. Before using these immunoglobulin preparations in radioimmune assays, DTE, iodoacetamide, and/or dehydroascorbic acid were removed by gel filtration.

Radiolabeling. Immunoglobulins produced by cell lines were biosynthetically labeled overnight in cysteine- and methionine-free medium containing ³⁵S-methionine and ³⁵S-cysteine (150 μCi each/10⁶ cells). Supernates were collected after centrifugation and then filtered through a 0.45 micron filter to remove remaining debris. The supernates were stored at -70 °C until use. Immunoglobulins purified as described in the preceding paper were iodinated by the iodogen method (Fraker and Speck 1978). Membrane immunoglobulins were radioiodinated by the lactoperoxidase method as described previously (Oi et al. 1980). The Non-idet P40 solubilized cell lysates were stored at -70 °C until used.

Immunoprecipitations. All antibodies used for immunoprecipitations were Igh-1^b immunoglobulins. Radiolabeled cell lysates and supernates were incubated with purified monoclonal antibodies (5 μg) for 30 min on ice. Antigen-antibody complexes were collected with anti-Igh-1^b 4.7 antibody (10 μg) coupled to CNBr-activated Sepharose 4B (Pharmacia). The precipitates were washed three times in a high salt

buffer as described previously (Oi et al. 1980). Precipitated material was analyzed by two-dimensional gel electrophoresis as described by Oi and co-workers (1978).

Radioimmune assays. Detection of anti-dansyl and anti-DNP antibodies by solid-phase radioimmune assay was done according to the procedure of Tsu and Herzenberg (1980). Allotope levels were measured by solid-phase competition assay (Huang et al. 1982). The antibody-blocking assay has been described (Oi and Herzenberg 1979). Briefly, antigen is coated onto the wells of the polyvinylchloride plate. Serial dilutions of anti-allotope antibody are allowed to bind for 1 h; then a second, radiolabeled allotope-specific antibody is added. If the epitopes recognized by the two antibodies are near each other or otherwise conformationally linked, the binding of the first antibody will hinder the binding of the second antibody.

Results

Thirty-three monoclonal antibodies were generated that react with allotopes on Igh-1, Igh-3, or Igh-4 molecules. A full description of the characteristics and strain reactivity of these antibodies is included in the preceding paper. To insure that the antibodies reacted with determinants requiring the immunoglobulin polypeptide for presentation, target antigens were extensively digested with pronase. Analysis of these samples in the assay for allotope levels indicated that each of the 24 allotopes examined had been destroyed.

Localization of Igh-1 allotopes on Igh-1^a and Igh-1^b molecules

The initial localization of the allotopes in Igh-1^a and Igh-1^b molecules was accomplished by testing proteolytic fragments of immunoglobulin molecules for reactivity with each monoclonal antibody (Oi and Herzenberg 1979). We have extended this analysis to include the newer Igh-1-specific antibodies using the antibody-blocking assay. Typical blocking patterns observed in these assays, as illustrated by the Igh-1^b-specific antibodies, are shown in Figure 1. Antibodies such as 19.8 and 4.7 (Figure 1a) do not influence each other's binding to antigen. Other antibodies fully inhibit each other's binding (for example, 19.8 and 20.1 in Fig. 1b), presumably reacting with topographically neighboring determinants. Antibodies which partially inhibit the binding of a second antibody were also observed (see 1A7 and 19.8 in Fig. 1c); and finally there are pairs of antibodies which show non-reciprocal effects on one another's binding (Fig. 1d). These analyses show that every Igh-1^b-specific antibody has a unique relationship to the other antibodies (Table 1).

An explanation for both partial and nonreciprocal blocking could be that Igh-1^b molecules are heterogeneous, even in the monoclonally derived myeloma and hybridoma protein preparations used in these analyses. This is not the case. We have affinity-purified serum Igh-1^b molecules from both SJL/J and BAB/14 sera with monoclonal antibody-immunoabsorbant columns and found that more than 99% of all molecules reactive with one Igh-1^b-specific monoclonal antibody are also reactive with other Igh-1^b-specific antibodies (data not shown).

The partial blocking phenomenon seen in Figure 1c (i. e., 1A7 partially blocks 19.8 binding to antigen) is difficult to explain. The co-binding assay indicates that these allotope-specific antibodies (like all others we tested) react with two sites on the Igh-1^b molecule. However, if binding of an antibody to one site on the molecule

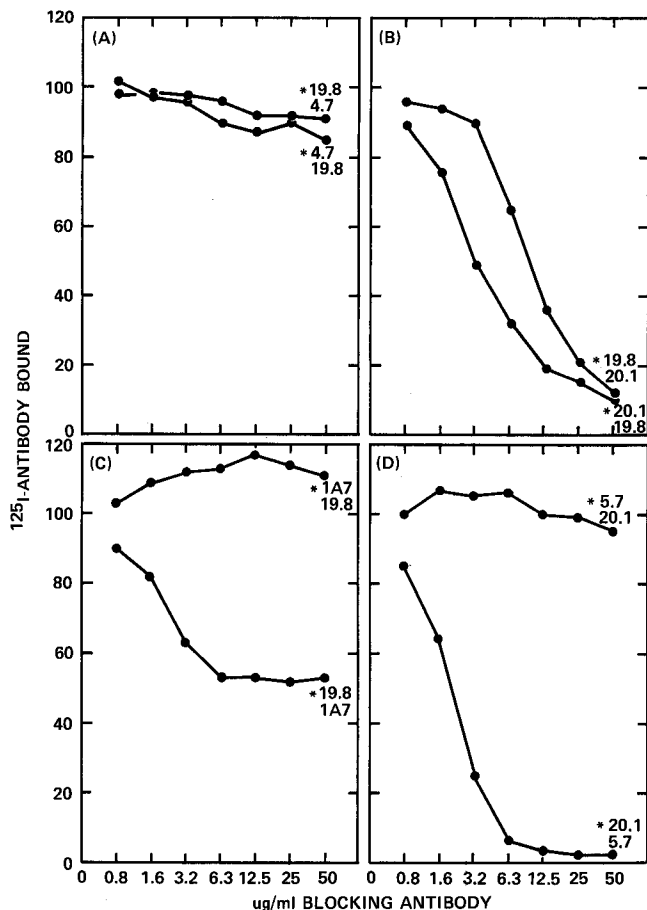


Fig. 1. Hybridoma blocking assays. The topographic relationship of Igh-1b allotypes was examined in the hybridoma-blocking assay of Oi and Herzenberg (1979) as described in *Materials and Methods*. The plate coat was Igh-1b myeloma protein CBPC 101. Serial dilutions of one antibody were added, as shown on the abscissa. Subsequent binding of a second radiolabeled antibody is expressed as the percentage of binding obtained when no blocking antibody was added. The pairs of antibodies examined are indicated next to each curve, with an asterisk marking that which was radiolabeled.

rendered the contralateral site less accessible, a second antibody (reactive with a topographically related determinant) might be blocked for the first site only. This would generate the partial-blocking phenomenon.

The nonreciprocal blocking shown in Figure 1d could be explained in either of two ways. The simplest is a binding-affinity difference between the two antibodies. In such a case, unless the affinity difference is quite large, increasing the amount of the "non-blocking" antibody should result in some inhibition. This was not observed. A second possibility is that the topography of the two allotypes is such that blocking in only one direction is possible. One allotype could be situated on a bulge on the molecule, while the second could be in a nearby hollow or cleft.

Table 1. Cross-blocking of Igh-1^b-specific antibodies

Blocking antibody	4.7	2.9	BG1	3.1	1A7	19.8	20.1	5.7
Radiolabeled antibody								
4.7	+*	p [†]	p	- [‡]	-	-	-	-
2.9	p	+	+	-	p	p	p	p
BG1	-	-	+	-	-	-	-	-
3.1	p	p	p	+	+	-	-	-
1A7	-	-	-	-	+	-	-	-
19.8	-	-	-	-	p	+	+	+
20.1	-	-	-	-	-	+	+	+
5.7	-	-	-	-	-	-	-	+

* +, complete blocking.

† p, partial blocking.

‡ -, no blocking.

Antibody to the first allotope then would block access of the second antibody to the cleft, but the converse would not necessarily be true.

Igh-1^a allotopes were analyzed in similar assays (Table 2). Although each has a unique two-dimensional gel-electrophoresis fingerprint, the three antibodies 9.8, 14.4, and 17.2 show identical blocking patterns, suggesting that they recognize the same allotope. Extensive analyses of inbred (see the preceding paper) and wild mice (Huang et al. 1982) confirm this hypothesis. Antibodies 8.3 and 31.6 fully inhibit each other's binding and presumably react with topographically related determinants. However, while antibody 8.3 partially blocks the binding of antibody 33.3, antibody 31.6 fails to block antibody 33.3. These data indicate that these antibodies are detecting related, but separate epitopes. The remaining seven antibodies have unique antibody-blocking patterns. The blocking analyses combined with our previous studies of immunoglobulin proteolytic fragments (Oi and Herzenberg 1979) indicate that the Igh-1^a allotopes detected here, with the possible exception of allotope 34.6, are all located in CH2.

Igh-3 allotopes

Cross-blocking analyses were also performed with antibodies generated against Igh-3^a molecules (Table 3). Each antibody, with the possible exception of antibody 35, shows a unique pattern, indicating that they detect different epitopes. Two of the antibodies were produced in rats against mouse IgG_{2b}; antibody 168.1 detects an isotypic marker in CH3, while 180.4 detects an allotypic marker in CH2 present on Igh-3 molecules of all allotypes except *f* and *p* (Huang et al. 1983, M. Scharff, personal communication). The blocking relationship of antibodies 36.2 and 168.1 suggests that allotope 36.2 is located in CH3. Because antibody 35.1 itself reacts with two of the Igh-3-specific antibodies, a full analysis with this antibody was not possible. However, the blocking studies in combination with strain and isotype analyses presented in the companion paper (Huang et al.) show it, too, reacts with a distinct determinant. Antibodies 23.1 and 24.1 recognize spatially distinct allotopes on Igh-3^b Fc when examined with the antibody-blocking assay (data not shown).

Table 2. Cross-blocking of Igh-1^a-specific antibodies

Blocking antibodies	30	9*	32	16	31	8	29	33	15	34
Radiolabeled antibody										
30	+ [†]	+	-	-	-	-	-	-	-	-
9	+	+	p [‡]	-	-	-	-	-	-	-
32	+	p	+	-	-	-	-	-	-	-
16	-	p	-	+	-	-	-	-	-	-
31	-	-	-	-	+	+	+	-	-	-
8	-	-	-	-	+	+	+	-	-	-
29	-	-	-	-	-	-	+	-	-	-
33	-	-	-	p	-	p	+	+	p	-
15	-	-	-	-	-	-	+	+	+	-
34	-	-	-	-	-	-	-	-	-	+

Antibodies are designated by hybridoma number (clone number is omitted).

* Identical blocking patterns were obtained with antibodies 9, 14, and 17.

[†] +, complete blocking.

[‡] p, partial blocking.

^{||} -, no blocking.

Table 3. Cross-blocking of Igh-3^a-specific antibodies

Blocking antibodies	180.4	16.3	35.1	168.1	36.2
Radiolabeled antibody					
180.4	+	p	-	-	-
16.3	-	+	ND	p	-
35.1	ND	ND	+	p	-
168.1	-	-	-	+	p
36.2	-	-	-	+	+

ND: Not determined. Antibody 16.3 (Igh-1^b anti-Igh-1^a/Igh-3^a) reacts directly with antibody 35 (Igh-1^d anti-Igh-1^a, Igh-1^b/Igh-3^a, Igh-3^b); antibody 35 also reacts with rat antibody 180.4 (see preceding paper).

Igh-4 allotypes

Table 4 presents blocking data using these Igh-4^a antibodies, demonstrating that each antibody reacts with a distinct determinant. Reactivity patterns with rat and other vertebrate immunoglobulins support this conclusion (Huang et al. 1983, Parsons and Herzenberg 1981). We have described previously that the four Igh-4^b antibodies appear to recognize different allotypes (Herzenberg et al. 1981). To date, all of the Igh-4^b-specific antibodies bind with relatively lower affinity than other allotope-specific antibodies, limiting our analysis of the anti-Igh-4^b molecules.

Allotopes on reduced and alkylated immunoglobulins

We reduced and alkylated Igh-1^b, Igh-1^a, and Igh-4^a proteins to study the presentation of allotypes on these conformationally altered molecules. SDS gel electrophoresis confirmed that the treated molecules were no longer in native

Table 4. Cross-blocking of Igh-4^a-specific antibodies

Blocking antibody	18.1	10.9	28.1
Radiolabeled antibody			
18.1	+	+	-
10.9	+	+	p
28.1	-	+	+

configuration, but existed as a mixture of 85K (HL), 55K (H), and 27K (L) molecules (data not shown). We then tested these proteins and proteins which were reduced and then oxidized to reform the disulfide bonds for allotope expression. All of the allotopes examined on Igh-1^a (9.8, 15.3, 14.4, 16.3, and 8.3) and Igh-4^a (18.1, 10.9, 28.1) molecules were preserved. Of the Igh-1^b allotopes, all were expressed on the reduced molecules, except the allotope recognized by the Igh-1^b-specific 4.7 antibody. This allotope has been localized previously to the hinge region of the Igh-1^b molecules (Oi and Herzenberg 1979).

Expression of allotopes on hybrid Igh-1^a/Igh-4^a molecules

A somatic cell hybrid cell line producing both an Igh-1^a DNP-specific antibody and an Igh-4^a dansyl-specific antibody was generated. Hybrid cell line 93-1.5 secretes a mixture of immunoglobulins representing random assembly of chains into tetramers of H2L2 molecules composed of the heavy and light chains from both parental cell lines. Since the heavy and light chains of the parental cell lines were clearly distinguishable by two-dimensional gel-electrophoresis analysis, we were able to determine which allotopes were expressed on the mixed H2L2 molecules produced by this cell line.

³⁵S-methionine- and ³⁵S-cysteine-labeled supernates of the two parental cell lines and the hybrid cell line were immunoprecipitated with a variety of allotope-specific antibodies. Precipitates were analyzed by two-dimensional gel electrophoresis and autoradiography. Autoradiograms were examined for the presence of the heavy chain that is not recognized by the monoclonal anti-allotope used for immunoprecipitation. Its presence indicates that the monoclonal anti-allotope recognizes the hybrid Igh-1^a/Igh-4^a molecule. If only the homologous heavy chain is seen, this indicates that the allotope-specific antibody only recognizes molecules with two homologous heavy chains, and not the mixed Igh-1^a/Igh-4^a molecule.

Figure 2a and b shows the heavy chains of secreted immunoglobulin from the parental cell lines 29-B1 and 27-4.4 precipitated by the anti-Igh-1^a 8.3 and anti-Igh-4^a 10.9, respectively. 29-B1 has a slightly larger and more basic heavy chain. The 27-4.4 heavy chain is a series of spots with identical charges, but different sizes. No bands were seen when the parental supernates were precipitated with the wrong allotope-specific antibody. When biosynthetically labeled 93-1.5 supernate was precipitated with Igh-1^a-specific antibodies, the 29-B1 (Igh-1^a) and 27-4.4 (Igh-4^a) heavy chains were co-precipitated (Fig. 2d). In addition, both parental light chains were precipitated (not shown). Similar findings were obtained with all Igh-1^a-specific antibodies tested (9.8, 8.3, 17.2, 16.3, 15.3, and 14.4). When Igh-4^a-specific

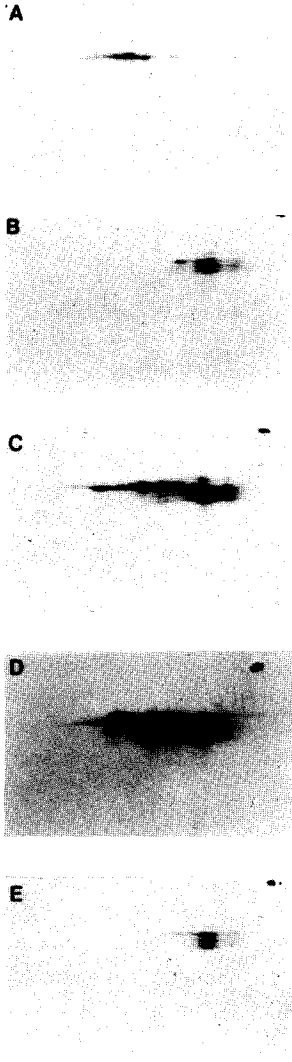


Fig. 2a-e. Allotypes presented on secreted mixed Igh-1^a/Igh-4^a molecules. Cells were metabolically labeled with ³⁵S and the supernatant incubated with allotope-specific antibodies. The antigen-antibody complexes were collected with second-step antibodies coupled to Sepharose and analyzed by two-dimensional electrophoresis. The first (*horizontal*) dimension was non-equilibrium pH gradient gel electrophoresis, and the second (*vertical*) dimension was electrophoresis through a SDS-polyacrylamide gel (10%). The supernatants and allotope-specific antibodies are as follows: **a** parental Igh-1^a line 29-B1/anti-Igh-1^a; **b** parental Igh-4^a line 27-4.4/anti-Igh-4^a; **c** hybrid Igh-1^a/Igh-4^a cell line 93-1.5/formalin-fixed *S. aureus*; **d** hybrid Igh-1^a/Igh-4^a cell line 93-1.5/anti-Igh-1^a; **e** hybrid Igh-1^a/Igh-4^a cell line 93-1.5/anti-Igh-4^a.

antibodies 18.1 and 10.9 were used to precipitate the labeled immunoglobulins, only the 27-4.4 heavy chain was precipitated (Fig. 2e). Both light chains were again seen. Thus, while the Igh-1^a-specific antibodies react with the mixed molecules, the Igh-4^a-specific antibodies do not.

The same experiment was performed with radioiodinated cell membrane extracts to determine (1) if mixed membrane immunoglobulin molecules were formed and (2) if the same allotypes were presented in similar fashion on membrane molecules. Figure 3a and b shows the radioiodinated heavy chains of secreted and membrane immunoglobulin of 29-B1 and 27-4.4, respectively. As previously reported, the membrane Igh-1^a is both larger and more acidic than the secreted form

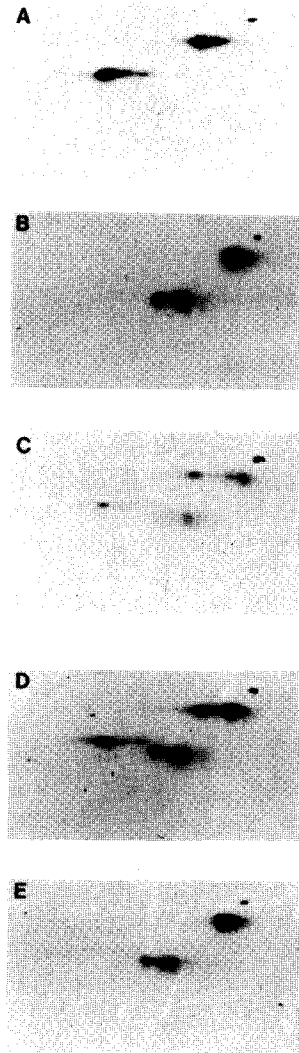


Fig. 3a-e. Allotopes presented on membrane mixed Igh-1^a/Igh-4^a molecules. Cells were surface labeled with ¹²⁵I by the lactoperoxidase method and lysed. The lysates were incubated with allotope-specific antibodies. The antigen-antibody complexes were collected and analyzed by two-dimensional electrophoresis, as in Figure 2. The lysates and allotope-specific antibodies are as follows: **a** parental Igh-1^a line 29-B1/anti-Igh-1^a; **b** parental Igh-4^a line 27-4.4/anti-Igh-4^a; **c** hybrid Igh-1^a/Igh-4^a line 93-1.5/formalin-fixed *S. aureus*; **d** hybrid Igh-1^a/Igh-4^a line 93-1.5/anti-Igh-1^a; **e** hybrid Igh-1^a/Igh-4^a line 93-1.5/anti-Igh-4^a.

of the molecule (Oi et al. 1980). The same relationship holds for the Igh-4^a membrane molecule of 27-4.4. The source of the radiolabeled secreted form may be molecules in the process of secretion or secreted chains pairing with membrane form chains (Goding 1982). Analyses of the hybrid cell line show that mixed Igh-1^a/Igh-4^a molecules are formed and inserted into the membrane. Furthermore, as on secreted immunoglobulins, Igh-1^a allotopes are preserved in the mixed membrane molecules (Fig. 3d), while Igh-4^a determinants are lost (Fig. 3e).

Discussion

Recent determinations of the protein or cDNA sequences of *Igh-1^a*, *Igh-1^b*, *Igh-3^a*, and *Igh-3^b* confirm extensive differences between alleles (Ollo et al. 1981, Dognin et al. 1981, Schrier et al. 1981, Ollo and Rougeon 1982). These sequences allow us to compare our serologic data, which is based on the topographical structure of the molecule, with primary sequence information. First, we can examine these sequences for regions in CH2 which are shared by *Igh-1^a* and *Igh-3^a*, but which are divergent in *Igh-1^b*. These might represent areas responsible for the 16.3 *Igh-1^a*/*Igh-3^a* determinant, which is known to be in CH2 and present on both IgG_{2a} and IgG_{2b} (Oi and Herzenberg 1979). Such regions occur at the beginning of CH2 and near the end of CH2. Other data, using Lepore-type hybrids of *Igh-1^a* and *Igh-3^a* with chimeric heavy chains (Oi et al., manuscript in preparation) indicate that determinant 9.8, which is closely related to 16.3 in blocking studies, is the most N-terminal of the *Igh-1^a* allotypic determinants (16.3 could not be analyzed in this system since it is on both subclasses). These data suggest the 16.3 allotype is also N terminal and probably occurs near the beginning of CH2.

While eight amino acid differences have been found between *Igh-1^a* and *Igh-1^b* in CH2, only two of these are not shared between *Igh-1^a* and *Igh-3^a* sequences. Since there are eight distinct *Igh-1^a* allotopes not found on *Igh-3*, but only two unique amino acid substitutions, clearly the conformation of the molecule plays an important role in presenting the allotopes. One possibility could be that while different antibodies recognize determinants based on the same amino acid substitution, they could involve different neighboring amino acids. Alternatively, if amino acid residues shared between *Igh-1^a* and *Igh-3^a*, but not *Igh-1^b*, were brought into contact with residues which differed between *Igh-1^a* and *Igh-3^a*, this might provide the specificity needed to restrict the allotopes to the *Igh-1^a* class. It is unlikely that any of the *Igh-1^a* allotopes are made by the combination of allotypic determinants on the two heavy chains, since there is no close contact between CH2 domains (Silverton et al. 1977).

We have found that *Igh-1^a* allotopes are preserved, while *Igh-4^a* allotopes are lost on hybrid *Igh-1^a*/*Igh-4^a* molecules. These molecules, in which primary structure is preserved while quaternary structure is altered, provide an ideal case in which to study the effects of conformation on the presentation of allotopes. We know that the *Igh-1^a* allotopes are located in CH2 and thus might be unlikely to be perturbed by the presence of a "wrong" heavy chain in the immunoglobulin molecules. Unfortunately, we do not know whether the *Igh-4^a* allotopes reside in CH2 or CH3. CH3 domains are more intimately related in the quaternary structure and more differences between IgG₁ and IgG_{2a} are in this domain than in others. It is also interesting that none of the *Igh-4^a* allotopes were lost in *Igh-1^a* or *Igh-4^a* molecules which were mildly reduced and alkylated. Thus, while the *Igh-4^a* allotopes require two IgG₁ heavy chains for presentation, it is not necessary that these chains be linked by disulfide bonds. The one allotope which was extremely sensitive to reduction and alkylation is *Igh-1^b* allotope 4.7. It is noteworthy that this determinant is usually lost during papain digestions and thus is located in or near the hinge region.

These data emphasize the role of conformation in presenting immunological determinants. Conformational considerations are very important when using monoclonal antibodies, where if the antigenic epitope is disturbed, reactivity is lost. However, polyclonal sera may be prone to this problem as well. For example, if a serum reacts with epitopes clustered in a region where tertiary and quaternary structure are important, a similar result could be obtained. Such a result might be expected for immunoglobulin subclass-specific sera since, of all domains, CH3 domains differ most. Experiments using antibodies as probes in situations where conformation may be altered must be interpreted carefully.

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