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LOCALIZATION OF MURINE Igh-1^a ALLOTYPIC DETERMINANTS BY USING A PANEL OF MOUSE MYELOMA VARIANT IMMUNOGLOBULINS[1]

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A number of monoclonal antibodies are available that are reactive with distinct mouse immunoglobulin allotypic determinants. By determining which ones are present on a panel of hybrid IgG2b-IgG2a immunoglobulins, we have localized some of the allotypic determinants present on the IgG2a heavy chain of the "a" allotype (Igh-1^a proteins). In particular, one group of determinants—Ig(1a)9.8 (20.6B8), 17.2 (20.19.2), and 14.4 (21.74.4)—has been placed in the C_H2 domain. A second group—Ig(1a)8.3 (20.8.3), 21.2 (20.11.2), and 15.3 (21.66.3)—is located in a segment spanning the C terminal 8 residues of the C_H2 domain and the complete C_H3 domain.

Most immunoglobulin isotypes from various inbred mouse strains are distinguishable by serologic determinants called allotype markers (1, 2). These allotypic markers have been used to examine various aspects of the biology of immunoglobulin genes, such as genetic linkage, allelic exclusion, and allotype suppression. Recently, a number of monoclonal antibodies reactive with distinct mouse immunoglobulin allotypic determinants (allotypes) were prepared (3). These monoclonal antibodies defined families of unique molecular determinants that alloantisera had previously defined as a single serologic determinant (4, 5). Furthermore, unique combinations of these allotypes were found on immunoglobulins of wild mice (6), thus describing new immunoglobulin alleles not represented in inbred mouse populations. To enhance the usefulness of these monoclonal reagents, we have partially localized some of the allotypic determinants present on the IgG2a heavy chain of the "a" allotype (Igh-1^a proteins) by determining which ones are present on a panel of hybrid IgG2b-IgG2a immunoglobulins.

MATERIALS AND METHODS

Monoclonal antibodies were prepared as described (7, 8). Briefly, SJL mice (Igh^b) were immunized with BALB/c (Igh^a) antibody to *Bordetella pertussis* complexed to killed *B. pertussis* organisms. Immune spleen cells were fused with the NS-1 variant of MOPC-21 with the use of 50% polyethylene glycol. Supernatants were screened for antibody activity by using a solid-phase radioimmunoassay (4). Cells from positive wells were cloned with the fluorescence-activated cell sorter (9). Hybridoma cell lines could be maintained as subcutaneous or ascites tumors in syngeneic mice (SJL × BAB/14 (Igh^b × Igh^b)). (BAB/14 is congenic with BALB/c at the immunoglobulin heavy chain gene complex.)

The MPC-11 (IgG2b,k) cell line has yielded several variant cell lines producing mutant immunoglobulin heavy chain molecules. The different mutant proteins produced by various variant cell lines have been grouped on the basis of charge, peptide maps, and chain assembly characteristics (10). Representatives of each group have been studied further by amino acid sequence analysis and nucleic acid studies. These groups are presented in schematic form in Figure 1.

The ability of monoclonal antibodies to bind to a variant protein was measured by a radioimmunoassay, or in some cases, by inhibition of hemagglutination. The radioimmunoassay was described previously (4). Briefly, microtiter wells were coated nonspecifically with monoclonal anti-Igh-1^a allotype antibody. Competition for binding sites is between radiolabeled IgG2a protein and unlabeled variant proteins. The two IgG2a proteins that were radiolabeled were 29-B.1 and GPC-8. Both of these proteins are of the BALB/c or "a" allotype; however, they behaved differently. For example, monoclonal Ig(1a)15.3 bound poorly, if at all, with the Fc fragment of GPC-8, but it bound well with that of 29-B.1, which was prepared by an identical procedure. The hybridoma protein 29-B.1 was derived recently from the BALB/cN/Hz subline, and GPC-8 was derived from a myeloma tumor of the BALB/c subline maintained in Australia, which has been separated from the BALB/cN subline since 1952 (Dr. Noel L. Warner, personal communication). The GPC-8 tumor is no longer viable; consequently, the putative structural differences between these two γ2a heavy chains cannot be further explored. The test results with 29-B.1 protein were consistent with findings obtained with a second type of assay (hemagglutination). Because 29-B.1 is more likely to be an appropriate representative of BALB/c IgG2a proteins, we based our interpretations only on the data derived with it as the competing antigen.

An assay based on inhibition of hemagglutination (11) was also utilized to screen two of the antiallotypic antibodies. Red cells were coated with a monoclonal BALB/c IgG2a anti-sheep red blood cell antibody (12). Such a reagent did not agglutinate unless additionally coated with a monoclonal antiallotype antibody. An appropriate dilution of the monoclonal antiallotype antibody was taken that would reproducibly give agglutination. Inhibition of agglutination was assessed after additions of serial dilutions of variant proteins.

RESULTS

Analysis of papain-digested 29-B.1 showed that the seven monoclonal antiallotype antibodies failed to bind to Fab but that all reacted with the Fc fragment. The use of MPC-11-variant proteins provided further localization. The MPC-11-variant proteins used fell into three groups based on peptide maps, charge, and assembly characteristics. One group was comprised of ICR 9.9.2.1, ICR 11.8, M 224, and M 319.2. Primary structural studies have shown that a representative of this group, ICR 9.9.9.2.1, quite likely has a complete γ2a-constant region (13) (Fig. 1). Nucleic acid studies of ICR 9.9.2.1 and other members of this group are consistent with the conclusion (14). One variant protein, ICR 16, has been shown to have a hybrid γ2b-γ2a heavy chain, with the junction between the C_H1 and the hinge regions (15) (Fig. 1). Thus, its Fc region is entirely γ2a-like, and it is expected to bind with monoclonal antiallotype antibodies exactly as those variant proteins in the first group. A third group of variants consists of ICR 11.19.3, ICR 11.19.2, ICR 4.68.66, and ICR 4.68.110. A representative of this group, ICR 11.19.3, has a γ2b-γ2a hybrid heavy chain (16), as depicted in Figure 1. If a monoclonal anti-Igh-1^a antibody binds to ICR 11.19.3, we can infer that the recognized determinant lies in the segment of γ2a sequence extending from N-340 to the C-terminus (numbering based on the MPC-11 heavy chain). Monoclonal antibodies that fail to bind to this group of variant proteins but do bind to the

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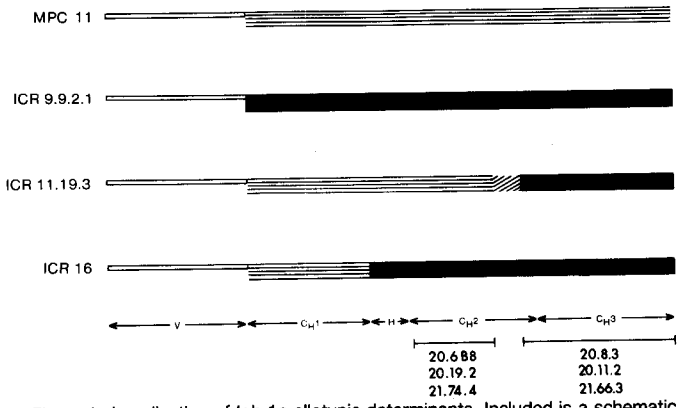

Figure 1. Localization of Igh-1a allotypic determinants. Included is a schematic of the structural organization of MPC-11 of four myeloma heavy chains produced by MPC-11 and three variants derived from it. γ2b-constant region segments are denoted (▭), and γ2a-constant region segments are denoted (▬). The hatched area (▨) in ICR 11.19.3 represents a segment of sequence (N316-339) comprising 24 amino acids and 72 nucleotides that is identical between γ2b and γ2a heavy chains. Papain cleavage occurs between residues N-239 and N-240 for MPC-11, ICR 11.19.3, and ICR 9.9.2.1, and between N240 and N241 for ICR-16. Allotypic determinants are localized as indicated. 20.6B8 = Ig(1a)9.8; 20.19.2 = Ig(1a)17.2; 21.74.4 = Ig(1a)14.4; 20.8.3 = Ig(1a)8.3; 20.11.2 = Ig(1a)21.2; 21.66.3 = Ig(1a)15.3. The numbering of MPC-11 is taken from the complete sequence of this molecule. The variable region includes 121 residues, which places the beginning of the constant region at N-122. The bottom line shows the general organization of the immunoglobulin gene.

other variants probably recognize determinants in the segment commencing at the papain cleavage site—residue N-244-245—(13) and terminating at residue N-315 in the C_H2 domain.

In Table I, we present the binding of seven monoclonal antiallotype antibodies to MPC-11 and MPC-11-variant proteins. Three monoclonals—20.8.3, 20.11.2, and 21.66.3—bind to all the MPC-11-variant proteins, but not to MPC-11 itself, which indicates that the determinants recognized by these antibodies lie in the C-terminal of the C_H2 domain or in the C_H3 domain. Three monoclonals—20.6B8, 20.19.2, and 21.74.4—bind to some MPC-11-variant proteins, but not to those in the third group, indicating that the determinants recognized by these antibodies most probably lie in the C_H2 domain. One monoclonal, 21.48.3, binds to all IgG2a-variant proteins as well as to MPC-11. The extensive segments of sequence identity between γ2b and γ2a heavy chains threading throughout the entire constant region preclude localization of this determinant at this time.

DISCUSSION

Our experiments show that MPC-11-variant immunoglobulins are useful reagents for localizing allotypic markers. These variant proteins have been used previously to determine which parts of the mouse heavy chain react with the Fc receptors on mouse macrophages (17). Although alterations in the molecules could cause a conformational change, it seemed more likely that the intact proteins may maintain normal conformation, thus preserving determinants that require stabilization from adjacent portions of the molecule.

Previous attempts to localize these allotypic determinants recognized by monoclonal antibodies were based on proteolytic cleavage of the target immunoglobulin and were difficult to interpret. Limited proteolytic cleavage of an IgG2a[a] protein by using *Staphylococcus aureus* V8 protease yielded fragments that, although smaller than the native H_2L_2, retained the ability to bind antigen (4). Such an observation implied that these fragments had lost C-terminal segments. That these fragments also maintained the ability to bind monoclonal antiallotypic antibodies led to the tentative conclusion that the determinants analyzed here may not lie in the extreme C-terminal segment of the C_H3 domain. However, because the *S. aureus* cleavage products are neither reproducible nor well defined, efforts to use them for more precise localization have been frustrating. Problems were also encountered when papain-cleaved molecules were used to locate sites of allotypic determinants. Papain cleavage of GPC8 led to destruction of the determinant recognized by monoclonal Ig(1a)15.3, and thus to a tentative localization of the determinant to the hinge region (18). However, papain cleavage of 29-B.1 did not destroy this determinant (18), and results with MPC-11 variants verified that the determinant actually was located between residue N-340 in the C_H2 domain and the C-terminal of the chain. This localization, taken together with serologic findings that showed that the determinant recognized by monoclonal Ig(1a)15.3 was present on secreted IgG2a and absent from membrane IgG2a (18), is compatible with current experiments that indicate that membrane and secreted IgG2a heavy chains, like membrane and secreted IgM heavy chains, differ in their C-termini (19–22). Well-characterized cyanogen bromide fragments of IgG2a heavy chains that maintain the ability to bind to Fc receptors (23) are unfortunately devoid of reactivity with antiallotype sera.

Recently protein (24) and DNA studies (25) have led to a complete description of γ2a-constant regions from two different allotypes. These sequences are quite similar in C_H1 and C_H2 domains (94% and 94% homology, respectively) and differ more extensively in the hinge region and C_H3 domains (71% and 72% homology, respectively). Clearly, these structural differences provide the basis of the serologic determinants we are examining. None of the determinants analyzed in this paper lies in the hinge region because the papain-derived Fc fragment, which contains all of them, commences in the C_H2 domain. The plethora of structural differences in C_H2 and C_H3 domains precludes an

TABLE I

Presence of allotypic determinants on variant Igh-3a/Igh-1a immunoglobulins[a]

	Group 1			Group 2			Group 3
	Ig(1a)8.3 (20.8.3)	Ig(1a)21.2 (20.11.2)	Ig(1a)15.3 (21.66.3)	Ig(1a)9.8 (20.6B8)	Ig(1a)17.2 (20.19.2)	Ig(1a)14.4 (21.74.4)	Ig(1a/3a)16.3 (21.48.3)
ICR 9.9.2.1.	+	+	+	+	+	+	+
ICR 11.8	+	+	+	+	+	+	+
M 224	+	+	+	+	+	+	+
M 319.2	+	+	+	+	+	+	+
ICR 16	+	+	+	+	+	+	+
ICR 11.19.2	+	+	+	−	−	−	+
ICR 11.19.3	+	+	+	−	−	−	+
ICR 4.68.66	+	+	+	−	−	−	+
ICR 4.68.110	+	+	+	−	−	−	+
29-B.1	+	+	+	+	+	+	+
MPC-11	−	−	−	−	−	−	+

[a] Results shown in this table were obtained by using the radioimmunoassay described in the text. Results obtained with antibody Ig(1a)8.3 and Ig(1a)9.8 were confirmed by using the hemaglutination inhibition assay also described in *Materials and Methods.*

exact correlation of amino acid sequence and serologic allotype determinants, although the experiments described here lead to a general localization separating two groups of determinants, as shown in Figure 1. A precise description of a given marker could be made by analyzing a variant γ2a heavy chain that had lost one determinant (e.g., by a rare point mutation) while retaining the rest. Even the partial localization of allotypic determinants facilitates the interpretation of experiments in which antiallotypic antibodies are used. For example, heavy chain class switches in hybridoma cells have been observed and assessed by using these monoclonal antibodies (26–28).

It is of interest that IgG2a immunoglobulins from certain wild mice (6) are identical to one group of MPC-11 variants (16), represented by ICR 11.9.3, that have lost C_H2 allotypic determinants. Because structural analysis of heavy chains from these wild mice has not been carried out, it is not known which sequences replace those carrying these determinants. It is possible that, like ICR 11.19.3, these wild mice heavy chains are hybrid in nature, resulting from a domain transfer event (29, 30), accomplished by crossing over or gene conversion between C_H genes.

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