LOCALIZATION OF MURINE IgH-1\textsuperscript{*} ALLOTYPE DETERMINANTS BY USING A PANEL OF MOUSE MYELOMA VARIANT IMMUNOGLOBULINS\textsuperscript{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\textsuperscript{*} proteins). In particular, one group of determinants-Ig(1a)9.8 (20.866), 17.2 (20.19.2), and 14.4 (21.74.4)-has been placed in the C2,2 domain. A second group-Ig(1a)3.3 (20.8.3), 21.2 (20.11.2), and 15.3 (21.6.8.3)-is located in a segment spanning the C-terminal 8 residues of the C2,2 domain and the complete C3 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 gene, 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 alcautzer and his colleagues 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\textsuperscript{*} 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\textsuperscript{a}) were immunized with BALB/c (Igh\textsuperscript{b}) 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 fluorescein-activated cell sorter (9). Hybridoma cell lines could be maintained as subclones or ascites tumors in syngeneic mice (SJL × BALB/c)F\textsubscript{1} (Igh\textsuperscript{b} × Igh\textsuperscript{a}). (10). Supernatants from these hybridoma cell lines were assayed with 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).

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, M224, and M319.2. Primary structural studies have shown that a representative of this group, ICR 9.9.9.2.1, quite likely has a complete γ\textsubscript{2a}-constant region (13). Thus, its Fc region is entirely γ\textsubscript{2a}-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 γ\textsubscript{2b}-γ\textsubscript{2a} hybrid heavy chain (16), as depicted in Figure 1. If a monoclonal anti-Igh-1\textsuperscript{*} antibody binds to ICR 11.19.3, we can infer that the recognized determinant lies in the segment of γ\textsubscript{2a} 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...
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 C2 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 C2 domain or in the C3 domain. Three monoclonals—20.68B, 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 C2 domain. The monoclonals 21.16.3, bind to all the variant proteins as well as to MPC-11. The extensive segment 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 protein by using Staphylococcus aureus V8 protease yielded fragments that, although smaller than the native IgG2a, 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 lie in the extreme C-terminal segment of the C3 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 Igl(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 C2 domain and the C-terminal of the chain. This localization, taken together with serologic findings that showed that the determinant recognized by monoclonal Igl(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 C1 and C2 domains (94% and 94% homology, respectively) and differ more extensively in the hinge region and C3 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 C2 domain. The plethora of structural differences in C2 and C3 domains precludes an

**TABLE I**

Presence of allotypic determinants on variant IgG-2a/IgG-1a immunoglobulins*

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1a8.3 (20.8.3)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IgG2a12.2 (20.11.2)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IgG1a15.3 (21.66.3)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IgG1a8.8 (20.68B)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IgG1a17.2 (20.19.2)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IgG1a4.4 (21.74.4)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Results shown in this table were obtained by using the radioimmunoassay described in the text. Results obtained with antibody IgG1a8.3 and IgG1a8.8 were confirmed by using the hemagglutination 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 y2a 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 antiallo- type 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 (see) are identical to one group of MFC-11 variants (16), represented by ICR 11.9.3, that have lost C2 allo- type 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 11.9.3, 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 Cv genes.

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REFERENCES


