THE STRUCTURE AND GENETICS OF MOUSE IMMUNOGLOBULIN HEAVY CHAIN CONSTANT REGIONS DEFINED BY MONOCLONAL ANTI-ALLOTYPE ANTIBODIES

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In 1964 we postulated a chromosomal region for immunoglobulin heavy chains based on the finding that newly discovered allotypes of IgG₂a and IgA were closely linked (1). At that same Cold Spring Harbor meeting Henry Kunkel presented similar evidence of linkage for human heavy chain genes (2). Since that time allotypes have been found for 7 of the 8 known heavy chain genes in the mouse. The loci thus defined are now called IgH-1 through IgH-7 for, respectively, \( \gamma_2a \), \( \alpha \), \( \gamma_2h \), \( \gamma_1 \), \( \delta \), \( \mu \), and \( \epsilon \). Only \( \gamma_2h \) has yet to be shown to be polymorphic. Of course, the \( V_H \) genes as well as all the \( C_H \) genes are known to be clustered in one region on chromosome 12 of the mouse (3).

With the advent of hybridoma we have generated a series of monoclonal antibodies which recognize allotypic determinants (allotopes) on the gene products of the IgH-1, -3, and -4 loci (4,5, and Parsons et al., in preparation) (see ref. 6 for nomenclature). In this communication we discuss the use of these antibodies to examine the structure of these polymorphic proteins. Several key findings have emerged. The polymorphisms, as typified by the IgH-1a and IgH-1b proteins, are structurally complex, with allotopes scattered along the molecule. We have found that natural populations of mice provide a reservoir of genetic heterogeneity at the IgH loci as revealed by the relatively high levels of polymorphism in these populations as well as the existence of novel IgH phenotypes, allotypes, and haplotypes. We have also found situations where the structure of one Ig domain affects the presentation of allotypic determinants on another domain. Finally, our recent finding of membrane IgG₂a (7) can be extended to other classes of IgG.

There are extensive polymorphic differences throughout the Fc regions of the IgH-1a and IgH-1b alleles of IgG₂a immunoglobulins. This was determined by mapping the allotypic surface topography of these molecules with monoclonal anti-allotype antibodies (Fig. 1). Five distinct determinants were found on IgH-1a molecules and eight on IgH-1b molecules. The mapping of these determinants was done in two ways. Anti-
Figure 1. Location of allotype determinants on IgG_{2a}.
As described in the text, Igh-la and Igh-lb allotypic determinants were roughly localized to immunoglobulin domains by examining antibody activity with various proteolytic fragments of the antigen. Studies examining the ability of each antibody to hinder the binding of other antibodies to antigen also aided us in clustering the allotopes. Anti-Igh la antibodies 14.4, 17.2, and 9.8 may recognize the same determinant. Antibodies 2.9 and BG1, 3.1 and 1A7 react with distinct, but closely related determinants. While only one heavy chain of each immunoglobulin is depicted, we do not wish to imply that the determinants are expressed on single heavy chains.

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body blocking assays (4) were used to determine whether individual monoclonal antibodies obscure determinants that are recognized by other monoclonal antibodies. In this case masking of determinants can be attributed to steric considerations or induced conformational changes. To roughly localize the regions of the IgG_{2a} molecules which display these allotypic determinants, we examined polypeptide fragments of the Fc regions for binding to the anti-allotype antibodies (4).
While conventional serology indicated a single allotype specificity unique to IgH-1b proteins (8), with monoclonal antibodies we have divided this into 8 distinct allotopes (allotypic determinants). Since almost every independently derived monoclonal antibody defined a new allotope, the number of allotopes must be very large. The extensive differences between the IgH-1a and IgH-1b alleles have been well substantiated by the recent nucleotide and amino acid sequence information (see articles of Bothwell et al., and Strosberg et al., this volume). In fact, these data demonstrate that more structural differences exist between the two IgH-1 allotypic molecules described than between IgH-1 (IgG2a) and IgH-3 (IgG2b) proteins.

The 20 monoclonal antibodies now available (including 2 kindly provided by M. Bosma, I.C.R., Philadelphia) divide the IgH-type strains into 5 pheno groups (Table 1): a,g,h,j; b; c; f; and d,e,n,o. After examining the allotopes of inbred strains, we decided to search in wild mice for further genetic variation at the IgH-loci (9). Sera from 122 wild mice (Mus musculus) were kindly provided by Dr. J. Klein (Max Planck Inst., Tübingen, W. Germany) and Dr. E. K. Wakeland (U. of Florida, Gainesville, Fl).

Most of the wild mice have IgH phenotypes similar to those of inbred strains or heterozygotes thereof. However, we have found new allotypes of the IgH-1 and IgH-4 loci. We have also found mice with unusual combinations of allotopes as well as new haplotypes which could be explained by recombination and/or gene duplication.

Table 1 shows reactivity patterns (Ig phenotypes) of some of these unusual wild mice. All of the mice from Poland that we have tested possess the IgH-a determinants of IgH-a or -d-like strains. In addition they have all eight IgH-1b allotopes, but not the IgH-4b allotope. In this population, the a-like and b-like determinants do not occur separately in individual mice, so it is unlikely that the animals are heterozygous. This would imply duplicated IgH-1 loci. Preliminary progeny testing from one of these Polish mice indicates that the IgH-1a and IgH-1b genes are segregating together, providing further evidence that the two a and b IgH alleles are on the same chromosome. The Polish mice are thus reminiscent of the Kyushu mice reported by Lieberman and Potter (10).

Interestingly, three Egyptian mice were found to possess only 3 of the 8 IgH-1b allotopes. These determinants have been localized to the CH3 domain of the IgH-1b molecule (Fig. 1). Although this variant IgH-1b molecule lacks the CH2 IgH-1b determinants, the molecular weight of its heavy
<table>
<thead>
<tr>
<th>Igh-Loci:</th>
<th>Igh-4</th>
<th>Igh-3</th>
<th>Igh-1</th>
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<tr>
<td>Determinants:</td>
<td>a b a b</td>
<td>a b</td>
<td></td>
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<td>Igh-4</td>
<td>1 1 1 1</td>
<td>1 2 2 2</td>
<td>8 1 9 1 1 1 1 1</td>
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<td>0 1 2 6</td>
<td>3 4 6 4 7 1 5</td>
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<td>Igh-Haplotypes</td>
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<td>f</td>
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</tr>
<tr>
<td>Poland</td>
<td>+ -</td>
<td>+ -</td>
<td>++ - - * +</td>
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*Not tested
chain indicates that no substantial deletion has occurred. We examined this molecule for other allotypic determinants and found that it also bears a determinant common to Ighe-1e and Ighe-3e (16.3). It thus appears that this molecule resulted from an Ighe-1e/Ighe-1b or Ighe-3e/Ighe-1b recombination. These and other interesting Ighe-haplotypes will be the subject of another communication (Huang. et al., in preparation). With mutation creating new alleles as indicated by the loss of allotypes and recombination assorting allotypes to form new allotypes, the Ighe gene complex shows an extensive polymorphism in the natural wild mice populations. It will be interesting to further explore the extent of polymorphism and try to determine what selective advantage, if any, it represents for the species.

Possible Domain Interactions. An interesting interaction between the Fab and Fc domains of individual molecules was discovered during our analysis of allotypic determinants. Individual monoclonal anti-Ighe-4b (IgG6 of allotype) antibodies react with different monoclonal Ighe-4b proteins (presumably derived from the same Ighe-4b gene) in quantitatively distinct patterns. A good example of this kind of reactivity pattern is the binding of anti-Ighe-4b 22.9 to different Ighe-4b proteins (Table 2). MOPC-245T, MOPC-300, and anti-DNP hybridoma 29-B5 react equally well with antibody 22.9. However, anti-dansyl hybridoma 44-26.2 reacts much more strongly. When the Fc fragment of this molecule was prepared, it reacted in a

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**TABLE 2**

**REACTIVITY OF ANTI-Ighe-4b 22.9 WITH VARIOUS Ighe-4b PROTEINS**

<table>
<thead>
<tr>
<th>Protein</th>
<th>50% Inhibition</th>
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<tbody>
<tr>
<td>MOPC-245T</td>
<td>3.0</td>
</tr>
<tr>
<td>MOPC-300</td>
<td>2.5</td>
</tr>
<tr>
<td>29-B5</td>
<td>2.6</td>
</tr>
<tr>
<td>44-26.2</td>
<td>0.45</td>
</tr>
<tr>
<td>44-26.2 Fc</td>
<td>4.3</td>
</tr>
<tr>
<td>44-26.2 Fab</td>
<td>†</td>
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</tbody>
</table>

*pmoles required for 50% inhibition of 22.9 binding to antigen (44-26.2)
†no inhibition at highest concentration tested
fashion similar to the other Igh-4b proteins. Mixtures of 44-26.2 Fab and Fc resemble the Fc rather than the intact molecule, demonstrating that the structural linkage of these two moieties is necessary to influence the expression of the Fc allotypic determinant. These data indicate that the variable region can alter the presentation of distal determinants, located as far away as CH2.

Serological heterogeneity within Fc fragments of different Igh-la proteins has also been observed. In this case, intact molecules of 29-B1, an Igh-la anti-DNP hybridoma protein, and GPC-8, also an Igh-la protein both react with anti-Igh-la 15.3. However, the Fc fragment of GPC-8 reacts poorly with 15.3, while the Fc of 29-B1 reacts as well as the intact molecule. Two other Igh-la Fc fragments were also examined: one behaves like GPC-8 and the other like 29-B1.

Two-dimensional gel analysis (11) of these Fc fragments reveal differences between the reactive and non-reactive Fc's (Fig. 2). No relative molecular weight differences are obvious from these analyses, but there are clear differences in the isoelectric points possibly due to deamidation differences. Any biological significance of the serological and charge heterogeneity is not known. Although glycosylation differences are possible, we do know that the determinant with which 15.3 reacts is pronase-sensitive and made in tunicamycin-containing cultures. Further work on the effect of these differences on a variety of immunoglobulin effector functions, such as complement or protein A binding and anisotropy decay studies, may provide more insight into this problem.

We have also reported that Igh-la is expressed on the surface of hybridoma cells in a form which is serologically and biochemically distinct from that which is secreted (7). Membrane Igh-la is approximately 10K daltons larger than secreted Igh-la, and was shown to lack the determinant recognized by antibody 15.3. Although we know that this determinant is located in the CH2 domain (4), the loss of reactivity with antibody 15.3 does not necessarily mean that the primary sequence of membrane CH2 differs from secreted CH2. However, it does imply that the topography is different. Our preliminary evidence indicates that the Fc fragment of surface Igh-la is both larger and more acidic than secreted Fc fragments made by the same cells.

We have looked for membrane IgG on hybridomas of other allotypes and isotypes. Fig. 3 shows the two-dimensional gel pattern of surface and secreted Igh-1b derived from an Igh-1b anti-eosin hybridoma. As in the case of Igh-la, the membrane Igh-1b is both larger (10K daltons) and more acidic. Membrane
Figure 2. Two-dimensional gel analysis of Igh-la Fc's. Purified Igh-la proteins 29-B1 and GPC-8 were digested for 10 min at 37°C with papain as previously described (4). The Fc was separated from Fab and intact molecule by gel chromatography. Two-dimensional gel analysis under reducing conditions was as described by O'Farrell (11), with isoelectric focusing as the first (horizontal) dimension and SDS gel electrophoresis (12.5% acrylamide) as the second dimension.
The gels were stained with Coomassie Blue and Crocein scarlet to reveal protein bands. The arrows indicate the Fc molecules. Other stained bands are marker proteins (Pharmacia): phosphorylase B (94K Mr), bovine serum albumin (67K Mr), ovalbumin (45K Mr), carbonic anhydrase (30K Mr), and soybean trypsin inhibitor (20K Mr). The carbonic anhydrase migrates similarly to the Fc's in the second dimension, but has a different isoelectric point.

2-a: GPC-8 Fc
2-b: 29-B1 Fc

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Figure 3. Two-dimensional gel analysis of membrane and secreted IgH-1b. IgH-1b hybridoma cells (149-1.5.5, an anti-eosin cell line), were washed, surface-labeled with 125I, and lysed as described by Ledbetter et al. (12). The immunoglobulin was precipitated with rabbit anti-mouse Ig coupled to Sepharose. After through washing the bound antigen was eluted and analyzed by two-dimensional gel electrophoresis as described by O'Farrell et al. (13). The first dimension was non-equilibrium pH gradient gel electrophoresis; the second dimension was SDS electrophoresis through 10% polyacrylamide gel. The position of molecular weight standards (see Fig. 1) is indicated on the right. Only the heavy chain portion of the gel is shown.

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Igh-1b possesses all 8 allotopes detected by the available anti-Igh-1b monoclonal antibodies. We have also found similar surface IgG on hybridoma cells producing IgG1. It should be noted that different cell lines express different levels of membrane IgG and that culture conditions, particularly variations in serum, affect this expression.
The availability of well-characterized monoclonal anti-allotypic antibodies will be useful in the task of explaining genetic and structural polymorphisms of murine immunoglobulins. We may expect new Ig C\(\gamma\) genes and arrangements of these genes in wild mouse populations. This information may increase our understanding of the evolution of Ig genes and the functional significance of allotypic and haplotypic variation. The differential regulation of isotype and allotype by T cells (14, 15) indicates the functional importance of allotypes and perhaps allotypic differences between membrane and secreted IgG's, IgE and possibly IgA. Finally, it is worth mentioning that allotypic determinants are equivalent to isotypic determinants in individual mouse strains. Monoclonal anti-allotypes are isotypic-specific probes in allotypically homozygous mice, and should thus have a valuable future in the study of the immune response and its regulation.

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REFERENCES