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Genetic 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 IgG_1, IgG_{2a}, and IgG_{2b}. In this communication we describe their reactivities with immunoglobulins of the inbred mouse strains. Comparison with serology charts indicates that many of these monoclonal antibodies detect allotypic specificities previously defined by conventional antisera; others define previously undescribed specificities. Strain and isotype distribution allows us to assign five new allotypic specificities to *Igh-1* and three new specificities to *Igh-3*. In addition, on the basis of reactivity with the monoclonal antibodies, we have defined a new *Igh* haplotype in SWR/J mice, Igh^p.

Introduction

Mouse immunoglobulin allotypes are serologically detected antigenic determinants that define the genetic polymorphisms encoded by the immunoglobulin heavy-chain constant-region gene complex (Herzenberg and Herzenberg 1978, Lieberman 1978, Shimizu et al. 1981). Individual antigenic determinants recognized by allotype-specific antibodies are called allotopes. Unique combinations of these allotopes define immunoglobulin alleles (allotypes), and combinations of these alleles define immunoglobulin haplotypes. A number of haplotypes exist in the inbred mouse strains, and still more extensive polymorphism is found in wild mouse populations (Lieberman and Potter 1965, 1969, Huang et al. 1982).

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Abbreviations used in this paper: Igh, immunoglobulin heavy chain: SDS, sodium dodecyl sulfate.

Allotypes have been defined in the past by the use of cross-strain immunizations to obtain the original antiserum, followed by cross-strain absorption to delineate the number of genetic specificities detected. From these cumbersome analyses emerged a picture of enormous complexity at the immunoglobulin heavy chain (*Igh*) loci. Not only is the number of alleles at any given locus large, but as recently confirmed by DNA sequence analyses, the differences between alleles can be substantial (Ollo et al. 1981, Dognin et al. 1981, Schreier et al. 1981, Ollo and Rougeon 1982). The use of monoclonal antibodies has facilitated the molecular and populations analyses of immunoglobulin gene polymorphism in the mouse (Oi and Herzenberg 1979, Huang et al. 1982) and more recently, in man (Zelaschi et al. 1983). In addition to revealing further genetic polymorphism in outbred populations, these antibodies provide convenient tools for analyses of genetic linkage, immune regulation, and the expression of immunoglobulin genes. In this report we describe the generation of and the isotype and strain reactivities of 33 monoclonal allotope-specific antibodies. Parsons and co-workers (1983) used these antibodies to examine the presentation of allotypic determinants at the molecular level.

Materials and Methods

Somatic cell hybridization. Hybridoma-antibody-producing cell lines were generated by methods described previously by Köhler and Milstein (1975) as modified by Oi and Herzenberg (1980). To obtain nearly all of the hybridomas used here, we used single-cell-cloning techniques and selection of antigen-specific antibody-producing cell lines using the fluorescence-activated cell sorter (Parks et al. 1979). Antibodies 1A7 and BG1 were gifts of Drs. M. and G. Bosma (Fox Chase Institute for Cancer Research). Rat monoclonal antibodies 2b-180.4 and 2b-168.1 were a gift of Dr. M. Scharff (Albert Einstein College of Medicine). All other antibodies described here were made in this laboratory and will be available from American Type Culture Collection; some have been described previously (Oi and Herzenberg 1979).

Immunoglobulin purification. Many different purification procedures were tested to determine the most reproducible method with highest yields. Monoclonal antibodies were purified from serum and ascites fluids from hybridoma and myeloma-tumor-bearing mice by gel filtration in AcA34 or AcA22 (LKB) equilibrated with 0.05M Tris-HCl, pH 8.1, 0.15M NaCl. Immunoglobulin-rich fractions then were bound directly to QAE Sephadex A-50 (Pharmacia) equilibrated with the same buffer. Purified immunoglobulin was eluted using a salt gradient to 0.5M NaCl. Some monoclonal immunoglobulins do not bind to this ion-exchange matrix under these conditions. In these cases, the flow through fraction contains the purified immunoglobulin molecules. This method avoids any precipitation of antibody in low-ionic-strength buffers and losses from dialysis procedures. Most of these antibodies were analyzed after reduction and alkylation by two-dimensional gel electrophoresis with a non-equilibrium pH gradient gel electrophoresis as the first dimension and sodium dodecyl sulfate (SDS) gel electrophoresis (10% acrylamide) as the second dimension (Oi et al. 1978). These two-dimensional gel analyses provided the standard by which all subsequent antibody batch purifications were judged.

Radioimmune assays Purified immunoglobulins were iodinated by the iodogen procedure (Fraker and Speck 1978). The radioimmune assays using monoclonal antibodies were originally described by Oi and Herzenberg (1979). Allotope levels were measured using a solid-phase competitive binding assay as detailed by Huang and co-workers (1982). To determine if two antibodies react with determinants on the same molecule, a solid-phase co-binding assay was used (Huang et al. 1982). In this assay one allotope-specific antibody is coated onto the plate and the antigen (in the form of purified protein or diluted deaggregated sera) is then bound by virtue of the antibody coat. Finally, radioiodinated allotope-specific antibody is added. If the second antibody binds, then it reacts with the same molecules as the first antibody.

The normal competition assay worked poorly when Igh-4^a-specific 28.1 antibody was used as a plate coat. Therefore, the co-binding assay was used to detect this determinant. The plate was coated with anti-Igh-4^a 18.1; test sera were added, and the 28.1 allotope was detected with radiolabeled 28.1.

Results

Thirty-three monoclonal antibodies were characterized as reactive with allotopes encoded for by the *Igh-1* (IgG_{2a}), *Igh-3* (IgG_{2b}), and *Igh-4* (IgG_1) loci (see Green 1979, and Table 1 for immunoglobulin genetic nomenclature). Each of the 24 antibodies tested has a unique two-dimensional gel electrophoresis pattern. Table 2 lists the pertinent characteristics of these hybridoma antibodies including specificity, isotype and allotype, and chain composition (as determined by the presence or absence of the NS-1 light chain on two-dimensional gels). The specificity designations in Table 2 refer only to the immunizing haplotypes Igh^a and Igh^b.

Genetics of the Igh-1 allotopes

The *Igh* haplotype distribution of the Igh-1 allotopes is summarized in Table 3. Reactivity with the Igh-1-specific antibodies divides the inbred mouse strains into five groups: (1) Igh^a, Igh^g, Igh^h, Igh^j; (2) Igh^b; (3) Igh^c, Igh^p; (4) Igh^d, Igh^e, Igh^n, Igh^o; and (5) Igh^f.

As described in the following paper, antibodies 9.8, 14.4, and 17.2 react with topographically related determinants on the IgG_{2a} molecules. These antibodies

Table 1. Distribution of alleles of the *Igh* loci in the *Igh* haplotypes

Haplotype	Prototype strains	Immunoglobulin locus and heavy-chain isotype						
		Igh-6 IgM	*Igh-5* IgD	*Igh-4* IgG_1	*Igh-3* IgG_{2b}	*Igh-1* IgG_{2a}	*Igh-7* IgE	*Igh-2* IgA
a	BALB/c	*a*	*a*	*a*	*a*	*a*	*a*	*a*
b	C57BL/10J	*b*	*b*	*b*	*b*	*b*	*b*	*b*
c	DBA/2	*a*	*a*	*a*	*a*	*c*	.	*c*
d	AKR/J	*d*	*a*	*d*	*d*	*d*	*a*	*d*
e	A/J	*e*	*e*	*a*	*e*	*e*	*a*	*d*
f	CE/J	.	*a*	*a*	*f*	*f*	.	*f*
g	RIII/J	.	*a*	*a*	*g*	*g*	.	*c*
h	SEA/J	.	*a*	*a*	*a*	*h*	.	*a*
j	CBA/H	*a*	*a*	*a*	*a*	*j*	*a*	*a*
n	NZB	*e*	*a*	*a*	*e*	*e*	.	*d*
o	AL/N	*e*	*e*	*a*	*d*	*d*	.	.
p	SWR/J*	.	.	.	*f*	*c*	.	.

Allotypes at each locus are as described by Parsons and colleagues (1981), Borges and co-workers (1981), and Herzenberg and Herzenberg (1978). (.) indicates not yet examined. There has been no polymorphism detected for IgG_3.

* A new *Igh* haplotype defined in this paper.

Table 2. Characteristics of monoclonal allotope-specific antibodies

Name*	Specificity†	Isotype/ allotype	Chain composition	Spleen donor
5.7	Igh-1^b	IgG_3	HL	BALB/c
4.7		Igh-4^a	HL	SJA/9
3.1		Igh-4^a	HLK	BALB/c
BG1		Igh-4^a	HL	BALB/c
2.9		Igh-1^a	HL	BALB/c
1A7		Igh-4^a	HL	BALB/c
19.8		Igh-4^a	HLK	BALB/c
20.1		Igh-1^a	HL	SJA/9
8.3	Igh-1^a	Igh-1^b	HL	SJL
9.8		Igh-1^b	HL	SJL
14.4		Igh-1^b	HL	SJL
15.3		Igh-1^b	HL	SJL
21.2		Igh-1^b	HL	SJL
17.2		Igh-1^b	HL	SJL
30.3		Igh-1^b	HL	SJL
29.9		Igh-4^b	HL	SJL
32.2		Igh-4^b	HL	SJL
31.6		Igh-4^b	HLK	SJL
33.3		Igh-4^b	HLK	SJL
34.6		Igh-4^b	HLK	SJL
16.3	Igh-1^a/Igh-3^a	Igh-1^b	HL	SJL
35.1	Igh-1^a, Igh-1^b/Igh-3^a, Igh-3^b	Igh-1^d	HL	AKR
36.2	Igh-3^a/Igh-3^b	Igh-4^d	HL	AKR
180.4		Rat IgG_{2b}	N. D.	Sprague-Dawley
23.1	Igh-3^b	Igh-1^a	HL	SJA/9
24.1		Igh-4^a	HL	SJA/9
22.9	Igh-4^b	Igh-4^a	HL	SJA/9
25.2		Igh-4^a	HL	SJA/9
26.5		Igh-4^a	HL	SJA/9
27.4		Igh-4^a	HL	SJA/9
10.9	Igh-4^a	Igh-1^b	HL	SJL
18.1		Igh-1^b	HLK	SJL
28.1		Igh-6^b	N. D.	SJL

* Hybrid number precedes the (.) and clone number follows.

† This refers only to the antigens used in the immunization of the mice from which these antibodies were derived. Reactivity of these antibodies with immunoglobulins of other haplotypes is shown in other tables and text.

N. D. = not done.

showed weak, but detectable reactivity with sera from groups 4 and 5 in the competition assay for allotope levels. Using the co-binding assay with antibody 9.8 as plate coat and radiolabeled anti-Igh-1^a 8.3 as detecting reagent, the presence of 9.8–like allotopes in group 4 sera was verified (group 5 was not tested). This result indicated that the 8.3 allotope is expressed on 9.8-bearing molecules in the group 4 strains, just as it is in group 1 strains. Since the reactivity of group 4 sera was high with antibody 8.3 and low with antibody 9.8 in the competition assay, this suggested that either the 9.8 allotope was altered in these strains or was present only on a subset of 8.3-bearing molecules. We therefore examined the reactivity of antibodies

9.8 and 8.3 with IgG_{2a} hybridoma proteins of BALB/c (*Igh-1*^{a}) and AKR/J (*Igh-1*^{d}) origin. Using the solid-phase co-binding assay, we showed that the hybridoma proteins behave similarly to serum Ig of the donor strain. Thus, while the Igh-1^a and Igh-1^d proteins demonstrate identical reactivities with 8.3 (Fig. 1a), the Igh-1^d proteins have reduced reactivity with 9.8 (Fig. 1b). A full titration with these and other proteins was studied with similar results (data not shown). These findings indicate that the reduced activity of 9.8 antibody toward Igh^d serum is not a result of an authentic 9.8 allotope being expressed on a subset of Igh-1 molecules, but rather that the Igh^d 9.8 allotope is somewhat different and now reacts only weakly with antibody 9.8.

Since antibody 16.3 detects an allotope common to Igh-1 and Igh-3 proteins of *Igh*^{a} origin (see Table 2), it was important to determine whether the determinant is present on both Igh-1 and Igh-3 gene products in other haplotype strains. Again, the co-binding assay was employed. Serum IgG_{2a} molecules were bound to the plate using antibody 15.3 as a plate coat. This antibody reacts with Igh-1 proteins of all haplotypes except *Igh*^{b}. As shown in Figure 2a, subsequent binding of radiolabeled 16.3 allowed us to determine that IgG_{2a} from DBA/2J (*Igh*^{e}) alone does not bear the 16.3 determinant. This serum showed levels of Igh-1 molecules (as detected with antibody 15.3) similar to BALB/c serum, indicating that the absence of the 16.3 allotope is not due to quantitative variation in IgG_{2a} levels or reduced affinity of Igh-1c for Igh-15.3-specific antibody. IgG_{2a} from all other Igh-type strains (except Igh^b, which completely lacks the 16.3 epitope) clearly possesses the 16.3 marker.

Igh-3 allotopes

Table 4 summarizes the Igh-3 allotope distribution among inbred mouse haplotypes. Again, since allotope 16.3 is present on both Igh-1 and Igh-3 proteins of Igh^a origin, we had to determine whether this allotope was present on Igh-3 molecules of other alleles. To this end, monoclonal rat anti-mouse IgG_{2b} 2b-180.4 was used to bind serum IgG_{2b} to the plates. Subsequent binding of radioiodinated antibody 16.3 by the immobilized IgG_{2b} of every haplotype (except Igh^b) indicates that the 16.3 allotope is present on IgG_{2b} of all *Igh*$^{non\text{-}b}$ haplotype strains (Fig. 2b).

The Igh^d, Igh^e, Igh^n, Igh^o haplotype sera possess both the Igh-3^b allotope 23.1 and the Igh-3a allotope 16.3 (Table 4). To determine whether these allotopes occur on the same molecule, we used the co-binding assay. Antibody 16.3 was used as the plate coat. Sera from various inbred strains were then allowed to react with this plate coat. Radioiodinated antibody 23.1, in 4% (v/v) BALB/c normal mouse serum (to inhibit binding of the radiolabeled antibody, itself an Igh-1a protein, to the plate-coat antibody) was added. As shown in Figure 3, the labeled antibody bound to wells corresponding to *Igh*^{d}, *Igh*^{e}, *Igh*^{n} haplotype sera (*Igh*^{o} was not tested). In these strains, therefore, allotope 23.1 is on at least some of the molecules which bear allotope 16.3, that is IgG_{2a} or IgG_{2b} (or both). When anti-Igh-1^a 8.3 was used as plate coat to bind serum IgG_{2a}, only background counts were obtained. Thus the 23.1 and 16.3 allotopes are co-expressed on IgG_{2b} of *Igh*^{d}, *Igh*^{e}, *Igh*^{n} haplotypes.

Table 3. Reactivity of Igh-prototype strain sera with Igh-1-specific antibodies

Antibody	5	4	3	2	BG	1A	19	20	8	31	14	9
Haplotype												
Igh^a, Igh^g, Igh^h, Igh^j	−	−	−	−	−	−	−	−	+	+	+	+
Igh^c, $Igh^{p\dagger}$	−	−	−	−	−	−	−	−	−	w	−	−
Igh^d, Igh^e, Igh^n, Igh^o	−	−	−	−	−	−	−	−	+	+	w	w
Igh^f	−	−	−	−	−	−	−	−	+	+	w	w
Igh^b	+	+	+	+	+	+	+	+	−	−	−	−

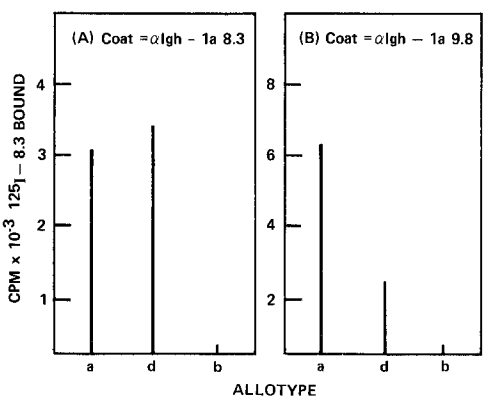

Fig. 1 A and B. Anti-Igh-1^a 9.8 detects a cross-reactive determinant on Igh-1^d. Solid-phase co-binding assays were used. Antibody 8.3 was the plate coat in **A** and 9.8 was the plate coat in **B.** Purified hybridoma proteins of Igh-1^a (29-B1), Igh-1^d (179-38) or Igh-1^b (149-1.5) allotypes were added at 25 μg/ml. Final step was ^{125}I-8.3.

Igh-4 allotopes

Table 5 shows the reactivity pattern of the Igh-4-specific antibodies with the sera of the inbred mouse strains and with rat immunoglobulins. Although the allotypic specificities recognized by the Igh-4^a-specific antibodies cannot be distinguished on the basis of reactivity with inbred mouse sera, reactivity patterns with rat immunoglobulins indicates that each antibody detects a distinct determinant. Mouse allotope 18.1 is found on rat IgG_1 molecules derived from the LOU strain, while 10.9 is not present on rat IgG of any subclass. Allotope 28.1 is found on both IgG_1 and IgG_{2a} molecules from the rat. Therefore, we conclude that on any single mouse Igh-4^a molecule, there are at least three distinct allotopes

We have described previously that the four Igh-4^b antibodies appear to recognize different allotopes (Herzenberg et al. 1981). To date, all of the Igh-4^b-specific antibodies bind with relatively lower affinity than other allotope-specific antibodies. This has limited the usefulness of these particular antibody reagents.

The Igh^p haplotype

SWR/J strain mice have been classified as *Igh-1*^{c} by conventional antisera and thus have been provisionally classified as Igh^c (Herzenberg and Herzenberg 1978). Analysis with monoclonal allotope-specific antibodies confirms that SWR/J do have the *Igh-1*^{c} allele at the IgG_{2a} locus (Table 3). However, at the *Igh-3* locus, the

17	30	32	15	33	16*	29	34
+	+	+	+	+	+	+	+
−	w	w	+	+	−	+	−
w	w	+	+	+	+	+	+
w	w	+	+	+	+	+	−
−	−	−	−	−	−	−	−

The result of each allotope assay was scored as "+", "w", and "−". "+" denotes that for a given assay condition, the test serum gives 50% or more inhibition than either no serum (RIA buffer alone), or serum with the wrong allotype. In most cases, the inhibition was greater than 95%. "w" indicates that for the given assay, the test serum gives weak but consistent inhibition (approximately 10%–20%). "−" indicates no significant inhibition (less than 10%). All weak reactivities were confirmed by co-binding assays. The antibody is the hybrid number as listed in Table 2 (clone number is omitted).

* The 16.3 allotype is present on Igh-3 proteins of all Igh-non-b strains, so all non-b sera react (Table 4). However, Igh-1^c and Igh^p lack this marker.

† Igh^p haplotype type strain is SWR/J as defined in this paper.

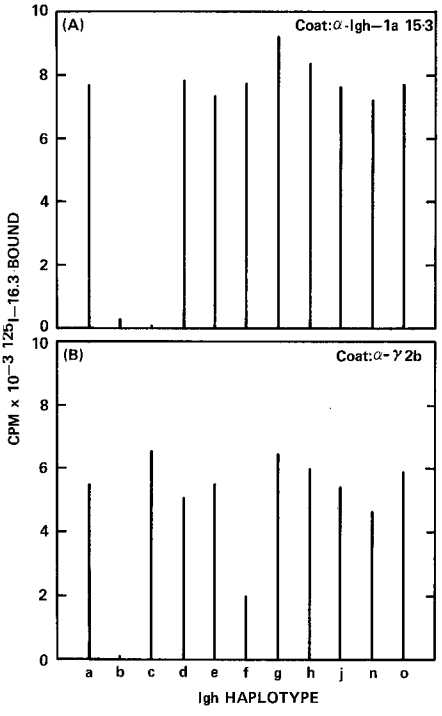

Fig. 2 A and B. Polymorphism of the 16.3 allotope on IgG_{2a} and IgG_{2b}. Strain distributions of Igh-1 and Igh-3 allotopes were determined by the co-binding assays as described in Fig. 1. **A** Polymorphism on IgG_{2a}; plate coat: anti-Igh-1 15.3; second step: ^{125}I-16.3. **B** Polymorphism on IgG_{2b}, plate coat: rat anti-mouse IgG_{2b} 2b-180.4. This antibody reacts only weakly with Igh^f. Second step: ^{125}I-16.3.

SWR/J mice type as Igh^f, lacking the allotope 180.4 that is found on Igh-3^c molecules (Table 4). Therefore, SWR/J possess an *Igh-1*^{c} allele and an *Igh-3*^{f} allele, defining a new haplotype designated Igh^p.

Table 4. Reactivity of Igh-type strain sera with Igh-3-specific antibodies

Antibody	180	16*	35	36	23	24
Haplotype						
Igh^a, Igh^c, Igh^g, Igh^h, Igh^j	+	+	+	+	−	−
Igh^b	+	−	+	+	+	+
Igh^d, Igh^e, Igh^n, Igh^o	+	+	−	−	+	−
Igh^f, Igh^p	W†	+	+	+	−	−

Methods are as described in Table 3.

* This antibody reacts with an allotope common to *Igh-1* and *Igh-3* alleles, except in the case of the Igh^c and Igh^p haplotypes where it reacts only with Igh-3 (IgG_{2b}) molecules.

† Reactivity was not detectable in the competition assay for allotope levels, but was weakly detectable by the cobinding assay.

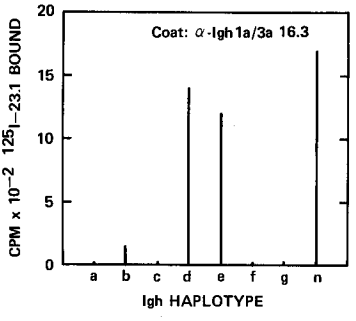

Fig. 3. Igh-3^a determinant 16.3 and Igh-3^b determinant 23.1 reside on the same molecule in Igh^a, Igh^e, Igh^n, Igh^o haplotypes. Antibody 16.3 (30 μl, 25 μg/ml) was the plate coat. Serum from the various haplotype strains, diluted 1/50 was then added and incubated for 1 h. Finally, radioiodinated antibody 23.1, in 4% BALB/c serum, was added. When molecules bear both 16.3 and 23.1 determinants, a positive signal is obtained.

Table 5. Reactivity of Igh-type strain sera with Igh-4-specific antibodies

Antibody	22	25	26	27	10	18	28
Haplotype							
Igh^a, Igh^c, Igh^d, Igh^e, Igh^f,Igh^g,							
Igh^h, Igh^n, Igh^o, Igh^p	−	−	−	−	+	+	+
Igh^b	+	+	+	+	−	−	−
rat IgG_1	−	−	−	−	−	+	+
rat IgG_{2a}	−	−	−	−	−	−	+
rat IgG_{2b}	−	−	−	−	−	−	−
rat IgG_{2c}	−	−	−	−	−	−	−

Discussion

The conventional serology charts show how each mouse immunoglobulin locus and allele is composed of combinations of allotypic specificities. These specificities were defined by taking individual alloantisera reactive with sera from several strains and absorbing it with sera from one of these strains. The antisera were then retested to determine whether any reactivities with other mouse sera remained. For example,

an antiserum reactive with both BALB/c and DBA/2J sera was first absorbed with DBA/2J sera until all reactivity with DBA/2J was removed. If any antibody reactivity with BALB/c sera remained, the antiserum was said to contain antibodies reacting with at least two specificities. One specificity consists of the determinants shared between BALB/c and DBA/2J, and the other consists of determinants unique to BALB/c. If the original antiserum was absorbed with BALB/c sera and some reactivity against DBA/2J sera remained, this would define a third specificity, i.e., those allotypic determinants unique to DBA/2J. By testing a library of individual alloantisera before and after a series of absorptions, specificity charts describing different immunoglobulin loci and alleles were laboriously constructed. In this manner, the *Igh-1* (IgG_{2a}) alleles were shown to possess a large number of allotypic specificities (Herzenberg and Herzenberg 1978, Lieberman 1978). In contrast, the *Igh-4* (IgG_1) alleles could only be found to have three specificities, defining three allotypes (Parsons et al. 1981). Since allotypic determinants could not be distinguished unless they were separated genetically, the complexity underlying these alleles could not be further defined. The following paper (Parsons et al. 1983) investigates allelic complexity at the molecular levels, using the monoclonal antibodies described here.

With the 33 monoclonal allotope-specific antibodies described in this paper, we have been able simply to define some of the alleles and haplotypes described by conventional serology. Since each monoclonal antibody defines a single antigenic epitope, properly called allotope, reactivity of each antibody with different mouse sera directly demonstrates the presence of the allotope on the immunoglobulin molecules of particular mouse strains. The assays used to construct the allotope charts presented in this paper were simple and easily manipulated because of the use of monoclonal antibodies. The monoclonal allotope-specific antibodies described here have defined new genetic specificities. The allotopes detected by antibodies 8.3, 34.6, and 9.8 represent determinants as yet unrecognized by conventional serology. As such, they are designated Igh-1.13, 1.14, and 1.15, respectively (Table 6). The cross-reactive allotope detected by 9.8 in Igh^d, Igh^e, Igh^f, Igh^n, Igh^o haplotype strains was shown to be a structure distinct from the specificity 1.15. Thus, this antibody also defines another specificity, 1.16.

Both the strain and isotype distribution of the allotope recognized by antibody 16.3 demonstrate that 16.3 detects a new allotypic specificity on Igh-1 (1.17) and Igh-3 (3.10). We can hypothesize that this shared specificity was present on both IgG_{2a}

Table 6. New Igh specificities found with monoclonal antibodies

Antibody	Igh specificity	Haplotype distribution
8.3	1.13	Igh^a, Igh^d, Igh^e, Igh^f, Igh^g, Igh^h, Igh^j, Igh^n, Igh^o
34.6	1.14	Igh^a, Igh^d, Igh^e, Igh^g, Igh^h, Igh^j, Igh^n, Igh^o
9.8	1.15	Igh^a, Igh^g, Igh^h, Igh^j
9.8	1.16	Igh^d, Igh^e, Igh^f, Igh^n, Igh^o
16.3	1.17	Igh^a, Igh^d, Igh^e, Igh^f, Igh^g, Igh^h, Igh^j, Igh^n, Igh^o
16.3	3.10	Igh^a, Igh^c, Igh^d, Igh^e, Igh^f, Igh^g, Igh^h, Igh^j, Igh^n, Igh^o, Igh^p
23.1	3.11	Igh^b, Igh^d, Igh^e, Igh^n, Igh^o
180.4	3.12	Igh^a, Igh^b, Igh^c, Igh^d, Igh^g, Igh^h, Igh^j, Igh^n, Igh^o

and IgG_{2b} molecules before the divergence of the non-Igh^b haplotypes since most mouse strains possess the 16.3 allotope on both isotypes. Since the divergence of the Igh^a and Igh^c haplotypes, however, $Igh\text{-}1^c$ has lost this determinant.

SWR/J strain mice have been typed as $Igh\text{-}1^c$ by conventional serology and thus have been provisionally classified as Igh^c haplotype (Herzenberg and Herzenberg 1978). Our monoclonal data confirms that SWR/J do have $Igh\text{-}1^c$ allele at the IgG_{2a} locus. However, according to Lieberman (1978), SWR/J has unassigned conventional specificities 21, 25, and is similar to Igh^g strain (prototype RIII/J). Unassigned specificity 21 was defined by an antiserum DBA/2 (Igh^c) anti-RIII/J (Igh^g). Since DBA/2 strain is the recipient for the antiserum defining specificity 21, DBA/2 should not have this specificity. Furthermore, since the Igh-1 molecules of DBA/2 and SWR/J are identical, the difference between Igh^c and Igh of SWR/J must be residing in Igh molecules other than Igh-1 molecules. Unassigned specificity 25 was defined by $(SJLJ \times BALB/cJ)F_1$ anti-CE strain (Igh^f). The presence of specificity 25 in SWR/J strain and donor CE strain supports our finding that the Igh-3 molecules of CE/J and SWR/J are similar. Unfortunately, we do not know in which isotypes these conventional specificities reside. Together, analyses with a panel of monoclonal allotope-specific antibodies indicated that SWR/J possess an $Igh\text{-}1^c$ allele and an $Igh\text{-}3^f$ allele and therefore define a new haplotype designated as Igh^p.

The geneology of inbred mouse strains reveals many common ancestors, and the geographical origins of the *Igh* haplotypes is somewhat obscure. Most of the antibodies described here react only with mouse immunoglobulins. The exceptions are anti-$Igh\text{-}4^a$ 18.1, which shows widespread reactivity with vertebrate immunoglobulins (Parsons and Herzenberg 1981) and antibody 35.1, which reacts with sera from various rodents and hooved animals (C.-M. Huang, unpublished results). It is still striking that the Igh^b allotypic determinants are so distinct, with only the IgG_{2b} determinant 23.1 appearing in other haplotypes of inbred strains. This might indicate that the Igh^b chromosome has had a separate evolutionary history for quite some time. However, we have evidence of *a*-like and *b*-like chromosomes in wild mice occupying the same geographical regions. Analyses of the allotypes of these wild mice revealed intermediate allotypes that could have resulted from recombination between $Igh\text{-}1^a$ (or $Igh\text{-}1^a$-like) allele with $Igh\text{-}1^b$ (or $Igh\text{-}1^b$-like) alleles (Huang et al. 1982).

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