0022-1767/82/1282-0661$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 128, No. 2, February 1982
Printed in U.S.A.

NEW IMMUNOGLOBULIN IgG ALLOTYPES AND HAPLOTYPES FOUND IN WILD MICE WITH MONOCLONAL ANTI-ALLOTOPE ANTIBODIES[1]

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Sera from 156 wild mice (*Mus musculus L.*) collected from parts of Eurasia, Northern Africa, and the Americas were tested in solid-phase radioimmunoassays for reactivity with 20 monoclonal anti-allotope antibodies directed against gene products of the *Igh-1*, *Igh-3*, and *Igh-4* loci. Most of the wild mice have Igh phenotypes similar to those of inbred strains or heterozygotes thereof, but frequently the wild mice showed unique combinations of allotypic determinants (allotypes) for a given immunoglobulin allele and unique combinations of allotypes on a given chromosome (haplotypes) not seen in inbred strains. We found new allotypes of the *Igh-1* and *Igh-4* loci. Mice from three regions (Poland, Taiwan, and Japan) possess combinations of allotypes indicating that recombination, gene conversion, or other gene duplication events have occurred in portions of the *Igh* constant region gene complex. Most interestingly, sera of three mice from Egypt possess immunoglobulin molecules appearing to result from an intragenic recombination event involving either *Igh-1d* or *Igh-3d* with *Igh-1b*. Two mice from Pakistan and one from Seychelles Island in the Indian Ocean also showed similar unusual phenotypes. These mice possess two CH2 IgH-1b and two CH2 Igh-1a/CH2 Igh-1d determinants, suggesting that these variant immunoblobulin arose from recombinations within the CH2 domain.

Immunoglobulin (Ig) allotypes of inbred mouse strains have been studied for more than 15 yr with conventional antisera (1, 2). Some interesting features have been revealed. Genetic polymorphisms have been found for seven of the eight closely linked Ig constant region genes (1, 3). These polymorphic loci, located on chromosome 12, are designated as *Igh-1*[4] to *Igh-7* in the order of the discoveries of their polymorphisms. The gene order, from 5′ to 3′ in the DNA, is as follows: μ(*Igh-6*), δ(*Igh-5*), γ-3, γ-1(*Igh-4*), γ-2b(*Igh-3*), γ-2a (*Igh-1*), ϵ(*Igh-7*), and α(*Igh-2*) (4). Among all the inbred strains of mice that have been analyzed, nine alleles have been found at the *Igh-1* locus, six alleles at *Igh-3*, and three alleles at *Igh-4* locus (5, 6). All together, 11 *Igh* haplotypes have been defined in laboratory inbred mice, and three have been defined in wild mice (see Ref. 3 for nomenclature). Recombination of the Ig heavy chain constant genes has not been observed among thousands of progeny from appropriate crosses (1, 7–9). The *Igh-b* haplotype appears to be quite distinct from other known haplotypes (especially *Igh-1b*). Geographic isolation of the *b* haplotype may be responsible for the extensive divergence of *Igh-b* relative to other *Igh* haplotypes (10).

Recently, we have used monoclonal antibodies (11–13) to study the Ig constant region genes and assign a number of allotypic determinants (allotopes) to restricted parts of the Ig molecules (14, 15). After examining the allotype makeup of inbred strains, we decided to survey allotypes in wild mice populations in a search for natural recombinants and other genetic variants at the *Igh* loci (16).

In this investigation, 20 monoclonal anti-allotope antibodies were used to analyze 156 sera from wild mice caught in many different localities around the world. Our studies revealed that the Ig are extremely polymorphic in wild mice populations. Furthermore, different allotypic compositions predominate in different geographic localities. Although most of the wild mice that we have examined exhibited Ig phenotypes that are found in the inbred strains or heterozygotes thereof, a substantial fraction (18%) of wild mice possess unusual Ig phenotypes. These unusual Ig phenotypes may be derived from mutations, recombinations, or gene duplications in the *Igh* loci.

MATERIALS AND METHODS

Inbred mice. Sera from the inbred strains were obtained from our animal colony at the Department of Genetics, Stanford University School of Medicine, and from The Jackson Laboratory, Bar Harbor, ME.

Wild mice. Sera from wild house mice, *Mus musculus L.*, were used in the analysis of Ig allotypes and haplotypes. The origins and sources of wild mice and the Igh phenotypes found in each locality are listed in Table I. They will be referred to by geographic origin and, when needed, by individual number.

Normal mouse sera. Mice were bled from the retro-orbital sinus, and the blood obtained was coagulated at room temperature for 1 hr. Most of the normal sera obtained were stored at −70°C and shipped to Stanford Univ. in dry ice. Sera, with 0.1% sodium azide as preservative, from Taiwan and Japan were shipped on ice and stored on arrival at −70°C.

Monoclonal antibodies. Monoclonal anti-allotope antibodies were produced and purified as previously described (14, 15). All together, 20 different anti-allotope antibodies to gene products of the *Igh-1*, *Igh-3*, and *Igh-4* loci (for IgG2a, IgG2b, IgG1, respectively) were used in this study (Table II). Two monoclonal antibodies, BG1 and 1A7, were a gift of Dr. M. J. Bosma (Institute for Cancer Research, Philadelphia, PA).

Allotope assay with radioimmunoassay (RIA). A solid-phase blocking assay, which was previously described (14, 15, 17), was used to determine the presence or absence in the testing sera of an allotope determinant detected by a specific monoclonal antibody. This assay is sensitive enough to eliminate quantitative variation in Ig levels as a reason for apparent absence of a determinant. Thirty microliters of pretitrated monoclonal antibodies in phosphate-buffered saline (PBS) were first coated onto the wells

Received for publication August 28, 1981.
Accepted for publication October 26, 1981.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported in part by Grants CA-04681, AI-08917, and AI-17966 from The National Institutes of Health.
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[4] Abbreviations used in this paper: Igh, immunoglobulin heavy chain; PVC, polyvinyl chloride; RIA, radioimmunoassay.

TABLE I

List of wild mice, localities of collections, and Ig-phenotypes

Species and Subspecies	Country and No. of Localities	No. of Mice	No. of Mice with Particular Ig-Phenotypes[a]
Mus musculus[b]	Chile (4)	33	24(d), 8(a × d), 1(a)
Mus musculus	Poland (1)	10	8(w9), 1(w8), 1(w10)
Mus musculus	Taiwan (4)	15	10(d), 3(f), 2(w8)
Mus musculus	England (8)	21	16(a), 4(f), 1(b × c)
Mus musculus	Germany (6)	17	16(a), 1(f)
Mus musculus	Spain (4)	7	7(a)
Mus musculus	Egypt (4)	10	5(a), 2(w4), 1(w5), 1(f), 1(b × f) or (b × d)
Mus musculus	Italy (1)	2	1(f), 1(d)
Mus musculus	France (1)	2	2(a)
Mus musculus	Greece (1)	2	1(c), 1(f)
Mus musculus	Indonesia (1)	4	4(d)
Mus m. spetus	Spain (1)	4	4(a)
Mus m. domesticus	Canada (2)	4	2(a), 2(d)
Mus m. castaneus	Philippines (1)	2	2(d)
Mus m. castaneus	Taiwan (1)	2	2(d)
Mus m. domesticus	Seychelles Isl. (1)	2	1(a), 1(w7)
Mus m. domesticus	Mauritius Isl. (1)	2	1(a), 1(f)
Mus m. molossinus	Japan (5)	10	2(a), 3(w12), 5(w11)
Mus m. molossinus	Pacific Isl. (1)	3	3(d)
Mus m. bactrianus	Pakistan (1)	2	2(w6)
Mus m. bactrianus	Afghanistan (1)	2	2(w13)

[a] (d) indicates phenotypes indistinguishable from those of inbred *d*, *e*, *n*, and *o* strains. (w4–w13) denotes unusual phenotypes observed in wild mice. Please refer to Table III and Table IV for detailed descriptions of phenotypes. The number indicates the mouse number of a given phenotype or genotype.

[b] No special efforts were made to identify the subspecies of *Mus musculus*.

TABLE II

List of monoclonal anti-allotype antibodies and their pertinent characteristics

Names	Specificity	Isotype/ Allotype	Antibody Concentration Used	Serum[a] Dilution Used	Sensitivity[b]
			μg/ml		μg/ml
18.1[c]	Igh-4a	1b	10	1/200	625
10.9	Igh-4a	1b	10	1/200	156
22.9	Igh-4b	4a	50	1/10	31
26.5	Igh-4b	4a	50	1/10	31
16.3	Igh-1a,3a	1b	25	1/50	625
23.1	Igh-3b	1a	10	1/50	156
24.1	Igh-3b	4a	5	1/50	156
8.3	Igh-1a	1b	10	1/100	313
16.3	Igh-1a,3a	1b	25	1/50	625
9.8	Igh-1a	1b	25	1/50	156
14.4	Igh-1a	1b	2	1/100	313
17.2	Igh-1a	1b	10	1/100	78
15.3	Igh-1a	1b	25	1/50	625
4.7	Igh-1b	4a	25	1/50	625
2.9	Igh-1b	1a	10	1/100	78
BG1	Igh-1b	4a	2	1/200	39
3.1	Igh-1b	4a	50	1/50	39
1A7	Igh-1b	4a	4	1/200	10
19.8	Igh-1b	4a	2	1/200	39
20.1	Igh-1b	1a	4	1/100	78
5.7	Igh-1b	IgG3	5	1/200	39

[a] Sera from both *Igh-a* strains (BALB/cJ and SJA/9) and *Igh-b* strains (BAB/14 and SJL/J) were used.

[b] The sensitivity for the allotope assay is defined as the amount of unlabeled antigen (designated as concentration: μg/ml) required to inhibit at least 40% of the total ^{125}I-Ag binding.

[c] The number which precedes (.) denotes the allotope number defined by an anti-allotope antibody, and the number after (.) represents the clone number of a given hybridoma.

of polyvinyl chloride (PVC) microtiter plates (Cooke Laboratory Products, Division of Dynatech Laboratories, Alexandria, VA), and the plates were incubated at room temperature for 1 hr. The unbound antibodies were removed, and the wells were washed three times with RIA buffer (PBS containing 1% bovine serum albumin [BSA]). This washing blocks any free protein-binding sites on the PVC plates. Then 20 μl of diluted normal mouse sera from wild or inbred mice, or RIA buffer alone, were added into the antibody-coated plates, and the plates were incubated for 30 min. ^{125}I-labeled purified Ig from a myeloma or hybridoma of the appropriate Ig allotype were then added to the plates. If a test serum contains molecules reactive with the coated anti-allotope antibodies, they will bind and subsequently block the binding of ^{125}I-labeled Ig. To test the sensitivity of each anti-allotope antibody, 20 μl of serial fourfold dilutions of unlabeled purified myeloma or hybridoma proteins of appropriate allotypes were used as the second step in allotope assays. The sensitivity of an allotope assay is defined as the amount of unlabeled antigen (designated as concentration: μg/ml) required to inhibit at least 40% of the total ^{125}I-Ag binding.

Because of the small amount of normal wild mice sera available for analysis and the apparent differences in affinity of the monoclonal anti-allotype antibodies, it was necessary to pretitrate both the coating concentration and the serum dilution for each antibody so that the minimum amount of serum required to detect inhibition could be determined. Table II summarizes the concentration of each monoclonal antibody, the dilutions of mouse sera used in this study, and the sensitivity of each assay.

Solid-phase co-binding assay. A modified solid-phase RIA was used to determine whether certain allotopes are expressed on the same molecules. Briefly, the first anti-allotope antibody was coated on a plate. Then a diluted serum was added and incubated for 1 hr. The unbound proteins were washed off, and the bound proteins were further tested for the presence of other allotypic determinants by incubating with a second ^{125}I-labeled anti-allotype antibody. Because the plate coat anti-allotope and the ^{125}I-labeled anti-allotope antibodies both possess their own allotypes, certain combinations of antibodies cannot be employed in this assay. If the coat antibody reacts with the ^{125}I-labeled antibody, this reactivity can be blocked with serum before addition of the radiolabeled anti-allotope antibody (final step).

In some cases, the antigen bound to a plate-coated antibody was ^{125}I-radiolabeled by using chloramine-T (1, 18). The labeled antigens were then eluted off the plate with 0.2 M glycine-HCl, pH 3.2. This procedure does not remove material directly bound to the plate, i.e., the anti-allotope plate coat and BSA. This method of labeling can specifically isolate trace quantities of the allotope-bearing molecules and protects the allotopes during iodination. Labeled eluated proteins were then tested for direct binding to a panel of monoclonal anti-allotope antibodies.

Radio-iodination of myeloma and hybridoma proteins. A solid-phase reagent, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodogen, Pierce Chemical, Rockford, IL), was used for iodination as previously described (14, 19). Free ^{125}I is removed from labeled protein by gel filtration using a calibrated, prepacked Sephadex G-25 column (PD-10 column, Pharmacia Fine Chemicals, Piscataway, NJ).

Polyacrylamide gel electrophoresis. Laemmli buffer system SDS-polyacrylamide gel (10%) electrophoresis (SDS-PAGE) under reducing conditions (25 mM dithioerythritol) was used for m.w. analyses (20). Gels were dried, stained, and autoradiographed with Kodak X-omat R film at −70°C with Cronex Lightning-Plus intensifying screens (Dupont Instrument, Wilmington, DE).

RESULTS

Allotope distribution in inbred Igh-prototype strains. Eleven Ig haplotypes, each represented by an "Igh-prototype" strain, have been defined among inbred mice by using conventional anti-allotype sera (5, 6). However, the available monoclonal antibodies divide these inbred strains into five haplotype groups, namely, *a,g,h,j* (referred to as "*a*" throughout the text); *b*; *c*; *f*; and *d,e,n,o* (referred to as "*d*" throughout the text). The reactivity of the monoclonal anti-allotope antibodies with inbred mouse strains has been described[5] and is summarized in Table III.

Antibodies 9.8, 14.4, and 17.2 do not react with *Igh-b* and *Igh-c* haplotypes. These antibodies show extremely weak reactivity with sera from *Igh-d,e,n,o* and *f* haplotype strains under the conditions employed in the allotope assay (Table III). However, testing with more concentrated serum or using the co-binding assay[5] has shown that the "*d*" group sera possess a cross-reactive determinant(s).

Allotope distribution in wild mice. To examine the allotypes of wild mice and to search for Ig variants, we have analyzed 156 mouse sera from around the world. Most of the wild mice have Igh phenotypes similar to those of inbred strains or F_1 hybrids. We found that 39% of mice have *Igh* chromosomes very similar, if not identical, to those of *a* group mice; 34% have *d*-like, and 7% have *f*-like *Igh* chromosomes. About 1% have a *c*-like Igh chromosome region, and less than 1% have a *b*-like one. About 18% of the wild mice have reactivity

[5] Parsons, M., V. T. Oi, C.-M. Huang, L. A. Herzenberg, and L. A. Herzenberg. Genetics of murine IgG allotopes detected by monoclonal antibodies: description of the antibodies and genetics of the allotopes. Submitted for publication.

TABLE III

Reactivity of monoclonal anti-allotope antibodies: inbred strains

Igh-loci		*Igh-4*				*Igh-3*			*Igh-1*													
Allotopes		a			b	a		b	a						b							
Allotopes[a]		18[b]	10	22	26	16[c]	23	24	8	16[c]	9	14	17	15	4	2	BG	3	1A	19	20	5
Strains and Haplotypes																						
BALB/c	*a*	+	+	–	–	+	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–
RIII/J	*g*	+	+	–	–	+	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–
SEA/J	*h*	+	+	–	–	+	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–
CBA/J	*j*	+	+	–	–	+	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–
DBA/2J	*c*	+	+	–	–	+	–	–	–	–[d]	–	–	–	+	–	–	–	–	–	–	–	–
CE/J	*f*	+	+	–	–	+	–	–	+	+	w	w	w	+	–	–	–	–	–	–	–	–
AKR/J	*d*	+	+	–	–	+	+	–	+	+	w	w	w	+	–	–	–	–	–	–	–	–
A/J	*e*	+	+	–	–	+	+	–	+	+	w	w	w	+	–	–	–	–	–	–	–	–
NZB/J	*n*	+	+	–	–	+	+	–	+	+	w	w	w	+	–	–	–	–	–	–	–	–
AL/N	*o*	+	+	–	–	+	+	–	+	+	w	w	w	+	–	–	–	–	–	–	–	–
C57BL/6J	*b*	–	–	+	+	–	+	+	–	–	–	–	–	–	+	+	+	+	+	+	+	+

[a] The assignment of allotopes to the gene products of the different Igh-loci has been described elsewhere [reference 14, 15, and footnote (5)].

[b] Only allotope names are listed, clone numbers are omitted.

[c] Allotope 16 is present at least on 1a and 3a myelomas, therefore representing a shared allotopes.

[d] By use of co-binding assay, allotope 16 was shown to be present on IgG2b (Igh-3) but not IgG2a (Igh-1) molecules in DBA/2J mice [footnote (5)]. The result of each allotype assay was scored as "+," "w," and "–." "+" denotes that for a given assay condition, the test serum gives at least 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 (less than 40% but greater than 20%). "–" indicates no significant inhibition (less than 10%).

TABLE IV

New Ig allotypes and haplotypes in wild mice

Igh-loci		*Igh-4*				*Igh-3*			*Igh-1*													
Allotopes		a			b	a		b	a						b							
Allotopes[a]		18	10	22	26	16	23	24	8	16	9	14	17	15	4	2	BG	3	1A	19	20	5
Wild Mice and Haplotypes[b]																						
Egypt																						
CRO	w4	+	+	–	–	+	–	–	+	+	w	w	w	+	–	–	–	–	–	+	+	+
THG	w5	+	+	–	–	+	–	–	+	+	+	+	+	+	–	–	–	–	–	+	+	+
Pakistan	w6	+	+	–	–	+	+	–	+	+	–	–	–	+	–	+	+	–	–	–	–	–
Seychelles	w7	+	+	–	–	+	+	–	+	+	+	+	+	+	–	+	+	–	–	–	–	–
Taiwan	w8	+	+	–	–	+	+	–	+	+	–	–	–	+	+	+	+	+	+	+	+	+
Poland	w8	+	+	–	–	+	+	–	+	+	–	–	–	+	+	+	+	+	+	+	+	+
Poland	w9	+	+	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Poland	w10	+	–	–	–	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Japan	w11	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Japan	w12	–	–	+	+	+	+	–	–	+	–	–	–	–	+	+	+	+	+	+	+	+
Afghanistan	w13	+	–	–	NT[c]	+	+	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–

[a] "+," "w," and "–"—please refer to Table III footnotes.

[b] The designations of new haplotypes in wild mice are tentative. However, the authors suggest that the new haplotypes found in wild mice should be given a number preceded with a letter w indicating wild mice origin. There are 3 wild mice Ig haplotypes defined by Lieberman and Potter (22), therefore, we started with w4.

[c] Not tested.

patterns distinct from the inbred strains, or combinations of inbred strains. The Igh phenotypes of wild mice in each locality are summarized in Table I. The unusual Igh phenotypes of wild mice are shown in Table IV.

We found variant Ig presumably coded for by *Igh-1* and *Igh-4* loci (Table IV). For example, the Polish mouse PLD 828 (w10) expresses Igh-4a allotope 18.1 but not Igh-4a 10.9. A similar Igh-4 phenotype was observed in two mice collected in Afghanistan (w13). Because this phenotype does not occur among the inbred strains, this mouse must possess a new allele, presumably at the *Igh-4* locus. Also, three Egyptian mice (w4, w5) possess Ig bearing only three of the eight Igh-1b allotopes. These mice, therefore, have a new *Igh-1* allele.

Wild mice with unusual allotope compositions that may be explained by recombination or gene duplication. The first interesting group contains mice from Poland and Taiwan. These mice possess the *Igh-1a*, *Igh-3d*, and *Igh-4a* allotypes (w9) or possess *Igh-1d*, *Igh-3d*, and *Igh-4a* allotypes (w8), therefore possessing *a*-like or *d*-like haplotypes. In addition, these mice have all of the eight Igh-1b allotopes but not the two Igh-4b allotopes. The absence of the two Igh-4b allotopes make it unlikely that these mice possess "modified" 4b-like variant molecules. These mice may be heterozygous for an *a* or *d* haplotype with a recombinant haplotype carrying *Igh-4a* and *Igh-1b*. Alternatively, these mice may possess a duplicated *Igh-1* locus (*Igh-1a* or *Igh-1d* and *Igh-1b* on the same chromosome).

Conversely, five Japanese (w11) mice possess the two 4b allotopes, only one of the two 3b allotopes (allotope 23), and all eight of the 1b allotopes. Because they lack Igh-3b allotope 24.1, these mice have a modified *b* phenotype. In addition, they have all of the 1a allotopes, including the shared 3a/1a allotope 16, but lack 4a allotopes. As in the inbred *d* haplotype, molecules expressing both Igh-3a 16.3 and Igh-3b 23.1 allotopes were shown in the co-binding assay.[5] These mice could be heterozygous for a modified *Igh-b* haplotype with a recombinant haplotype carrying *Igh-4b* and *Igh-1a*. Alternatively, these mice may have two *Igh-1* loci per chromosome (*Igh-1a*

linked to *Igh-1b*), reminiscent of the Kyushu wild mice reported by Lieberman and Potter (21, 22). The latter hypothesis is supported by the finding of another group of Japanese mice (w12) that possess the same "modified *b* haplotype" in conjunction with the 1a/3a determinant only. These mice could have the proto-haplotype that evolved to the unusual duplicated genes.

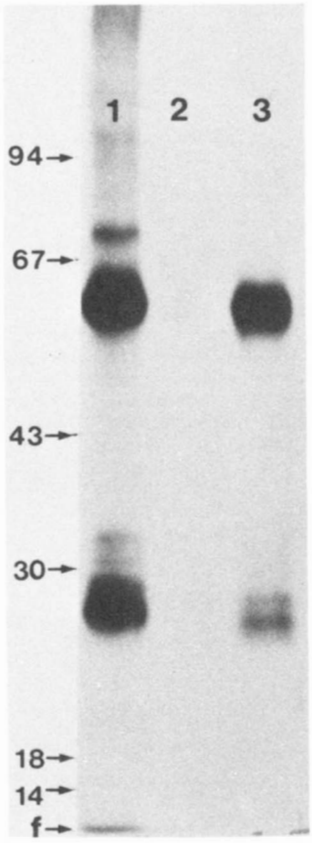

Figure 1. SDS-PAGE electrophoresis of 5.7-allotope-bearing molecules. Molecules possessing the 5.7 allotopes were first bound to the antibody 5.7 coated plate, ^{125}I-radiolabeled, and then eluted off the plate with pH 3.2 buffer (see *Materials and Methods*). The eluates were analyzed by SDS-PAGE and autoradiography. Lane 1, SJL/J serum; Lane 2, BALB/c serum; Lane 3, CRO 551 serum. The position of m.w. markers is indicated to the left of the gel: phosphorylase b, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, a-lactalbumin. The dye front is indicated by "f".

Wild mice with allotope compositions that may be explained by intragenic recombinations. A most interesting finding was obtained from Egyptian mice. Three Egyptian mice (w4, w5) were found to be reactive with only three (5.7, 19.8, 20.1) of the eight anti-1b anti-allotope reagents. Two mice, CRO 447 and CRO 551, were collected in Cairo, and one mouse, THG 632, was collected from a town about 100 miles north of Cairo. The three distinct antibodies have been found to detect allotopes localized in the CH3 domain of the Igh-1b encoded Ig (23). Therefore, it appears that these molecules have at least some of the CH3 domain of b allotype but without the CH2 b allotopes. As such they could result from an intragenic (interdomain) recombinantion or a domain deletion. To test these hypotheses, the variant serum was added to an anti-1b-5.7-coated plate, and bound molecules were radiolabeled on the plate as described in *Materials and Methods*. The labeled Ig were eluted and analyzed by 10% SDS-PAGE under reducing conditions. The results indicated that the 5.7-bearing Ig are composed of heavy and light chains that have m.w. identical to the normal Ig (IgG2a) heavy and light chains (Fig. 1). Thus, a large deletion is not involved in this phenotype.

The radiolabeled eluate was also analyzed for its capacity to bind directly to a panel of monoclonal anti-allotope antibodies. The results are shown in Table 5*a*. Confirming that these unusual Ig bear only three of the eight 1b allotopes, the radiolabeled eluate bound strongly to the previously mentioned three anti-Igh-1b antibodies (detecting CH3 domain) but did not bind to the other five anti-1b antibodies. This eluate also bound strongly to antibody 16.3, which detects the shared 1a/3a allotopes located in CH2. Because the eluate failed to bind to the other five anti-1a antibodies, it appeared that this molecule resulted from a nonhomologous crossing over between *Igh-1b* and *Igh-3a*. However, using the co-binding assay with 5.7 plate coats (Table 5*b*), followed by the variant serum and various radiolabeled anti-allotope antibodies, we observed that the determinants recognized by monoclonal antibodies 9.8 and 14.4 are also present on this unusual molecule. Because under the normal conditions of our assay we did not detect these 9.8 and 14.4 allotopes, these probably represent the cross-reactive Igh-1d determinant previously described[5] (see above). If this is the case, then the variant Ig would represent a recombinant between Igh-1b (CH3) and Igh-1d (CH2).

Similar recombinant molecules were also found in mice from

TABLE V

Igh-1b allotypes 5, 19, 20, and Igh-1a/3a allotope 16 are on the same molecule in Egyptian CRO 551 mice

a. Eluate Assay:

Locus Specificity	-4b-		-4a-		3a	-3b-		Anti-allotope Antibodies -----1a-----						----------1b----------							
	18	10	22	26	16	23	24	8	16	9	14	17	15	4	2	BG	3	1A	19	20	5
Eluate[a]																					
CRO 551	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	+	+	+
SJL/J	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+
BALB/cJ	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] Radiolabeling of CRO 551 serum (SJL/J and BALB/cJ as control sera) bound on anti-1b (5.7) coated plate and direct binding of the eluate to a panel of monoclonal anti-allotope antibodies.

b. Co-binding Assay: Plate Coat 5.7 (IgG3 anti-1b)

Locus and domain specificity	—1bCH3—[a]			1bCH2	Radiolabeled Anti-allotope Antibodies 3b	3a/1a	1ad	1a	1a	1a	4a
Serum	5	19	20	BG	23	16	8	15	9	14	10
CRO 447	+	+	+	–	–	+	–	.[b]	+	+	–
CRO 551	+	+	+	–	–	+	–	.	+	+	–
SJL/J	+	+	+	+	–	–	–	–	–	–	–
BALB/cJ	–	–	–	–	–	–	–	–	–	–	–
(SJL × BALB)F_1	+	.	.	+	–	–	–	.	.	.	.

[a] Allotopes 5, 19, and 20 are located on the CH3 domain of Igh-1b molecules.
[b] Not tested.

TABLE VI

Igh-1b allotopes 2, BG, and Igh-1a allotopes 8 and 15 are on the same molecule in Pakistanean and Seychelles Island mice. Monoclonal antibody BG1 was used as the plate coat in the co-binding assay

Locus and Domain Specificity	Radiolabeled Anti-allotope Antibodies									
	1ad[a] 8	*1ad* 15	*3a/1a* 16	*1a* 14	*1a* 17	*4a* 9	*3b* 23	*1bCH3* 5	*1bCH2* BG	*1bCH2* 2
Serum										
154 (Seychelles)	+	+	–	–	–	[b]	–	–	+	+
172 (Pakistan)	+	+	–	–	–	[b]	–	–	+	+
173 (Pakistan)	+	+	–	–	–	[b]	–	–	+	+
BALB/cJ	–	–	–	–	–	[b]	–	–	–	–
SJL/J	–	–	–	–	–	[b]	–	+	+	+

[a] Allotope detected in given allotypes.

[b] Cannot be determined by using this technique, because the allotype of the plate coating antibody BG is Igh-4a, therefore, when radiolabeled anti-41 9.10 is added, the radiolabeled reagent will bind to the plate coat. However, by using another anti-1b antibody, 2.9, as plate coat, allotope 9 was shown not to be on the same molecule as allotopes BG, 2, 8, and 15.

Pakistan and the Seychelles Islands in the Indian Ocean. These mice possess two closely spaced 1b determinants detected by antibodies BG1 and 2.9 on the CH2 domain of IgG2a in inbred b allotypes strains (23), but lack the other six Igh-1b determinants. By co-binding assay, these molecules have been found to carry the two CH2 1a/1d determinants (allotopes 8 and 15) but not the other three CH2 1a determinants (allotopes 14, 16, and 17) (Table VI). Such a molecule could result from a recombination within CH2 exons.

It is interesting to note that three 1a allotopes, 9, 14, and 17, were present in the Seychelles mouse by use of the allotope (RIA blocking) assay (Table IV). However, by use of co-binding assay, these allotopes were not present on the same molecule that expressed two of the eight Igh-1b allotopes (Table VI). These findings indicate that the Seychelles mouse may represent a heterozygote with one chromosome similar to that of the Pakistanean mice and the other chromosome coding for Ig molecules bearing allotopes 9, 14, and 17.

DISCUSSION

Analyses of Ig allotypes in wild mice populations were initiated years ago by Lieberman and Potter (21, 22) using Ouchterlony immunoprecipitation and electrophoresis with conventional anti-allotype antisera. The resolution of the methods then available prevented further progress. The large number of allotope-specific monoclonal antibodies, coupled with special techniques we have developed over a period of years, and recombinant DNA methodology invite a return to Ig genetic studies of wild mice. In this study, we used 20 monoclonal anti-allotope antibodies to reexamine the Ig allotype polymorphisms in wild mice collected from many different areas around the world. Many of the allotopes recognized by these antibodies have been localized to specific domains of the Ig molecules (14, 15, 23).

We have demonstrated that the Ig allotypes in natural wild mouse populations are extremely complex. Although most of the wild mice that we have tested have Ig phenotypes similar to those of inbred laboratory mice, a surprisingly high frequency of mice showed unusual Ig phenotypes. Because the available monoclonal anti-allotype antibodies were produced in mice of *a* haplotype against *b* haplotype proteins, or vice versa, this survey may greatly underestimate the complexity of Ig haplotypes. It is expected that if anti-allotope antibodies produced by other combinations (i.e., *d*-anti-*a*, *d*-anti-*b*, etc.) become available, one will find even more Ig allotypes and haplotypes both in inbred and natural wild mice populations.

The phenotypic frequencies of *Igh* allotypes and *Igh* haplotypes showed variation in their geographic distribution, ranging from zero or low frequencies in one area to high frequencies in another area. One example is allotope 23: with a high frequency in wild mice from Asia, Chile, and Poland, and negligible frequency in mice from England, Germany, Spain, France, Italy, and Egypt. Another example is the high frequency of *b* or *b*-like haplotypes in wild mice caught in Japan. In the human allotype system, it has been shown that there are striking differences in Ig haplotype composition and frequency in various populations (24, 25). Some haplotypes are characteristic of a particular race or population. Thus similar findings are also observed in wild mice populations. The occurrence of such geographic variation implies that the allotypes of Ig may be rapidly changing, and it could well be that the "Ig allotypes" themselves may have some important yet unknown functions. The differential regulation of isotype and allotype by T cells (26, 27) indicates the functional significance of allotypic and haplotypic variations. In the major histocompatibility complex system, it has been shown that the H-2 and Ia molecules are extremely polymorphic in the wild mice, and it has been speculated that the polymorphism of these molecules are vital to the survival of the individuals and the species (28–31).

One surprising finding is that even among the mice of unusual phenotypes, the two mice caught in Taiwan (w8) are phenotypically indistiguishable from the one caught in Polant (w8), and the two mice caught in Pakistan (w6) are phenotypically extremely similar to the one caught in the Seychelles Islands (w7) (Table IV). Whether the serologically indistinguishable phenotypes found in geographically distinct populations result from identical haplotypes is not known. Progeny testing by mating these wild mice with appropriate well-defined inbred strains should reveal the underlying allotypes and haplotypes of these wild mice. Expanding the battery of monoclonal anti-allotope antibodies (including antibodies to other Igh-loci, such as IgM and IgA) for phenotype analysis could also be informative.

Alternatively, biochemical comparison of serologically indistinguishable molecules by peptide mapping or two-dimensional gel electrophoresis or analysis of their constant region genes by DNA restriction mapping or sequencing analyses could also be fruitful. If the same alleles do occur in geographically distinct localities, there are several possible explantations: structural constraints on possible Ig variants, rapid dispersal of haplotypes through migration, or selective advantage for certain alleles or haplotypes.

With the recent recombinant DNA technology, the Ig heavy chain constant region genes of BALB/c have been well studied. It has been established that this gene complex consists of eight closely linked constant region genes in the order of μ, δ, γ-3, γ-1, γ-2b, γ-2a, ϵ, and α (4). Moreover, the DNA nucleotide sequences coding for the heavy chain hinge and constant region domains are encoded by tightly linked exons separated by noncoding intervening sequences, introns (32, 33). It has also been shown by both DNA and protein sequencing that

there is tremendous homology between Ig isotypes (classes), and especially between IgG2a and IgG2b CH2 domains (34–37). Furthermore, there are more homologies between the CH2 domains of *Igh-1a* and *Igh-3a* than the CH2 domains of *Igh-1a* and *Igh-1b.* These domain homologies between nonallelic but neighboring genes could lead to unequal crossing over. Intradomain or interdomain recombination or unequal crossing over between Igh-1 (IgG2a) and Igh-3 (IgG2b) in cultured cell lines have also been reported (38, 39).

It is interesting that we observed putative duplicated IgG2a in three separate wild mouse populations, i.e., in Poland, Taiwan, and Japan. Preliminary progeny analysis from one of the Polish mice, PLD 826, shows that the Igh-1a and Igh-1b allotopes segregate together, suggesting that *Igh-1a* and *Igh-1b* loci are on the same chromosome. Furthermore, the Japanese mice possessing Igh-1a and Igh-1b allotypes have most recently been shown to have duplicated *IgG2a* genes by use of Southern blot analysis (Dr. T. Honjo, personal communication). The homology between allelic genes may facilitate interdomain and intradomain recombinations as observed in Egyptian CRO mice, the Seychelles mouse, and Pakistanean mice. In the case of CRO mice, an intragenic recombination could have occurred by unequal crossing over or gene conversion. The *b* haplotype chromosome, which was thought to be rare, was also found in this population. The presence of *b* haplotype chromasomes in this locality supports the possibility of recombination events between *a* and *b* haplotypes. Differences in Ig phenotypes observed in wild mice populations may also be explained by a single-step mutation as observed in the Polish mouse PLD 828 (w10), which lacks an allotope 10.9.

Wild mice with unusual Ig phenotypes are currently being bred to inbred strains to reveal the contributing haplotypes. We are also developing homozygous strains for further analysis of the unusual chromosomes at the DNA level. It will be interesting to further explore the extent of Ig polymorphism and to try to determine what selective advantage, if any, it represents for the species. The availability of well-characterized monoclonal anti-allotope antibodies will be useful in the task of explaining genetic and structural polymorphisms of murein Ig. For example, we have already shown that membrane Igh-1a is structurally and allotypically distinct from secreted Igh-1a (40). We may expect new *Igh* 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 variations.

Acknowledgments. The authors wish to thank Doctors Leonore A. Herzenberg, Vernon T. Oi, and Paula B. Kavathas for valuable discussions, and Mrs. Huei-Jen Su Huang for excellent technical assistance. We would also like to thank Drs. Jan Klein and P.-A. Cazenave for the supply of some of the wild mice sera. The original collections of wild mice from the following persons are also acknowledged: Profs. R. J. Berry and M. Newton, Department of Genetics and Biometry, University College London, London, England; Drs. J. H. Nadeau and F. Figueroa, Max-Planck Institute for Biology, Tuebingen, West Germany; Dr. Z. Zaleska-Rutczynska, University of Warsaw, Poland; Dr. J. Vives, Hospital Clinico y Provincial, Barcelona, Spain; Dr. H. Hoogstraal, United States Naval Medical Research Unit No. 3, Cairo, Egypt; Dr. K.-Y, Wang, National Taiwan Normal University, Taipei, Taiwan.

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