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# A Chromosome Region for Gamma$_{2a}$ and Beta$_{2A}$ Globulin H Chain Isoantigens in the Mouse

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In the mouse, two gamma globulin isoantigens (allotypes, groups) controlled by alleles of a single gene locus have been reported (Kelus and Moor-Jankowski, 1961; Wunderlich and Herzenberg, 1963; Dubiski and Cinader, 1963; Dray, Lieberman, and Hoffman, 1963). We will show here that this locus is actually highly polymorphic (multi-allelic) and that the allele products are serologically cross-reacting. The cross reactions of the $\gamma$-globulins determined by the various alleles have shown that this locus is complex, and thus is reminiscent of the Gm and Rh loci in man and the H-2 locus in the mouse (Steinberg, 1962; Race and Sanger, 1958; Stimpfling and Snell, 1962). We will also describe a second locus specifying isoantigens of $\beta_{2A}$-globulins and show that this locus is genetically closely linked to the first locus.

## MATERIALS AND METHODS

We have not systematically investigated methods of immunization for production of isoantisera to gamma globulin isoantigens; however, we have found the following useful in preparing the antisera used for this study:

(1) Schedule. A primary subcutaneous injection in Freund's complete adjuvant, followed by a resting interval of approximately four weeks and then from three to eight weekly or biweekly intraperitoneal injections. After three or four injections, animals are bled and tested on a schedule compatible with their injections, so that at least one week elapses between injection and bleeding.

(2) Amount of Antigen. Immunization with 0.01 to 0.03 ml of whole serum or 10 $\mu$g of a purified fraction per injection has been generally more successful than with amounts of 0.1 ml (100 $\mu$g) or more. Antisera have been obtained with as little as one $\mu$l per injection.

(3) Source of Antigen. Whole normal serum may be used as antigen, but we have achieved better results by using whole immune serum taken from animals producing anti-tissue isoantisera, particularly if the anti-tissue isoantiserum reacts with the tissues of the mouse being immunized to produce the anti-gamma globulin antiserum. BALB/C mice are immunized to C57BL/10 gamma globulin by injection of C57BL/10 anti BALB/C tissue antiserum.

Individual samples of antisera from immunized animals were tested for precipitation on Ouchterlony plates with normal serum of several strains and also with purified serum proteins. Those sera with like patterns of reactivity were then pooled.

Most of the precipitating isoantisera which we have obtained in this fashion react with immunoglobulins. Some precipitate with other serum proteins as reported elsewhere (Erickson et al., 1964; Wortis, unpublished) and will not be discussed here.

Ouchterlony (1962) tests were carried out in agar buffered at pH 8.2 with 0.05 M barbital on microscope slides. One to two $\mu$l samples were employed in holes made with a 15 gauge needle. Immunoelectrophoresis was as described by Scheidegger (1955) but at pH 8.2.

In genetic tests, sera were obtained from progeny at least 8 weeks old to insure low levels of residual maternally transmitted proteins and expression of the animals own phenotype.

Iodine labeling with an average of 0.3–1 atom $I^{125}$ per molecule was performed by the method of Greenwood, Hunter, and Glover (1963).

## RESULTS

The first two anti $\gamma$-globulin isoantisera prepared in our laboratory were C3H/Hz (C3H) anti C57BL/10Hz (C57BL) and C57BL anti C3H. Each of these antisera reacted with its corresponding immunizing serum to form a single line of precipitation on Ouchterlony slides and a single arc of precipitation in immunoelectrophoresis corresponding to the gamma globulin arc seen with a polyvalent rabbit anti-mouse-serum (see Fig. 1). As all samples of serum from C3H and C57BL precipitated with their homologous antiserum and all samples of serum from mice of the $F_1$ hybrid cross between these two strains were positive with both antisera, we concluded that the inbred strains (as would be expected) are homozygous for the gene or genes

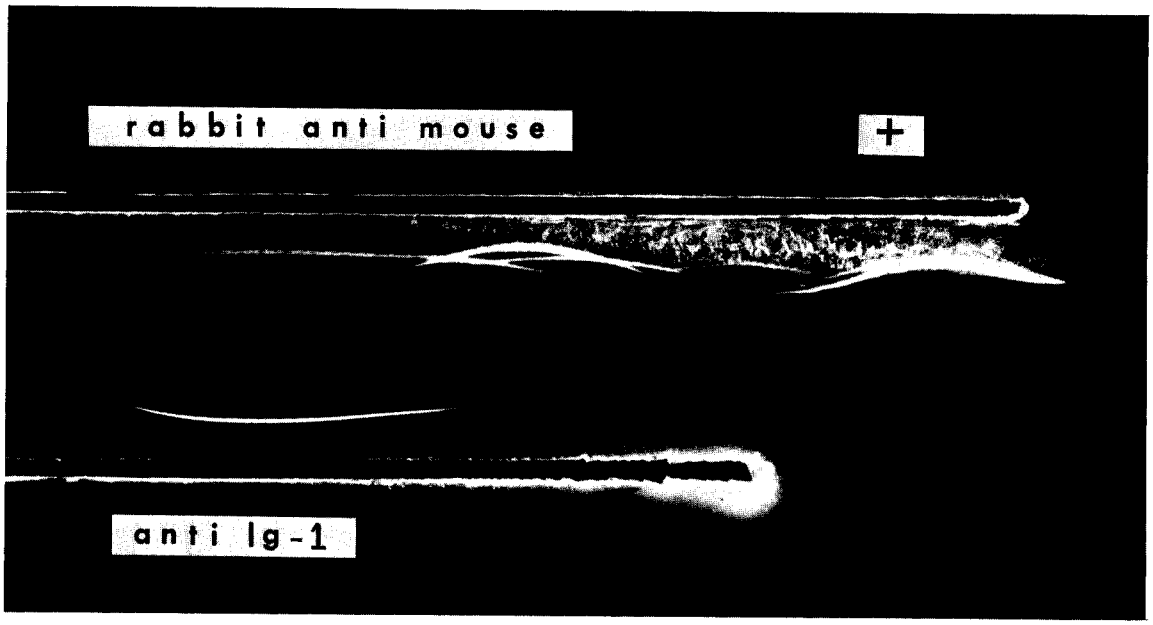


FIGURE 1. Immunoelectrophoresis of C57BL serum. Electrophoresis at pH 8.2 (see methods). Rabbit anti-mouse-whole-serum in upper trough and BALB/C anti C57BL in lower trough. Diffusion was stopped before precipitation of proteins in the mouse antiserum by the rabbit antiserum.

specifying the antigens, and that the antigens behave as codominant characters in the heterozygote.

The results of testing reciprocal backcross and $F_2$ progeny with the two isoantisera (lines 1, 2 and 3 of Table 1) indicate that these two antigens are specified by genes at a single locus (or chromosome region). We will use the name Ig-1 for this locus. (A more complete discussion of the definition and nomenclature of the Ig-1 locus is presented in the appendix.)

Other anti $\gamma$-globulin isoantisera were soon obtained from other combinations of antigen donor and recipient strains. These precipitated with sera from some, *but not all*, of the strains which precipitated with our original anti C3H and thus identified different gamma globulin isoantigens. From this information alone, we can not decide whether these new antisera react with antigens determined at different loci from Ig-1 or with different antigenic specificities determined at the Ig-1 locus itself. Table 1, lines 4 and 5 presents the results of the test crosses from which we must conclude; (1) that all the specificities are determined at the same locus and (2) that four alleles at this locus have been found.

We summarize from the data so far presented by saying that there are at least four alleles at the Ig-1 locus and that the antigens determined by at least three alleles have more than one antigenic specificity and are immunologically cross-reacting.

To further analyze the cross reactions of the $\gamma$-globulin isoantigens of the inbred mouse strains, we collected a panel of sera through the kindnesses of a number of laboratories. We have tested 64 of these by the Ouchterlony method with each of the antiserum types used in the progeny testing and have found that the strains can be divided into the four groups shown in Table 2. Each group contains strains which have the same distribution of positive and negative reactions with the four typing antisera. It should be noted that strains in different groups do in some cases react with the same antiserum. For example, some C57BL anti C3H antisera precipitate with all the strains except those in the Ig-$1^b$ group.

Because it is possible to differentiate the $\gamma$-globulins of strains within groups, we have assigned a type strain for each allele. These are as follows:

| Allele | Type Strain |
|---|---|
| Ig-$1^a$ | C3H/HeJ |
| Ig-$1^b$ | C57BL/10J |
| Ig-$1^c$ | DBA/2J |
| Ig-$1^d$ | AKR/J |

We can, in fact, further subdivide these strain groupings by several means, including (1) producing new antisera and testing $\gamma$-globulins from all the strains with each one, (2) absorbing already available antisera with each of the strain sera (or $\gamma$-globulins) and testing qualitatively for

TABLE 1. SEGREGATION OF GENES DETERMINING GAMMA GLOBULIN-ISOANTIGENS

| CROSS | | PROGENY | | | |
|---|---|---|---|---|---|
| Parent strains | Parent *Ig-1* genotypes | Tested with | Result | Total tested | Progeny *Ig-1* genotype |
| (C3H × C57BL)$F_1$ × C57BL | $\frac{a}{b} \times \frac{b}{b}$ | anti Ig-1a | +51<br>−39 | 90 | $\frac{a}{b}$<br>$\frac{b}{b}$ |
| (C3H × C57BL)$F_1$ × C3H | $\frac{a}{b} \times \frac{a}{a}$ | anti Ig-1b | +233<br>−209 | 442 | $\frac{b}{a}$<br>$\frac{a}{a}$ |
| (C3H × C57BL)$F_1$ × | $\frac{a}{b} \times \frac{a}{b}$ | (a) anti Ig-1b<br>(b) anti Ig-1b<br>anti Ig-1a | +202*<br>−<br>+ } 78** | 280 | $\frac{b}{a} + \frac{b}{b}$<br>$\frac{a}{a}$ |
| (C3H × DBA/2)$F_1$ × B10 . D2 | $\frac{a}{c} \times \frac{b}{b}$ | (a) anti Ig-1a<br>anti Ig-1c<br>(b) anti Ig-1a<br>anti Ig-1c | +<br>− } 78<br>−<br>+ } 71 | 149 | $\frac{a}{b}$<br>$\frac{c}{b}$ |
| (AKR × DBA/2)$F_1$ × C57BL | $\frac{d}{c} \times \frac{b}{b}$ | (a) anti Ig-1c<br>anti Ig-1d<br>(b) anti Ig-1d<br>anti Ig-1c | +<br>− } 71<br>+<br>− } 82 | 153 | $\frac{c}{b}$<br>$\frac{d}{b}$ |

Anti Ig-1a = C57BL anti C3H
Anti Ig-1b = BALB/C anti C57BL/6
Anti Ig-1c = C57BL anti DBA/2
Anti Ig-1d = DBA/2 anti AKR

* Not tested for segregation of Ig-1a. ** Sera from only 51 of the 78 Ig-1b negative animals were available for testing with anti Ig-1a. All were Ig-1a positive.

TABLE 2. DISTRIBUTION OF Ig-1 ANTIGENS IN INBRED MOUSE STRAINS

| Ig-$1^a$ | | | Ig-$1^b$ | |
|---|---|---|---|---|
| BALB/CGa | C58/J | POLY 1/Ao | B10.D2(new)/Hz | SJL/J |
| BDP/J | DE/J | POLY 2/Ao | B10.D2(old)/Hz | SM/J |
| BSL/Di | F/Ao | PRUNT/Ao | C57BL/H | STA/Je |
| BUB/Bn | H-2G/Go | SEA/Gn | C57BL/Ka | WB/Re |
| CBA/J | JK/Bi | SEC/Gn | C57BL/6J | WH/Re |
| CE/J | MA/J | ST/J | C57BL/10Hz | WC/Re |
| CHI/Ao | MA/MyJ | STR/N | H-2H/Go | WK/Re |
| C3H/Hz | N/Ao | T6/H | H-2I/Go | 58N/Sn |
| C3H.SW/Hz | P/J | 129/RrGa | HG/Hu | 101/R1 |
| C57BR/cdJ | PBR/Ao | | LP/J | |
| C57L/J | PL/J | | | |

| Ig-$1^c$ | | Ig-$1^d$ |
|---|---|---|
| DBA/1J | JB/Di | AL/N |
| DBA/2J | RF/J | AKR/J |
| DA/Hu | RIII/J | A/J |
| FZ/Di | STB/Je | NZB/B1 |
| I/Ao | SWR/J | |

residual precipitins by Ouchterlony test with each of the other strains, and (3) a quantitative analog of 2 which determines cross reactions by measuring inhibitions of precipitation of labeled reference antigens. This is an adaptation of a method of estimating total $\gamma$-globulin (Weiler, Hofstra, Szentivanyi, Blaisdell, and Talmage, 1960).

In screening the normal serum panel to recognize new alleles, we have found the third procedure to be most useful. We will present a detailed description and validation of this procedure elsewhere. A brief description here will be adequate for our purpose.

By agar-gel or starch-Pevikon-block electrophoresis, we prepare the slow migrating $\gamma_2$-globulins from a type strain (e.g. C3H) and trace label this fraction with $I^{125}$. We then choose a standard concentration of this labeled reference antigen and, with several antisera, determine the amount of antiserum which will precipitate most, but not all, of the precipitable counts. That is, we determine conditions under which precipitation is occurring in slight antigen excess near equivalence.

Under such conditions, when an identical unlabeled antigen is added, the number of counts precipitated is diminished in relation to the amount of unlabeled antigen. Thus, a large excess of unlabeled antigen completely inhibits the precipitation of $I^{125}$ labeled antigen. Only partial inhibition by large amounts of other test antigens is an indication that the test antigen is not identical to the labeled reference antigen, but is able to react with a portion of the antibody population, preventing that (cross-reacting) portion from precipitating the labeled antigen. To illustrate this method, an example of the inhibitions by a few strains with one antiserum and one labeled antigen is shown in Table 3.

TABLE 3. INHIBITION OF PRECIPITATION OF A REFERENCE ANTIGEN

| Inhibitor serum | % Inhibition |
|---|---|
| C57BL/10Hz | 0 |
| C3H/Hz | 102 |
| DBA/2J | 5 |
| AKR/J | 29 |
| DE/J | 15 |
| SEC/Gn | 49 |
| BSL/Di | 68 |
| A/J | 90 |

Antigen, $\gamma_2$-globulin fraction, 0.04 $\mu$g; inhibitor serum, 2 $\mu$l; antiserum C57BL anti C3H, 0.2 $\mu$l; diluent, 5% normal rabbit serum, 3% bovine serum albumin in 0.05 M Tris, pH 7.6. Total volume 100 $\mu$l. Incubated 3 hr at 37°, sedimented at 10,000 rev/min.

Antiserum: C57BL anti C3H
$I^{125}$-Labeled Antigen: C3H

By Ouchterlony testing of our panel of sera with the antisera we have available, only four allele groups of strains were distinguished. Nevertheless, although we are in the midst of the analysis of cross reactions using the inhibition of labeled antigen, we have evidence of several more groups which can be antigenically differentiated from C3H. Each strain or group of strains which has $\gamma$-globulin antigenically distinct from all the other strains defines a new allele. Of those strains listed in Table 2 as Ig-$1^a$, at least CE/J, DE/J, BDP/J, BSL/J, SEA/Gn, SEC/Gn, P/J and N/Ao are antigenically distinct from C3H by the inhibition test. CE/J and DE/J, and SEC/Gn and SEA/Gn appear to constitute two groups. There are differences between the rest of these strains but further work is needed to decide how many and which allele groups can be formed from them. In addition, we have clearly distinguished AL/N and AKR/J respectively from A/J and NZB/Bl, showing that the Ig-$1^d$ group is really at least two allele groups. The Ig-$1^b$ and Ig-$1^c$ groups have not yet received much attention and remain undivided. We can predict that these groups contain strains with alleles different from the type strain of each group.

## CROSS REACTIONS AND ANTIGENIC SPECIFICITIES

Having established the existence of a number of alleles at the Ig-1 locus, many of which determine molecules which partially cross-react with the others, we can now begin to analyze these cross reactions. A compact representation of cross reactions among antigen molecules is to consider that each one has several antigenic specificities, some of which are also represented on other antigen molecules. By screening of antisera from individual animals, C57BL anti C3H antisera have been found, some of which precipitate with C3H but not DBA/2 $\gamma$-globulin, and others of which precipitate both C3H and DBA/2 $\gamma$-globulin. We reason from this that C3H has one specificity which it does not share with DBA/2 and a second specificity which it does share with DBA/2. Similarly, some C57BL anti DBA/2 antisera have been found which precipitate DBA/2 but not C3H while others precipitate $\gamma$-globulin of both strains. This indicates that DBA/2 has at least one specificity which it does not share with C3H. The antigenic specificities of C3H and DBA/2 thus defined, plus further specificities added by similar studies with AKR and C57BL antigens and more antisera, are represented in Table 4. The number of specificities defined is always the minimum number compatible with the cross reactions observed. As more cross

reactions are worked out with more strains, this table will be extended.

The existence of shared specificities leads us to predict, as confirmed by the pattern in Fig. 2, that on an Ouchterlony plate with C3H and DBA/2 sera in adjacent holes, the line of precipitation between C3H and a central hole containing the

TABLE 4. SPECIFICITIES IN Ig-1 ALLELES

| Type strain | Allele | Specificities |
|---|---|---|
| C3H | Ig-1[a] | 1 2 — — — 6 |
| C57BL | Ig-1[b] | — — — 4 — — |
| DBA/2 | Ig-1[c] | — 2 3 — — — |
| AKR | Ig-1[d] | 1 2 — — 5 — |

cross-reacting C57BL anti C3H partially fuses and partially spurs over the precipitation line between the DBA/2 hole and the central hole. (See Ouchterlony, 1962, for general discussion of significance of spurs.) The difficulty in seeing spurs when cross-reacting sera are compared on Ouchterlony plates points out another reason for the greater usefulness of the inhibition test, using labeled antigen as described above, in recognizing cross reactions.

TABLE 5. PRECIPITATION OF LABELED ANTIGEN BY HOMOLOGOUS AND CROSS-REACTING ANTISERA

| Antiserum | | % of labeled Ig-1a (C3H) $\gamma_2$-globulin fraction ppt'd |
|---|---|---|
| Type | $\mu$l added | |
| Ig-1b (C57BL) anti | 1 | 75 |
| Ig-1a (C3H) | 2 | 74 |
| Ig-1b (C57BL) anti | 1 | 75 |
| Ig-1c (DBA/2) | 2 | 71 |
| Both antisera together | 1 + 1 | 72 |
| | 2 + 2 | 72 |

Each tube contains 0.04 $\mu$g $I^{125}$ labeled $\gamma_2$-globulin fraction and indicated antiserum volume. Total volume = 0.1 ml, diluent = 5% normal rabbit serum and 3% bovine serum albumin in 0.05 M Tris buffer, pH 7.6.

In this discussion of the antigenic analysis, we have tacitly made two corollary assumptions, namely that the antigenic specificities are (1) all on the same molecules of $\gamma$-globulin, and (2) determined at the same locus.

Assumption (1) is supported by our finding that a labeled $\gamma$-globulin is precipitated to the same extent by antisera made against this $\gamma$-globulin type or by antisera made against a cross-reacting $\gamma$-globulin. For example, as shown in Table 5, the

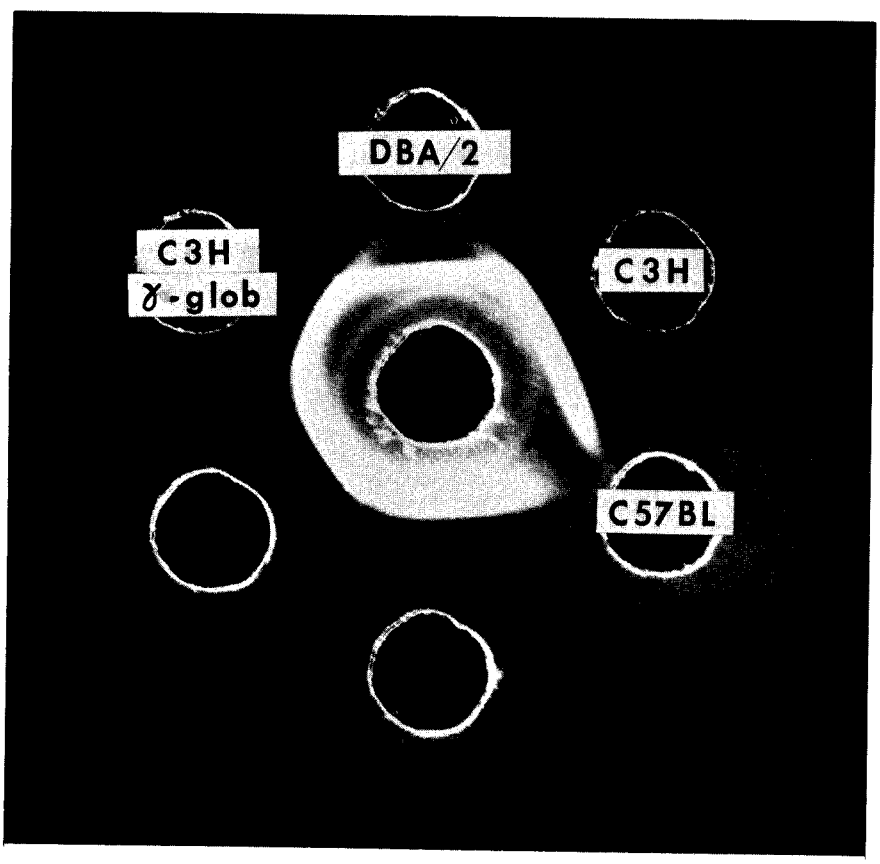


FIGURE 2. Cross reaction of DBA/2(Ig-1[c]) with C3H(Ig-1[a]). Center well contains C57BL (Ig-1[b]) anti C3H(Ig-1[a]).

labeled C3H $\gamma$-globulin which is precipitated with an anti C3H serum (made in C57BL) is equally precipitated by an anti DBA/2 serum (also made in C57BL) or by a mixture of both antisera. This experiment shows that each C3H molecule has at least one antigenic specificity shared by DBA/2. However, these results do not preclude the possibility that different 7 S $\gamma$-globulin molecules in a serum may be carrying different specificities.

Assumption (2), that the antigens are determined at a single locus, requires a statement of the meaning of the term "locus" in a mammal. "Locus" in higher organisms has come to be synonymous with "cistron" in microbial genetic terminology, that is, the region of chromosome coding for the structure of a polypeptide chain. The original definition of locus as a region not separable by crossing-over has been neglected whenever it became inconsistent with the newer structural definition (Lewis, 1952; Benzer, 1961). Thus, for our purposes here, the association of the various Ig-1 antigens with a single polypeptide would be the most cogent evidence for terming *Ig-1* a locus. We have already provided evidence that the Ig-1 antigenic specificities are on the same protein molecules and the demonstration that they are associated with only one constituent polypeptide of these molecules will be discussed below. Genetic recombination, crossing-over, may be found between *Ig-1* alleles. Though we have not observed one in several crosses with a total of several hundred progeny (data in Table 1), it may well be that previous cross-overs account for the large number of cross-reacting alleles at this locus, as has been postulated for other complex loci (Dunn, 1964). We are now looking for direct evidence of recombinations of the antigenic specificities by both Ouchterlony scoring of test cross-progeny with "unicomponent" antisera and by the inhibition of $I^{125}$ labeled $\gamma$-globulin precipitation method described earlier.

### Molecular Location of Isoantigens

Evidence for the association of *Ig-1* antigens with a single polypeptide has been obtained by Mishell and Fahey (1964). Fahey, Wunderlich, and Mishell (1964a and b) have found two classes of 7 S $\gamma$-globulins in the mouse which they call $\gamma_1$ and $\gamma_2$-globulins. The $\gamma_2$-globulins have been shown to consist of 2 subclasses, $\gamma_{2a}$ and $\gamma_{2b}$. These classes and subclasses are different from one another only in that portion of the molecule containing part of one of the constituent polypeptide chains (the H-chain). That is, each of these classes of $\gamma$-globulins has an H polypeptide unique to it. Using a C57BL anti C3H antiserum, Fahey et al. (1964b) have found that the *Ig-1* antigens are associated only with $\gamma_{2a}$. With the reservations that $\gamma_{2a}$ may be further subdivisible into subclasses and that the Ig-1 specificities of other strains were not tested by Fahey et al., we may conclude that *Ig-1* is a locus determining the H polypeptide chain of $\gamma_{2a}$-globulin.

### Second Locus

Some anti DBA/2 isoantisera form two lines of precipitation on Ouchterlony tests with DBA/2 serum. One of these is due to reaction with $Ig\text{-}1^c$ present on $\gamma_{2a}$ (7 S) globulin. The second line is due to reaction with a serum protein which the following evidence shows is $\beta_{2A}$ ($\gamma_{1A}$): it has an electrophoretic mobility on agar-gel and starch-block identical with $\beta_{2A}$; it sediments in zonal sucrose gradient centrifugation partly in the 7 S region and extending almost to the 19 S region; it is absent in newborn and nursling serum: and finally, it is completely precipitated with a specific rabbit anti-mouse $\beta_{2A}$ (kindly provided by Dr. John Fahey).

We have now found a serum which recognizes a $\beta_{2A}$ isoantigen in the C3H strain. Genetic tests entirely analogous to those with *Ig-1* antigens show that these $\beta_{2A}$ isoantigens are also controlled by genes at a single locus, designated *Ig-2*. Only the C3H and DBA/2 alleles at this locus are so far identified directly. There is at least one more allele to be recognized, as a number of strains exist which have neither the C3H or the DBA/2 antigen.

The linkage of *Ig-1* and *Ig-2* has been investigated in the cross in Table 6. No recombinations of alleles at these two loci have been seen in 149 animals tested, an indication of quite close linkage.

Screening of the normal sera from our panel of 64 strains showed (Table 7) that the Ig-2a antigen is found only in some strains carrying the $Ig\text{-}1^a$ allele and in no others, while the Ig-2c antigen is

Table 6. Linkage of Ig-1 and Ig-2

| Cross | Progeny | |
|---|---|---|
| $(C3H \times DBA/2)_{F_1} \times C57BL$ | Parental Types | |
| | $\frac{Ig\text{-}1^a \quad Ig\text{-}2^a}{Ig\text{-}1^b \quad -}$ | 78 |
| $\frac{Ig\text{-}1^a \quad Ig\text{-}2^a}{Ig\text{-}1^c \quad Ig\text{-}2^c} \times \frac{Ig\text{-}1^b \quad -}{Ig\text{-}1^b \quad -}$ | $\frac{Ig\text{-}1^c \quad Ig\text{-}2^c}{Ig\text{-}1^b \quad -}$ | 71 |
| | Recombinant Types | |
| | $\frac{Ig\text{-}1^a \quad Ig\text{-}2^c}{Ig\text{-}1^b \quad -}$ | 0 |
| | $\frac{Ig\text{-}1^c \quad Ig\text{-}2^a}{Ig\text{-}1^b \quad -}$ | 0 |
| | Total tested | 149 |

TABLE 7. DISTRIBUTION OF Ig-2 ANTIGENS AMONG THE 64 STRAINS TESTED

| *Ig-2* Allele | Distribution |
|---|---|
| *Ig-2*$^a$ | All *Ig-1*$^a$ strains except: CE/J, DE/J, JK/Bi, N/Ao, PBR/Ao, PL/J, PRUNT/Ao. |
| *Ig-2*$^c$ | All *Ig-1*$^c$ strains. |
| Silent allele(s) | All other strains. |

See Table 2 for *Ig-1* antigen distributions.

found in all the strains carrying *Ig-1*$^c$ and in no others. The distributions of the alleles at the two loci are clearly not independent. It could well be asked, then, what is the evidence that these are two loci? Could it not be that the antigenic configuration detected on $\gamma_{2a}$-globulin and $\beta_{2A}$-globulin are different phenotypic manifestations of the same gene; i.e., pleiotropic effects of this gene?

Earlier in this discussion we defined a locus as a region of the chromosome specifying the structure of a single polypeptide chain. We presented evidence reported by Mishell and Fahey (1964) that Ig-1 antigens are associated with the F-papain pieces, which contain part of the H chain, and therefore the portion of the molecule known to be unique to 7 S-$\gamma_{2a}$ gamma globulins. Mishell and Fahey (personal communication) have now localized the Ig-2 antigens to the "F" pieces (from papain digestion) of $\beta_{2A}$ and thus, these antigens are also associated with the $\beta_{2A}$ class specific polypeptide chain, the analog of the H chain. Thus, the Ig-1 and Ig-2 antigens in the two immunoglobulin classes are associated with different polypeptide chains and cannot be different expressions of a common polypeptide chain.

A more remote possibility, which we cannot rule out, is that Ig-1 and Ig-2 are the same locus which codes for an enzyme which modifies $\gamma_{2a}$ and $\beta_{2A}$ H chains respectively, rather than for the structure of the H chains themselves. On this hypothesis, each allele would have to determine an enzyme molecule which could simultaneously convert $\gamma_{2a}$ and $\beta_{2A}$ into antigens different from each other and from those determined by other alleles. It is difficult to see, among other things, how such a mechanism could account for the cross reactions of the Ig-1 antigens.

Thus, we visualize Ig-1 and Ig-2 as two loci controlling class-specific polypeptides of two immunoglobulins. We can look forward to the definition of loci for other of these polypeptides as well as for a locus for the common (L chain) polypeptide. (See Appendix.)

## HEMOGLOBIN ANALOGY

On the basis of (1) frequently observed simultaneous depression of levels of two or more immunoglobulin classes in human patients with hypogammaglobulinemia and their relatives and (2) the then-available evidence on structural subunits of the immunoglobulins, Fudenberg, Heremans, and Franklin (1963) suggested that the immunoglobulins might be under genetic controls similar to those of the hemoglobins.

Taking into account more recent structural knowledge of the immunoglobulins, the analogy with the hemoglobins is striking. In each group of proteins, all classes within the group have one common polypeptide chain ($\alpha$ for hemoglobin and L for immunoglobulins) and each class has a unique polypeptide chain which specificies the class. Table 8 illustrates this chain composition analogy. (The possibility suggested from recent evidence [Todd, 1963] in the rabbit that there is a third, probably common, chain in the immunoglobulins does not interfere with the analogy for two chains.)

In man, the L and H chain antigenic differences (and probably, but not necessarily, the polypeptide structures) are controlled by two unlinked gene loci, Inv and Gm (Steinberg, 1962). In the mouse, we have not yet found a gene to identify with the L chains but have identified H chain loci for two immunoglobulin classes, $\gamma_{2a}$ and $\beta_{2A}$. As discussed above, these loci, *Ig-1* and *Ig-2* are closely linked. In human hemoglobins the three "class specific" polypeptides are controlled by closely linked genes as well (Zuckerkandl, 1964). Thus, the hemoglobin analogy proposed by Fudenberg, Heremans, and Franklin is extended by the genetic evidence from these investigations with the mouse.

TABLE 8. ANALOGY OF HEMOGLOBIN AND IMMUNOGLOBULIN POLYPEPTIDE COMPOSITION

| Hemoglobins | | | Immunoglobulins | | |
|---|---|---|---|---|---|
| Protein | Polypeptide | | Protein | Polypeptide | |
| Hemoglobin | Common | Specific | | Common | Specific |
| A | $\alpha$ | $\beta$* | $\gamma_{2a}$ | L | $H^{\gamma_{2a}}$† |
| $A_2$ | $\alpha$ | $\delta$* | $\gamma_{2b}$ | L | $H^{\gamma_{2b}}$† |
| F | $\alpha$ | $\gamma$* | $\gamma_1$ | L | $H^{\gamma_1}$ |
| | | | $\beta_{2A}$ | L | $H^{\beta_{2A}}$† |
| | | | $\beta_{2M}$ | L | $H^{\beta_{2M}}$ |

* Determined by closely-linked genes.
† Determined by closely-linked genes.
The nomenclature for the H-chains is an ad hoc one for use in this table.

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## APPENDIX

A notation for mouse immunoglobulin loci and their phenotypes has now been generally adopted and several notations have now been published (see introduction). In this paper, we have followed the recommendations resulting from a discussion between members of the Committee on Standardized Genetic Nomenclature for Mice (see Committee, 1963) and some of those workers who have published on immunoglobulin isoantigens of mice. The recommendations are that loci concerned with immunoglobulins be given the class symbol Ig followed by a number, i.e., Ig-1, Ig-2, etc., in order of discovery, and that alleles be assigned lower case letters as superscripts. This notation is analogous to that used for the human gamma globulin groups except that a class symbol has not been adopted for the human immunoglobulins.

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