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The Chromosome Region for Immunoglobulin Heavy Chains in the Mouse: Allelic Electrophoretic Mobility Differences and Allotype Suppression

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It is only about three years since it was first recognized explicitly that there are separate genes for the various immunoglobulin heavy chains that distinguish the heavy chain classes. At the 1964 Cold Spring Harbor meeting, evidence for the close linkage of these genes was presented for man (Kunkel et al., 1964) and the mouse (Herzenberg, 1964). We then reported that the two genes for a γG-globulin and γA-globulin marked by allelic antigenic differences or allotypes did not recombine in 78 backcross animals tested, indicating quite close linkage. Further, on examining the alleles for the two loci in some 80 inbred strains of mice we found a complete association between γA and γG alleles except in a few cases where there was too little serum γA to detect the allotype by the relatively less sensitive Ouchterlony test then being used. When we employed the more sensitive inhibition of I^{125} labeled antigen precipitation (Herzenberg et al., 1965), complete association was observed (Herzenberg and Warner, 1967).

The mouse allotypic antigens on γG and γA are localized to the respective Fc fragments (Mishell and Fahey, 1964). They are distinct and generally non-cross-reacting for the two classes. This is in sharp contrast to the rabbit H chain allotypes which are associated with the Fd fragments and are quite similar (cross-reacting) for γA and γG as well as γM (Oudin, 1966).

Soon after the discovery of Ig-1 and Ig-2, Lieberman and Potter, and we added a third gene, Ig-3, coding for the H chain of γG_{2b}-immunoglobulin to the H chain gene cluster, again using antigenic or allotypic markers for the genetic scoring (Lieberman et al., 1965; Herzenberg, unpubl.). Close to 2,000 progeny have now been tested between both laboratories without a single crossover having been found, indicating that the map distance between any two elements tested is less than 0.1 centiMorgan (map unit).

For the other two recognized H chain classes of mouse immunoglobulins neither we nor, to our knowledge, anyone else has been able to find allotype differences by serological methods. Therefore we turned to other approaches to show genetic differences in γG_1 and γM-globulins. Recently a medical student in our laboratory, John Minna, made use of allelic electrophoretic mobility differences of γG_1-globulins and γG_1 Fc fragments to link the gene (designated Ig-4) for this H chain class to the other H chain genes in the mouse (Minna et al., 1967).

In Fig. 1 the precipitin arcs obtained on immunoelectrophoresis are depicted for γG_1 whole molecules and Fc fragments obtained by papain digestion. By developing with specific heterologous anti γG_1 sera, no purification of γG_1 is necessary to obtain these patterns. Papain digestion and electrophoresis is simply carried out on whole serum. The ease of scoring three phenotypes (fast, slow, and fast-slow) is shown in Fig. 2.

Two hundred and one F_2 progeny were scored for Ig-4 in this manner (Table 1) and for Ig-1 serologically. No recombinant types were found, providing evidence for close linkage of Ig-4 to Ig-1, Ig-2, and Ig-3.

ELECTROPHORETIC MARKERS FOR OTHER CLASSES

Although anti-allotype sera for the three classes of immunoglobulins controlled by the Ig-1, Ig-2, and Ig-3 genes have been prepared, reasonable amounts of strong precipitating antisera are sometimes quite difficult to obtain. Therefore we thought it would be useful to see if genetic immunoelectrophoretic mobility differences could be found for these classes.

Rabbit antisera specific for each class were made by immunization with purified myeloma proteins or Fc fragments of these proteins and absorption of the antisera with myeloma proteins of the other classes. With an adequate supply of purified myeloma proteins, these specific antisera are readily produced. Papain digests of the immunoglobulins in whole sera from a number of mouse strains were then subjected to electrophoresis and developed with these specific antisera. All the types of immunoelectrophoretic patterns obtained are shown in Fig. 3.

Within each of the three γG classes determined by the genes Ig-1, Ig-3, and Ig-4, two different

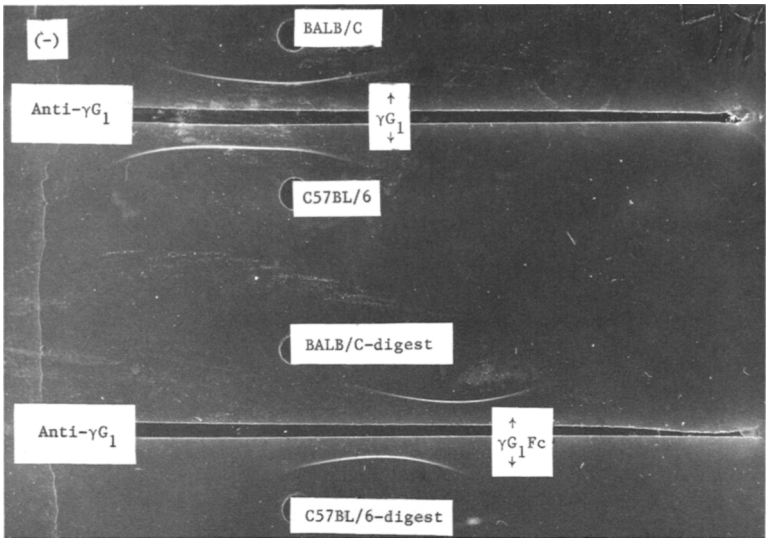

FIGURE 1. Electrophoretic mobilities of Ig-4 (γG_1) intact molecules and Fc fragments.

The upper two wells contained whole normal serum and the lower two wells contained 1 hr papain digests of these sera. Electrophoresis was carried out in 1% Ionagar No. 2 in 0.05 M barbital buffer, pH 8.2 for 90 min at 5 v/cm. Fifty to 75 μl of specific rabbit anti-γG_1 serum was then allowed to diffuse from troughs for 18 to 24 hr.

mobility types were found. In the γA class, determined by Ig-2, at least three readily distinguishable mobility types were found. Insufficient yields of γM Fc fragments precluded attempts to find mobility differences for this class of immunoglobulin.

TABLE 1. LINKAGE OF Ig-4 WITH Ig-1 (Hence Ig-2 and Ig-3)

	Ig-4^a	Ig-4^a/Ig-4^b	Ig-4^b
Ig-1^a	53	0	0
Ig-1^a/Ig-1^b	0	100	0
Ig-1^b	0	0	48

Ig-1 alleles were scored serologically. Ig-4 alleles were scored by electrophoretic mobility. Ig-1 determines γG_{2a}, Ig-4 determines γG_1. Progeny scored were from

(C3H·SW/SnHz × C57BL/10SnHz)F_1

or (BALB/cJ × C57BL/10)JF_1.

The associations between the electrophoretic mobility types and allotypes for the three immunoglobulin classes in which both were found were tested in two ways: progeny testing and strain typing. The results of progeny testing are shown in Table 2. To obtain the data in the first section of Table 2, 24 progeny of an F_2 generation selected to contain 12 of each homozygous allotype and 12 allotype-heterozygotes were scored for γG_{2a} Fc electrophoretic mobility. A complete concordance is seen. For the second part of Table 2 progeny of the cross (Ig-3^c/Ig-3^d) × (Ig-3^d/Ig-3^b) were scored serologically for allotype and then for γG_{2b} Fc electrophoretic mobility. Again complete concordance of the two methods of scoring is seen. Finally, γA Fc electrophoretic mobility is completely associated with serological allotype in progeny of the cross illustrated in the last part of Table 2.

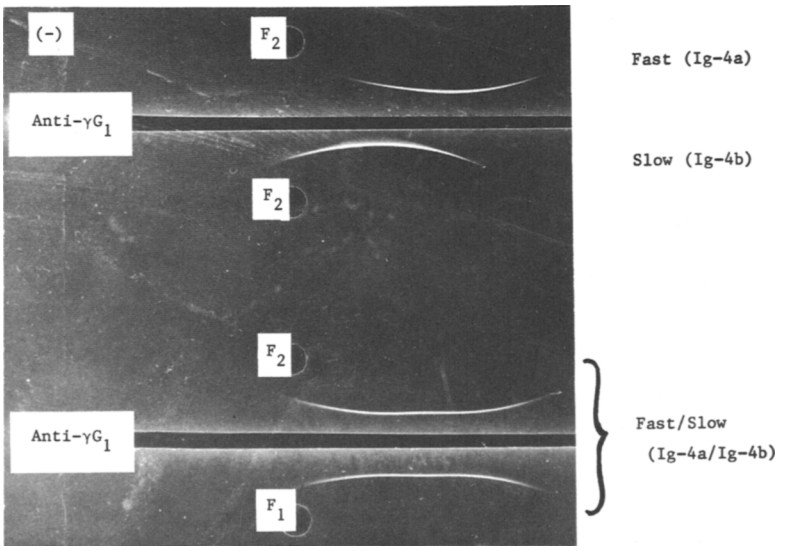

FIGURE 2. Phenotypes of Ig-4 (γG_1) Fc fragments.

In the wells were placed papain digests of whole serum from individual F_2 and F_1 progeny of the cross C3H·SW (Ig-4^a) × C57BL/10 (Ig-4^b). The arcs developed with rabbit anti-γG_1 sera show the fast, slow, and mixed phenotypes. Conditions for immunoelectrophoresis as in Fig. 1.

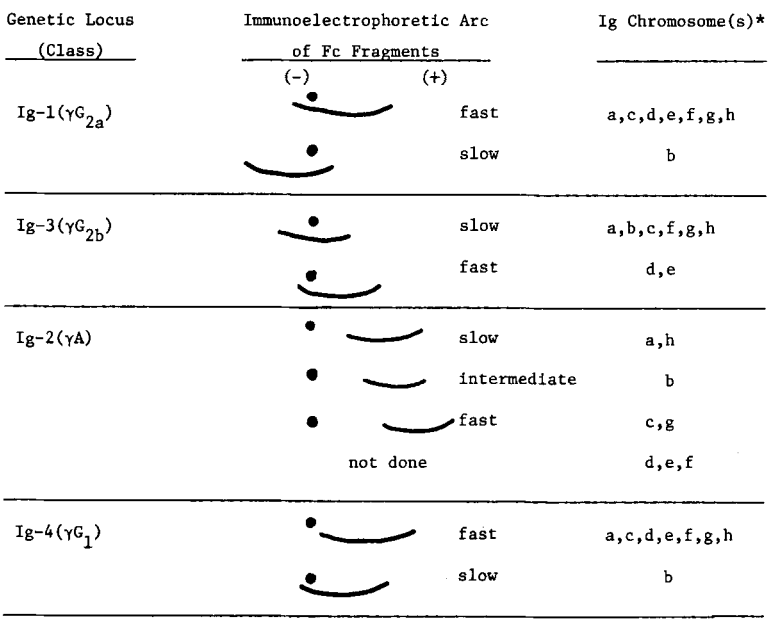

FIGURE 3. Electrophoretic mobility markers for distinguishing some immunoglobulin allotypes. The arcs are drawn to scale from immunoelectrophoretic patterns obtained with appropriate antisera as in Fig. 1.

* Defined by Ig-1 allele in type-strains: a= BALB/C, b= C57BL/10, c = DBA/2, d = AKR, e = A/J, f = CE, g = RIII, h = SEA.

Strain typing for electrophoretic mobility was carried out in 36 inbred strains chosen so as to have representatives from each of the eight Ig-1 allele groups. These representative strains were as unrelated in strain history as possible within these groups and as closely related as possible between groups. Within each group, all strains were indistinguishable electrophoretically from the respective type strains shown in Fig. 3. Thus again we see no evidence that crossing-over in the H chain region has occurred during the development of the currently available inbred strains of mice.

In concluding this part of my presentation, I should point out that these mobility differences serve as useful genetic markers for immunoglobulins and the cells producing them. For many purposes electrophoretic mobility may provide a convenient substitute for serological determination of allotype.

The second part of this presentation is work

TABLE 2. IDENTITY OF PROGENY SCORING BY ALLOTYPE AND ELECTROPHORETIC MOBILITY

Genetic locus (class)	Cross[1]	Allotypes of progeny	Fc electrophoretic mobility[2]		
			Fast	Fast-Slow	Slow
Ig-1 (γG_{2a})	$\frac{Ig\text{-}1^a}{Ig\text{-}1^b} \times \frac{Ig\text{-}1^a}{Ig\text{-}1^b}$	$Ig\text{-}1^a/Ig\text{-}1^a$	12	0	0
		$Ig\text{-}1^a/Ig\text{-}1^b$	0	12	0
		$Ig\text{-}1^b/Ig\text{-}1^b$	0	0	12
	Fast/Slow × Fast/Slow				
			Slow-Inter		Fast-Inter
Ig-2 (γA)	$\frac{Ig\text{-}2^a}{Ig\text{-}2^c} \times \frac{Ig\text{-}2^b}{Ig\text{-}2^b}$	$Ig\text{-}2^a/Ig\text{-}2^b$	17		0
		$Ig\text{-}2^c/Ig\text{-}2^b$	0		13
	Slow/Fast × Inter				
			Slow		Slow-Fast
Ig-3 (γG_{2b})	$\frac{Ig\text{-}3^c}{Ig\text{-}3^d} \times \frac{Ig\text{-}3^b}{Ig\text{-}3^b}$	$Ig\text{-}3^c/Ig\text{-}3^b$	16		0
		$Ig\text{-}3^b/Ig\text{-}3^b$	0		20
	Slow/Fast × Slow				

[1] Strains used in crosses: γG_{2a} and γG_1—(C3H·SW/SnHz × C57BL/10SnHz)F_1 ⊗(BALB/cJ × C57BL/10J)F_1⊗
γA —(B10·D2/SnHz × DBA/2J)F_1 × C3H/HeJ
γG_{2b} —(DBA/2J × AKR/J)F_1 × C57BL/10J

[2] Relative electrophoretic mobilities within each class (i.e. fast, slow, intermediate) were scored independently of other classes with the aid of class-specific heteroantisera. A cross for each class was chosen where the allotypic differences were associated with different electrophoretic mobilities of Fc fragments. From progeny of these crosses, a group of animals previously scored for allotype were chosen for mobility studies in approximately equal numbers of each progeny genotype.

primarily of my wife Leonore A. Herzenberg, done in the laboratory of Dr. Robert C. Goodlin and with the technical assistance of Miss Edna Rivera. The remarkable phenomenon of allotype suppression was described several years ago in rabbits by Dray (1962). The most recent extensions of this work are to be found in this volume in the articles by R. Mage and by S. Dubiski. Dray originally found that female rabbits, homozygous for one allotype, immunized against a second allotype and then mated with males homozygous for this second allotype, gave birth to offspring in which the production of the paternal allotype was suppressed. This suppression was later shown to last for months and even years and to be quantitatively variable in different rabbits (Mage and Dray, 1965).

We have found a similar suppression of allotype synthesis in heterozygous mice (Herzenberg and Herzenberg, 1966). More recently we have shown that allotype suppression may be obtained in homozygotes as well as in heterozygotes. Figure 4 shows the time course of the appearance of Ig-1^b allotype in suppressed and nonsuppressed Ig-1^a/Ig-1^b heterozygotes and in suppressed and nonsuppressed Ig-1^b homozygotes. Since in the mouse, γG_{2a}-globulins carrying the Ig-1 allotype are transferred from mother to young for roughly the first 14 days of the suckling period, Ig-1^b homozygotes used for this study were transferred at birth to either immunized or nonimmunized foster mothers, none of whom had the Ig-1^b allotype. The suppression seen results from the anti-allotype antibody taken up during suckling on an immune nurse. Calculations based on the curves in Fig. 4 show that the absolute synthetic rates of Ig-1^b are suppressed during the entire period of observation presented in this figure.

Suppression after the neonatal stage can be obtained by injection of antiserum at any time until the animal has synthesized substantial amounts of the allotype to be suppressed. A particularly striking example of this is shown in Fig. 5. Ig-1^b homozygotes were foster nursed on mothers lacking the Ig-1^b allotype. By 25 days of age these foster nursed animals had only very low levels of Ig-1b. They were then injected with 30 μl of anti-Ig-1b serum. As can be seen from Fig. 5, there is a profound and continuing suppression of Ig-1b synthesis in the animals injected with anti-Ig-1b, as compared to the controls who were injected with normal serum.

Allotype suppression thus works on some control mechanism(s) regulating the levels of immunoglobulins. We have shown that the catabolism of the particular immunoglobulin is not increased, so this regulation must be at some step or steps in the synthetic process. The duration of acute suppression in the mouse has so far been observed to be measurable in weeks, rather than in years as in the rabbit. Any hypothesis attempting to explain allotype suppression must account for this difference in severity and persistence. The following scheme, which we offer as a framework for further studies, has concepts drawn freely from many sources (see e.g., the review by Makinodan and Albright, 1966).

For convenience in writing, the following deals only with H chain control. It is meant to refer

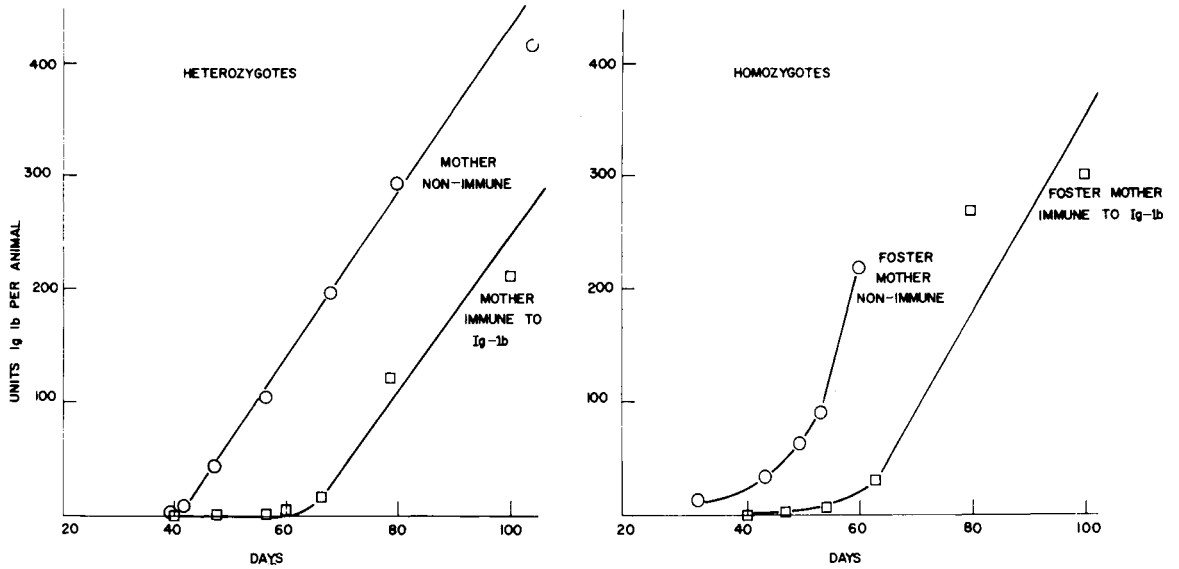

FIGURE 4. Allotype suppression in homozygotes and heterozygotes. A unit of Ig-1b equals the amount of Ig-1b in 1 μl of a standard pool of C57BL/6 adult normal serum. Units per animal are calculated from the determined units per ml serum and the estimated gamma globulin space (8.8% body weight). Points are averages of 3 litters of approximately 6 animals per litter. "Days" equals age of animal.

equally to L chains. Let us suppose that the embryonic immunoglobulin stem cell is totipotent with respect to class and allotype of immunoglobulin its progeny can produce. It contains two chromosomes, both of which carry the loci for all classes of immunoglobulin, but each of which, in an allotypic heterozygote, carries a different set of alleles at the immunoglobulin loci. As differentiation toward immunoglobulin production progresses, one of the two immunoglobulin chromosomes (or immunoglobulin chromosome regions) of the embryonic stem cell, either paternal or maternal at random, becomes irreversibly inactivated. This might be much in the same fashion as X-chromosome inactivation (Lyon, 1961). Further differentiation then leads to the partial derepression of one of the H chain immunoglobulin loci on the active chromosome, and the production of a few (perhaps only one) molecules of the immunoglobulin class specified at the derepressed locus. At least one of these immunoglobulin molecules then remains associated with the cell, identifying the derepressed locus and (directly or indirectly) repressing the other H chain loci. After some time, possibly after cell division, a different immunoglobulin H chain locus on the active chromosome may become derepressed, bringing about repression of the old locus and replacement of the old immunoglobulins, thus recommitting the cell to production of a different class of immunoglobulin. This tentative commitment will then hold until the next revolution.

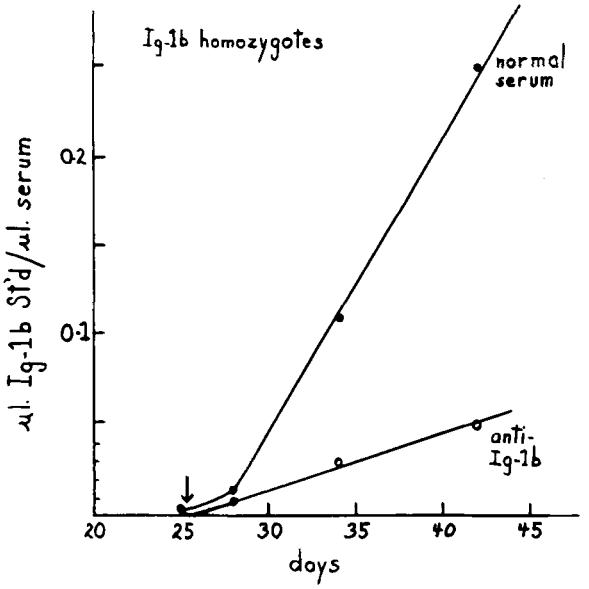

FIGURE 5. Allotype suppression in 4 week-old homozygotes. Suppression was obtained by one ip injection of 0.03 ml of BALB/c anti-Ig-1b serum per animal at 25 days of age. Controls received a similar injection of BALB/c normal serum. All animals were foster nursed from birth on Ig-1a foster mothers to obtain low levels of maternally derived Ig-1b.

We postulate that there is a self-replenishing stem cell population in the immunologically mature individual consisting entirely of cells which (1) have one H chain immunoglobulin chromosome active and the other irreversibly inactivated, (2) have one H chain locus on the active chromosome partially derepressed, (3) have one or more molecules specified by the derepressed locus associated with the cell but, (4) are only tentatively committed to the immunoglobulin they are producing, and periodically shift to derepression of another locus on the active chromosome, and production of a different immunoglobulin.

The identifying immunoglobulin on the stem cell (perhaps situated in association with the cell membrane) is accessible to external reactants, such as antigens and anti-allotype antibody. However, whereas combination with antigen may lead to differentiation of the tentatively committed cell into the irreversibly committed, fully derepressed plasmacytoid line (i.e. to immunization), combination of the identifying immunoglobulin with anti-allotype antibody results in the removal of the cell from the population able to give rise to the allotype, either through direct killing or through transformation into a different cell type (lymphoblastoid transformations?). This removal may be analogous to the mechanism by which tolerance is produced by appropriate antigenic stimulation.

The difference in duration of suppression between mouse and rabbit is predicted by this model. In the mouse the allotype antigens with which the suppressing antiserum reacts are on one class of immunoglobulins, i.e., they are determined at only one locus on the immunoglobulin chromosome, Ig-1b, which codes for γG_{2a}-globulin. Therefore, only cells producing Ig-1b are attacked. Cells producing other immunoglobulins (e.g., γG_{2b} or γG_1) which are determined by other loci on the same chromosome (e.g., Ig-3^b and Ig-4^b) will not be affected by the suppressing antiserum since it does not react with these proteins. Since γG_{2a}-globulins form less than 50% of the total immunoglobulins found in mouse serum, it is reasonable to expect that in a suppressed mouse there will be a sizeable population of unaffected cells with the "b" type chromosome active.

Once the suppressing antiserum disappears, any cells from the above population which shift and derepress the Ig-1^b locus will now survive. Some will go on to be stimulated to further differentiation and the production of large amounts of Ig-1b globulin. The deficit of Ig-1b cells caused by the period during which no cells were able to differentiate into full Ig-1b production will, however, remain and be evident as a decreased rate of Ig-1b production for a long period.

In contrast, in rabbits the antigens with which the suppressing antiserum reacts are present on the several classes of immunoglobulin (γG, γA, and γM) comprising more than 90% of rabbit immunoglobulins (Dray et al., 1963). Therefore most, if not all of the stem cells with the active chromosome carrying the affected allotype would be removed. When the suppressing antiserum disappears, very few cells would be available then to shift and repopulate, leaving a more or less permanently allotype-suppressed rabbit as the result.

This model would also explain why suppressed homozygous mice recover more quickly from suppression than do heterozygous mice, since both chromosomes in the homozygote would yield cells capable of shifting to production of the suppressed allotype.

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DISCUSSION

R. MAGE: I was not planning to discuss the suppression of homozygotes, but since you have mentioned your results with mice, I will say that we can obtain suppression in rabbits homozygous at the *b* locus; of b^4 b^4 and b^5 b^5 type. We did not succeed in suppressing homozygotes with immunized foster mothers as you have, but used a trick of producing homozygous offspring of heterozygous mothers allotype-suppressed for the allotype of the sire in the mating. Thus the injected antibody had to overcome a low antigen concentration in the newborns derived from the maternal suppressed allotype. Dr. Dubiski has independently performed similar experiments and since we both have similar results, I am beginning to believe mine. We find a considerable amount of γG-immunoglobulin (order of 0.5 mg N/ml) in rabbits 4–16 weeks of age which has only a small amount of detectable *b* locus determinant (b-positive). We think they have b-negative light chains, possibly analogous to the λ type chains that L. Hood thinks are present in the rabbit. Between 8–16 weeks of age, in our hands, the b-positive type appeared and rose in concentration to account for most of the immunoglobulin-G in the serum during the next four weeks, so this suppression was relatively short-lived. I do not know the details of Dubiski's data and am sorry he is not here now. I expect he will be telling us more about this tomorrow evening.