GENETIC VARIATIONS IN SOMATIC CELLS

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SUPPRESSION OF A γG-GLOBULIN ALLOTYPE IN MICE BY ANTI-ALLOTYPE ANTIBODIES

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In the mouse, maternal antibodies are passed to the young first intrauterinely and then, for about 2-1/2 weeks, via the milk.[1] During this period, the young mouse synthesizes very little, of its own γG-globulin.[2] At about 4 - 5 weeks of age, γG-globulin carrying the allotype (iso-antigen) determined by the mouses own genome reaches detectable levels in the serum, and after that steadily increases as the mouse matures.

We immunized female BALB/C mice ($Ig\text{-}1^a$ allotype) to the gamma globulin of C57BL/6 ($Ig\text{-}1^b$) animals,[3] and then mated these females to males either heterozygous or homozygous for the $Ig\text{-}1^b$ allele. The appearance of the $Ig\text{-}1^b$ allotype in the progeny of these matings was delayed (in some cases considerably) beyond 8 weeks of age.

The data in Table 1 show that only 14% (7/50) of progeny of immune mothers tested in this series are positive $Ig\text{-}1^b$ at 8 weeks of age whereas 100% (42/42) of progeny from non-immune mothers are positive. (Positive is defined here, and in Table 2, as having more than 0.5% of the level in a standard adult $Ig\text{-}1^b$ (C57BL/6) serum.)

TABLE 1.

Percentage of Offspring Showing $Ig\text{-}1^b$ at 8 Weeks of Age

Mother	Positive * for $Ig\text{-}1^b$	Total Tested	% Positive
Immune to $Ig\text{-}1^b$	7	50	14%
Non-immune	42	42	100%

* Positive defined as 0.5% of a standard adult homozygous $Ig\text{-}1^b$ serum level.

Strains used in these crosses were: females BALB/CCrg1Ga; males C57BL/10Hz, C57BL/10-bbHz and $(C57BL/6J \times DBA/2J)F_1$

In Table 2 a more complete study of appearance of antigen in these progeny with time is presented. The control progeny (from non-immune mothers) are found positive at the earliest test, (between 4 and 6 weeks), whereas the suppressed progeny (from immune mothers) are nearly all negative at 8 weeks, some being negative as long as 12 weeks. The two litters (7 progeny) already positive at 8 weeks were born to immunized mothers with very weak anti-$Ig\text{-}1^b$ titers.

Homozygous $Ig\text{-}1^b$ offspring, foster nursed on mothers immune to $Ig\text{-}1^b$ also are delayed in production of the allotype when compared to control foster nursed animals of the same genotype. (See Table 3). The poor yield of litters with suppressed animals (3/7) may be attributable to the varied elapsed time before transfer of the litters to the foster mother was made. This interpre-

tation is supported by the finding of a similar delay in appearance of Ig-1^b in a single homozygous Ig-1^b animal developed from an egg transplanted at the blastocyst atage[4] to an immune mother and subsequently nursed on that mother.

TABLE 2.

Appearance of Ig-1^b Antigen in Heterozygous Ig-1^b Animals Exposed to Maternal Anti-Ig-1^b

Parental Ig-1 Genotype			Weeks											
Mother	Father*	No. of Litters	4	5	6	7	8	9	10	11	12	13	14	15
a/a Immune†	b/b	2			0/15**	0/15	0/15	15/15						
		1			0/7	0/7	0/7			7/7				
	b/c*	2					7/7							
		1					0/7			7/7				
		1					0/6				6/6			
		1					0/3		0/3			3/3		
		1					0/2		0/2				2/2	
		1					0/3				0/3			3/3
a/a non-immune	b/b	2	3/3											
		3		12/12										
		2			11/11									
	b/c*	1				5/5								
		2					11/11							

* Only progeny carrying the (paternal) Ig-1^b allele included.

** Number positive/number tested. Positive defined as 0.5% of a standard adult homozygous Ig-1^b serum level. An animal once positive remains positive. Tests on litters after all animals are positive are not shown here.

† Has demonstrable anti-Ig-1^b activity.

Strains used in these crosses were: females BALB/CCrg1Ga; males C57BL/10-bbHz and (C57BL/6J x DBA/2J)F_1

"Positive for Ig-1^b" is defined above as having more than 0.5% of the level of a standard adult Ig-1^b (C57BL/6) serum. Defining 1 unit of Ig-1^b antigen as 1 µl of the standard serum or its equivalent, 0.5% of the standard is equal to a total of about 8 units of antigen in a 17 g. animal. This is equivalent to 40 µg per animal of gamma globulin carrying Ig-1^b. It is possible, however, to detect the presence of roughly 1 unit (5 µg) of antigen in the same size animals by changing from the immuno-diffusion technique used to collect most of the data in Tables 1 and 2 to the inhibition of precipitation of I^{125}-labelled antigen for antigen estimation.[5] Using this method of measurement, we find antigen in normal Ig-1^b heterozygous animals (mother Ig-1^a) appearing at about 5 weeks of age. Suppressed animals however do not have any measurable antigen until at least 7 to 8 weeks of age. In fact, maternal antibody is detectable in these animals until that time, and sometimes longer.

TABLE 3.

Appearance of Ig-1^b Antigen in Homozygous Ig-1^b Animals Exposed to Maternal Anti-Ig-1^b

	No. of Litters	Days 23	28	35	40	43	46	49	55	61
Immune Foster Nurse †	1				0/5**			0/5	5/5	
	1				0/6		2/6		5/6	6/6
	1				1/5			5/5		
	1					3/3				
	1					4/4				
	1						6/6			
	1							7/7		
Non-immune Foster Nurse	1	4/5	5/5							
	1	0/6	6/6							
	1	3/5	3/5	5/5						
Immune Egg Recipient			0/1	0/1		0/1		1/1		

** Number positive/number tested. Positive defined as 0.5% of a standard adult homozygous Ig-1^b serum level. An animal once positive remains positive. Tests on litters after all animals are positive are not shown here.

† Has demonstrable anti-Ig-1^b activity.

Foster mothers and egg recipient were BALB/CCrglga.
Progeny and egg donor were C57BL/10Hz or C57BL/10-bbHz

Data showing total units of Ig-1^b per animal is plotted, as a function of age, Figure 1 for normal heterozygotes (control) and for the heterozygous progeny of mothers immune to Ig-1^b (suppressed). Each curve represents the average of determinations of three litter (approximately) six animals per litter. In the litters tested, the suppressed animals are roughly 3 weeks behind the controls in Ig-1^b level, and even at 15 weeks have not yet "caught up".

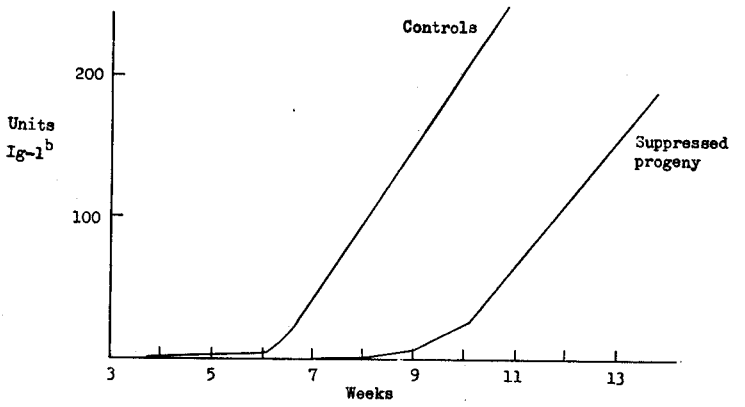

FIGURE 1

Strains used in this cross were: females BALB/CCrg1Ga; males C57BL/10Hz, and C57BL/10-bbHz. One unit Ig-1^b equals 1 µl of a standard C57BL/6J adult normal serum.

Since maternal anti-Ig-1^b is present in the suppressed progeny until shortly before the appearance of the Ig-1^b antigen, we felt that the delay in antigen appearance might be due to the complexing of maternal antibody with newly synthesized antigen and the subsequent removal of these complexes by the animal. The data in Figure 2 show this not to be the case.

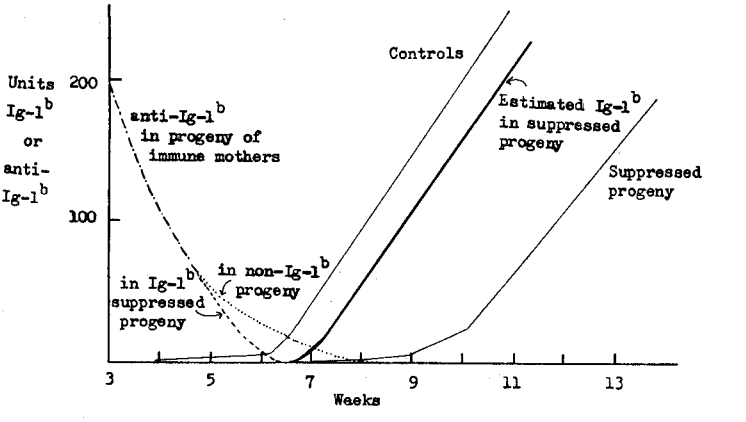

FIGURE 2

Strains used in this cross were: females BALB/CCrg1Ga; males C57BL/10Hz and C57BL/10-bbHz. One unit Ig-1^b equals 1 µl of a standard C57BL/6J adult normal serum. One unit of anti-Ig-1^b equals that amount of antibody which neutralized one unit of Ig-1^b antigen.

The amount of residual maternal anti-Ig-1^b in progeny decreases from time of weaning with a half life of 5 to 7 days. Taking as one unit of anti-Ig-1^b that amount which reacts with one unit of Ig-1^b antigen, and a half-life of 7 days, we estimated the amount of anti-Ig-1^b remaining in progeny of immune mothers as a function of time. The curve shown in Figure 2 for anti-Ig-1^b level agrees with experimental data obtained on antibody levels in non-Ig-1^b, 6 - 8 week old, progeny of these mothers.

If maternal anti-Ig-1^b is complexing with the newly synthesized Ig-1^b antigen and causing its removal, then, in Ig-1^b progeny the antibody level should begin decreasing as soon as antigen begins to be synthesized. If antigen is synthesized at the same rate as the controls the antibody level should drop to zero by 6-1/2 weeks. After that time Ig-1^b should begin to appear. Even allowing a lag in rate of production similar to that seen in the controls, the amount of antigen (see curve in Figure 2) expected to be present in suppressed progeny is far greater than that which is actually found. For reference in Figure 2, the curves for control and suppressed are reproduced from Figure 1.

Over 130 units of antibody would be necessary to remove enough antigen to give the observed result. This would be equivalent to a serum titer 1/3 as high as normally displayed by hyperimmune animals at the height of their response, much higher than could be expected at the result of residual maternal antibody at 6 - 8 weeks of age. We have found low levels of maternal antibody present past the eighth week of age in suppressed progeny. Were antigen being synthesized at the normal rate by suppressed progeny at this age, less than two days synthesis would have been enough to completely absorb the maternal antibody still circulating.

These estimations rest, of course, with the determination of the volume of antiserum which neutralizes one unit of antigen. Determination of this equivalence was done <u>in vitro</u>, where 5 µl of a standard antibody pool was found to be necessary to neutralize 1 µl of Ig-1^b standard; and <u>in vivo</u>, where just under 5 µl of antibody was necessary to remove 1µl of injected antigen from circulation of normal adult (10 week Ig-1^a) animals. To test these conclusions, the amount of residual maternal antibody in several offspring of an immune mother was estimated according to the curve in Figure 2, and the equivalent Ig-1^b antigen calculated. This amount of antigen, plus about 5 µl extra, was injected into the progeny. After about 24-hours the progeny sera were tested for antibody and antigen, Roughly 4 µl of Ig-1^b antigen were found in each animal, just as predicted.

On the basis of these experiments we feel it is reasonable to conclude that Ig-1^b progeny of mothers immune to Ig-1^b do not synthesize gamma-globulin of Ig-1^b allotype for several weeks after weaning.

Discussion

Suppression of synthesis could be occurring either at the cellular or subcellular level. Pernis et al. have brought forth convincing evidence that individual mature plasma cells of rabbits heterozygous at the Ab allotypic locus produce either one or the other allotype.[6]

(Opposite results have been reported by Colberg and Dray).[7] The findings of Pernis et al are consistent with experience, in humans and mice, that the gamma globulin synthesized by plasma cell tumors (myelomas) do not carry both allelic antigens, even though the tumors arose in a heterozygous individual. It is tempting to speculate that anti-Ig-1^b antibody either prevents the development of, or selects against, the cells committed to production of Ig-1^b.

Suppression of synthesis of an allotype due to immunization of the mother was originally reported by Dray, in the rabbit.[8] There, the time course of suppression appears to be quite different from that which we find in mice, since in rabbits the suppressed animals remain low in paternal type antigen for extremely long periods (over a year in the reported case). One possibility for the different findings in the two species is that the rabbit, being relatively immunologically mature at birth, responds to all the "natural" antigens before the disappearance of maternal antibody, whereas, the mouse has its major recruitment of cells for "spontaneous" antibody synthesis during the period after disappearance of maternal antibody. The deliberate immunization of animals while they are still suppressed and analysis of the relative contribution of the allelic genes to the antibody produced could answer this question. However, it should be remembered the mechanism producing suppression may be fundamentally different in the two species.

Finally, Lieberman and Dray have reported that maternal immunization to paternal allotypes drastically reduces the fertility of the females in the cross.[9] We find no such reduction, our immunized and non-immunized females being equally fertile and showing no difference from the general reproductive behavior of animals of their strain. In our original crosses, half of our females were mated to heterozygous males, (Ig-1^b/Ig-1^e) and the progeny typed for both antigens. As expected, we found a 1 to 1 ratio of Ig-1^b progeny to Ig-1^c, indicating that maternal immunization to Ig-1^b does not create a selective pressure against production of Ig-1^b offspring.

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