

TOLERANCE AND IMMUNITY TO MATERNALLY DERIVED
INCOMPATIBLE IgG_{2a}-GLOBULIN IN MICE*

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Several studies have demonstrated the presence in serum of antibodies to hereditary γ -globulin factors (Gm) in man. A high incidence of anti-Gm antibody was found in the sera of patients following multiple (1) and even single (2) blood transfusions, and in patients receiving γ -globulin injections for allergy or slight hypogammaglobulinemia (3). Furthermore, deliberate immunization of man has also been shown to lead to the production of antibody to Gm factors (3).

Gamma G globulins which carry the Gm antigens have been shown in man to cross the maternal fetal barrier in both directions (4). Therefore, Gm incompatibility between mother and fetus could lead to anti-Gm production by the mother in a manner analogous to the production of anti-Rh antibodies in an Rh negative mother of an Rh positive child; and/or it could lead to production of antibody in the child directed against maternal Gm factors (SNagg). Evidence for each of these possibilities has recently been advanced (5-9). A similar result has been observed in pigs, in that some sera from normal pigs were found to contain antibodies to γ -globulin isoantigens, presumably resulting from immunization with maternally derived incompatible γ -globulin (10).

In view of the present detailed knowledge of the multiple antigenic specificities of mouse allotypic antigens (11-15) and the availability of many inbred mouse strains, it was felt that a study of the possible consequences of maternal fetal incompatibility of the immunoglobulin allotypes could be undertaken in this species. Since maternal γ -G globulin not only crosses the placenta in mice but also crosses the gut until the young are about 16 days of age (16), and since newborn mice can be rendered tolerant by neonatal injection with appropriate doses of some antigens (17-19), particular emphasis was placed in this study on examining the recipients for both tolerance and immunity to the maternally derived immunoglobulins.

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Materials and Methods

Mice.—Mice of the inbred strains C57Bl/10Hz, BALB/cCrgl/Ga, B10.D2 (C57Bl/10-H2^d), 129/J, and DBA/2 were from colonies maintained in this laboratory. Hybrid mice of the cross (C57Bl/6 × A/J) were obtained from the Jackson Laboratories, Bar Harbor, Maine.

Maintenance of Mice.—Breeding of various crosses of inbred strains and foster nursing of various progeny mice were performed as previously described (20). Serum samples were taken at various intervals by incision of the tail artery; after clot retraction, sera were separated and stored at -20°C .

Allotypes.—The strains used in these experiments have the following immunoglobulin allotypes: Ig-1^a—BALB/c, 129/J; Ig-1^b—B10.D₂, C57Bl/6, C57Bl/10; Ig-1^c—DBA/2; Ig-1^a—A/J (11).

Experimental Groups.—Three separate groups of strain combinations were used in these experiments.

1. Female (C57Bl/6 × A/J)F₁ mice were mated to male DBA/2 mice and the progeny bled at various intervals after weaning.

2. A combined group of mice were all tested for the production of anti-Ig-1^a: (a) Female (BALB/c × B10.D2)F₁, mated to male C57Bl/10Hz (female previously immunized to 129/J spleen cells for production of anti-H-2^b); (b) Inbred C57Bl/10 or B10.D2 foster nursed on 129/J or BALB/c females immune to H-2^d; (c) Inbred C57Bl/10 or B10.D2 foster nursed on normal 129/J or BALB/c females.

The progeny from groups (a), (b), and (c) were bled for serum at various intervals and tested for anti-Ig-1^a antibody. Since no significant difference was observed between these three groups, the results are pooled from these mice.

3. Female BALB/c mice were mated to 129/J males. At birth one-half of each litter were foster nursed on C57Bl/10 females and the other half returned to the original BALB/c mother. At 6 wk of age all animals were injected with 10 μl of a C57Bl/10 anti-H-2^d serum emulsified in complete Freund's adjuvant, and at 8 and 9 wk each animal received 20 μl of the same serum intraperitoneally. At 10 wk of age all animals were bled for serum. From 12–18 wk of age, approximately half of the animals which had failed to respond to the immunizing injection of C57Bl/10 anti-H2^d by producing anti-Ig-1b antibody, were given weekly injections of 10 μl of C57Bl/10 serum intraperitoneally. At 19, 21, and 22 wk the immunizing injection of C57Bl/10 anti-H2^d serum was repeated and all mice were bled at 23 wk of age.

Serological Techniques.—Screening for progeny Ig-1^b immunoglobulin was done by agar gel diffusion with a potent anti-Ig-1^b serum raised in BALB/c mice by immunization with a C57Bl/10 anti-H-2^d serum.

Detection of Immunoglobulin Allotype Antigens in Serum.—Specific assays for determining the presence of Ig-1^a, Ig-1^b, and Ig-1^c globulin were set up with the inhibition of precipitation of ¹²⁵I-labeled immunoglobulin technique as previously described (11, 12, 15) using specific alloantisera to appropriate allotypic antigens.

Detection of Alloantibodies.—Sera were tested for their ability to precipitate ¹²⁵I labeled immunoglobulin of appropriate allelic type by the previously described method (11, 12, 15). In all cases 5 μl of test serum were used and each serum sample was repeatedly tested on three to five different occasions.

Immunoglobulin Preparations. Purified immunoglobulins for the above assays were prepared by either agar-block electrophoresis (pH 8.2 in Veronal buffer) from normal C57Bl/10 serum (for Ig-1^b) or by starch-block electrophoresis from myeloma-containing serum (RPC-5 line for Ig-1^a and GPC-7 for Ig-1^c [12]). All proteins were iodinated by the method of Greenwood et al. (21).

RESULTS

Spontaneous Antibody Formation in Intercross Progeny.—In the first experimental group, (C57Bl/6 × A/J) F_1 females were mated to DBA/2 males: C57Bl/6 = Ig-1^b, A/J = Ig-1^e, DBA/2 = Ig-1^c. Progeny were bled at 8 wk of age and the sera tested in Ouchterlony agar gel diffusion against specific anti-Ig-1^b and anti-Ig-1^e antisera. On the basis of these reactions 18 out of 31 mice were identified as genotype b/c and the remaining 13 shown to be genotype e/c. Further serum samples were taken from each mouse at 13, 17-21, 26, 32, 38, and 46 wk of age, and all samples were then tested for the presence of antibodies to the allotype of maternal γ -globulin not synthesized by the progeny mice, i.e., b/c mice tested for anti-e, and e/c mice tested for anti-b.

TABLE I
Incidence of Antibody to Maternally Derived Incompatible γ -Globulin in Intercross Mice

Progeny (Ig-1) genotype	Total No. of mice	Mice with antibody, at age for bleeding for serum, <i>wk</i> :									
		13	17	18	19	20	21	26	32	38	46
b/c	18	0*	7	33	39	35	19	42	43	—	35
e/c	13	15*	38	61	75	50	36	50	22	11	11
b/c and e/c	31	10†	25	48	55	58	58	62	62	62	62

* Per cent of mice with antibody at particular serum sample.

† Cumulative per cent of mice of both groups with antibody at or before the particular serum sample.

The data in Table I show that 56% and 77% of mice of the two genotypes make antibody to the incompatible maternal type γ -globulin at some stage in their life. The titers produced by these animals, however, were quite low, which made it necessary to do extensive testing of each serum before classifying it as positive. Addition of the maximum permissible amount of test serum (5 μ l) only precipitated between 8 and 25% of the labeled antigen, therefore each serum sample was tested three to five times. If a serum sample gave more than 8% precipitation of the labeled antigen on two or more out of three or four tests, or three or more out of five tests, it was scored as containing antibody. The degree of precipitation of the Ig-1^e antigen by sera from the b/c mice was in general lower than that of the Ig-1^b antigen by sera from the e/c mice. Samples of the strongest antisera were pooled and tested for antibody activity in Ouchterlony gel diffusion but no precipitation lines were ever observed.

The sera from the e/c mice were also assayed for the presence of maternal Ig-1^b globulin. Levels were related to a standard pool of adult C57Bl/10 serum where 0.2% of the standard (0.02 mg/ml) or greater indicates the significant presence of antigen. As expected, the concentration of maternal antigen is

high just after weaning, declines rapidly at first, and then more slowly until it is below the threshold for detection. The age of mice at this disappearance of maternal antigen varies between 6 and 12 wk. Appearance of antimaternal

TABLE II
Incidence of Antibody to Maternally Derived Incompatible γ -Globulin in Back-Cross and Foster-Nursed Mice

	Age of mice at serum sample, wk:						
	7	8	9	10	11	12	13
No. of mice tested	48	45	49	24	28	25	14
No. of mice with maternal antigen	30	20	13	2	1	0	0
No. of mice with antibody	2	4	10	7	6	7	1
Per cent of mice with antibody	4	11	20	29	22	28	7
Cumulative per cent of mice with antibody	3	10	22	22	32	32	34

TABLE III
Tolerance to Ig-1b Induced by Presence of Maternal Immunoglobulin

Allotype of progeny	Allotype nursed on	Age (wk)				
		0-3	6-9	9-16	19-22	
		Challenged* with Ig-1b	Responded with anti-Ig-1b	Ig-1b level	Challenged with Ig-1b	Responded with anti-Ig-1b
Ig-1a†	Ig-1a (natural mother)	40	40			
Ig-1a	Ig-1b§ (foster mother)	33	0	Maintained	14	1
				Allowed to decay¶	15	15

* Ig-1b challenge protocol: day 1, 10 μ l C57Bl/6 anti-H-2^d antiserum presented subcutaneously in Freund's adjuvant; days 21 and 28, 20 μ l of same antiserum diluted to 0.2 ml in phosphate-buffered saline; serum drawn for test at day 35.

† (BALB/c \times 129)F₁ animals (Ig-1a/Ig-1a) were used.

§ C57Bl/10, (Ig-1b) foster mothers were used.

|| Maintenance dose of 10 μ l of normal C57Bl/6 serum given at 10-12, 14 and 16 wk.

¶ Half-life of Ig-1a is approximately 5 days.

allotype antibody generally follows the disappearance of maternal allotype by 1-2 wk.

Serum samples taken between 6 and 15 wk of age from the b/b progeny of a back-cross of female b/a \times male b/b and from foster-nursed inbred b/b mice nursed on Ig-1^a mothers were tested for Ig-1a and for anti-Ig-1a. Since both groups showed similar results, the data were combined for presentation here.

The cumulative percentage of mice producing antibody to the maternal allotype rises from 3% at 7 wk to 32% at 11 wk and 34% at 13 wk. The number of mice making antibody at any given test is highest at about 10–12 wk (Table II).

Tolerance prior to Production of Antibody to Maternal Allotype.—73 Ig-1^a (BALB/c) newborns were divided into two groups; one group nursed on natural Ig-1^a mothers and the other on Ig-1^b (C57Bl/10) foster mothers. At 6, 8, and 9 wk both groups were challenged with Ig-1^b. All of those (40/40) nursed on Ig-1^a mothers (and therefore having no circulating Ig-1^b) gave a strong anti-Ig-1^b response. However, none (0/33) of the animals nursed on Ig-1^b mothers made detectable antibody (Table III). The latter, nonresponding group was then divided again; 14 animals were given maintenance injection of Ig-1^b (10 μ l of C57Bl/10 serum, intraperitoneally) and 15 controls were given 10 μ l of BALB/c serum weekly. The remaining 4 animals had died. At 19, 21, and 22 wk, all animals were again challenged with Ig-1^b. Only 1 of the 14 whose serum level of Ig-1^b had been maintained made detectable anti-Ig-1^b, whereas all of the controls were now able to make a normal, strong anti-Ig-1^b response.

DISCUSSION

The newborn mammal is apparently protected from a variety of pathogens in its environment by a legacy of maternal immunoglobulins. These immunoglobulins, transferred either across the placenta, across the gut during nursing, or by both routes, stay around for the first few weeks of life; during this time the animal is able to build up a competent immunity system, rendering him immunologically self-sufficient. This survival advantage, however, would be seriously compromised if the immunoglobulins were polymorphic and if they themselves were immunogenic (i.e., if they carried allotypes) unless the offspring were unable to respond to (were tolerant of) the incompatible maternal globulins, since an antibody response to maternal immunoglobulins would lead very rapidly to their elimination. In other words, here is a natural situation where tolerance to a foreign protein antigen is essential if a genetic polymorphism is to be maintained, or conversely, where a genetic polymorphism makes necessary the development of a system of tolerance to foreign antigen.

As the data in this paper show, the young mouse behaves toward the incompatible immunoglobulin received from his mother in the same way as he does toward other foreign soluble protein antigens introduced at birth (22); as long as the level of antigen is high enough to maintain tolerance, he will not respond with antibody production, even if additional antigen is presented in what would otherwise be a strongly immunizing regimen. Once the antigen level drops below that necessary to maintain tolerance, he shows a transient, weak antibody response and is then able to mount a strong immune response to the antigen when challenged.

It is likely that human infants exposed to incompatible maternal immunoglobulins undergo the same sequence of events. Steinberg and Wilson (6) have reported that individuals who produce antibody (SNagg) to Gm or Inv determinants on human gamma globulins in the absence of an external stimulus (for example, transfusion) were exposed to incompatible maternal globulin. Speiser (9), using the prospective approach, showed that a very large percentage (probably close to 100%, considering the difficulties in detection) of normal infants exposed to incompatible maternal gamma globulins have at least a weak transient antibody response to these globulins sometime between 3 months and 3 yr of age. This correlates well with our studies in mice where roughly 60% of mice at risk showed naturally occurring antimaternal antibody. Considering the difficulties, as in Speiser's study, of substantiating the relatively weak antibody responses, this 60% must be taken as a low estimate, the true incidence probably being closer to 100%.

Our demonstration of tolerance before formation of antimaternal antibody in mice appears to contradict Steinberg and Wilson, who state that in untransfused SNagg donors "it seems probable that . . . immune tolerance has not resulted from the presence of the (maternal) antigen in large quantity prior to and immediately after birth" (6). The contradiction is resolved, however, when one recognizes that tolerance to an antigen persists only as long as the antigen persists (22, 23), and therefore, that finding antibody production in an animal reveals nothing about whether the animal had been tolerant previously.

The direct demonstration of tolerance before the emerging immune state in newborn children is clearly impractical. The generalization about man from our findings in mice, however, is strongly supported by the data of both Steinberg and Wilson (6) and Speiser (8, 9) on the timing of antimaternal antibody production. The immunologic question still to be answered is why some, and not other, humans persist in producing good titers of antimaternal globulins well into adulthood in the apparent absence of additional exposure to antigen.

SUMMARY

Progeny mice were confronted with maternal γ -globulin of a different allotype by either back-cross mating, intercross mating, or by foster nursing. In all cases, many mice subsequently produced alloantibodies directed against the incompatible maternal type of IgG_{2a}-globulin.

In one series of experiments, immunologic tolerance to the maternally derived γ -globulin was demonstrated to exist in the period before formation of spontaneous antibody. The state of tolerance was then lost, unless maintenance injections of foreign γ -globulin were given.

These studies demonstrate in a natural situation that maternally derived foreign proteins can first induce a state of immunological tolerance which is followed, after disappearance of the antigen, by a state of immunity. As such,

this parallels the experimental induction of tolerance to foreign proteins by neonatal injections.

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