

Transmission of γ G Antibodies from Maternal to Fetal Circulation in the Mouse* (33592)

NICOLA CARRETTI¹ AND ZOLTAN OVARY²

New York University Medical Center, New York, New York 10016

The γ G immunoglobulins of the mouse possess different biological activities and, whereas the γ G1 may sensitize tissue of the homologous species for anaphylactic reaction (1, 2), the γ G2a and γ G2b antibodies are not able to do so. On the other hand, although γ G2a and γ G2b may both fix complement (3), only the γ G2a is capable of sensitizing guinea pig tissue for anaphylactic reactions (4).

The passage of immunoglobulins from mother to fetus was studied in many species. It is of interest to note that not all classes of immunoglobulins are capable of passing the placental barrier (5–10) and that the integrity of the Fc fragment is necessary in the case of rabbit γ G (5, 6).

The purpose of the present paper is to report the results obtained in mice with two classes of γ G antibodies: the γ G1 and the γ G2 classes.

Materials and Methods. Animals. Young adult female Swiss Webster mice weighing approximately 25 g were used for immunization and for passive antibody transfer experiments.

Antigens. Keyhole limpet hemocyanin (Hcy) (Pacific Bio-Marine Supply Co., Venice, Calif., lot SPH 79), 5 \times crystallized egg albumin (Ea) (Pentex, Inc., Kankakee, Ill., lot F61), crystalline bovine plasma albumin (BSA) (Armour, Kankakee, Ill., lot A69908) were conjugated with dinitrophenylsulfonic

acid (Eastman Kodak) under alkaline conditions as previously described (11). The following dinitrophenylated (DNP) protein preparations were used: DNP₁₆₀Hcy, DNP₃₄EA and DNP₃₇BSA. (The numbers refer to the groups of DNP per molecule of carrier protein—the molecular weight of Hcy was taken to be 10⁶.)

Antibody preparation. Mice were immunized by injecting intraperitoneally (i.p.) 0.5 ml of a mixture containing 0.25 ml of DNP₁₆₀Hcy (200 μ g/ml) and 0.25 ml of Freund's complete adjuvant (Difco Labs., Detroit, Michigan). The animals were boosted i.p. with 0.5 ml of the above mixture once weekly on the days 7, 14, and 21 after the primary injection. Three days before the last booster 0.1 ml of a 1:5 dilution of an Erlich ascites tumor was injected i.p. This tumor was grown in Swiss Webster mice and was taken from a donor with large ascites injected 7 days before with the tumor. Seven days after the final booster the animals were killed and the ascitic fluid was collected, pooled, and stored at -20° until use. It was assayed for antibody activities as described below.

Passive immunization of pregnant mice. Pregnant mice near term were injected, in the tail vein, with 0.8 ml of the pooled ascitic fluid and the mothers were bled to death under ether anesthesia from the axillary vessels. The serum obtained from each mother was stored separately at -20° until use. Immediately after the bleeding of the mother the pregnant uterus was extracted and the fetuses were delivered by cesarean section. The interval between antibody injection into the mother and the bleeding and delivery of the fetuses varied between 3 hr to 2 days. The fetuses were bled and the blood from each litter was pooled. The sera obtained

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¹ Research Fellow at New York University, School of Medicine, Department of Pathology from the NATO and the CNR (Consiglio Nazionale delle Ricerche), Roma. Present address: Clinica Ostetrica, Padova, Italy.

² Health Research Council Career Scientist of the City of New York.

TABLE I. PCA in Mice with the Original Ascitic Fluid, the Sera of Mothers and of Fetuses.*

Dilutions	Ascitic fluid	Mothers' sera					Fetuses' sera				
		3	6	18	19	26	3	6	18	19	26
1:2	nd	nd	nd	nd	nd	nd	0	8	9	8	7
1:5	nd	13	15	11	12	14	0	5	6	5	5
1:10	15	9	13	10	10	13	0	tr	0	0	0
1:20	nd	8	10	8	7	10	nd	nd	nd	nd	nd
1:40	13	6	10	5	5	10	nd	nd	nd	nd	nd
1:80	11	5	tr	tr	0	8	nd	nd	nd	nd	nd
1:500	7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

* The numbers represent the diameter (mm) of PCA reactions (av in 4 mice); tr = trace reactions; and nd = not done.

from the fetuses were also stored at -20° until use.

Assay of antibody activity. The original immune ascitic fluid, the sera from individual mothers, and the sera from each litter of the mothers were checked for γ G2a and γ G2b antibody activity by passive hemolysis of DNP₃₇BSA coated sheep erythrocytes, for γ G1 activity by passive cutaneous anaphylaxis (PCA) in mice, and for γ G2a activity by PCA in guinea pigs. Passive lysis was performed as previously described using guinea pig serum as source of complement (12). The titer was considered to be the reciprocal of the dilution giving 100% lysis. PCA in mice was performed as described in Ref. (13) and PCA in guinea pigs as described in Ref. (14). The titer was considered to be the reciprocal of the last dilution giving PCA reactions. The sensitization period (SP) was 1–1.5 hr in mice and 4–6 hr in guinea pigs. Mice were injected with 1 mg of DNP₃₄Ea as challenging antigen and guinea pigs were injected with 500 μ g of DNP₃₄Ea.

The immune ascitic fluid was analyzed by density gradient. Ultracentrifugation in sucrose gradient (12.5–37.5% sucrose) was done in a Spinco preparative ultracentrifuge, model L, using a swinging bucket type SW 39 for 16 hr at 32,000 rpm and 5-ml Lusteroid tubes. Guinea pig γ G1 anti-BSA antibody was used as 7 S marker and a sample of human macroglobulin was used as 19 S marker.

Results. As both γ M and γ G2 fix complement, it was important to analyze the im-

mune ascitic fluid by density gradient ultracentrifugation to see if it contained γ M antibody. Passive lysis was detected with contents of tubes 5, 6, and 7 from the bottom where the 7 S marker was also present. No passive lysis was detected in the contents of tube 1 (bottom) where the 19 S marker was present. It was concluded, therefore, that this hyperimmune ascitic fluid did not contain γ M anti-DNP antibody.

Representative experiments comparing the biological activities of the original ascitic fluid, that of the sera of mothers, and that of the pool of the liters of fetuses are presented in Tables I–III. In the sera of the mothers, the lytic antibody had a titer 4 times less (160 as compared to 640) and the titer of antibodies capable of giving PCA in mice (γ G1) or PCA in guinea pigs (γ G2a) had a titer 5 times less (80 as compared to 500) than in the original ascitic fluid.

As shown by these experiments, mouse γ G1 and γ G2 antihapten antibodies present in the serum of passively immunized pregnant mice are both transmitted to the fetus in detectable amounts.

Discussion. It is known that only the γ G2 class is capable of complement fixation, whereas the γ G1 is the antibody which sensitizes the homologous species for anaphylactic reactions (1, 2). The configurations necessary for these biological activities are probably present on the Fc portion of these molecules as the L chains are common in all immunoglobulin classes. The presence or absence of these configurations did not appear to be

TABLE II. PCA in Guinea Pigs with the Original Ascitic Fluid, the Sera of Mothers and of Fetuses.*

Dilutions	Ascitic fluid	Mothers' sera					Fetuses' sera				
		3	6	18	19	26	3	6	18	19	26
1:5	nd	18	18	20	15	17	0	12	12	12	15
1:10	nd	17	15	18	15	15	nd	nd	nd	nd	nd
1:20	20	15	14	15	13	10	0	tr	6	0	0
1:40	nd	12	11	12	9	5	nd	nd	nd	nd	nd
1:80	17	9	7	9	6	4	nd	nd	nd	nd	nd
1:400	9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1:500	tr	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

* The numbers represent the diameter (mm) of PCA reactions (av in 4 guinea pigs); tr = trace reactions; and nd = not done.

necessary for the passage from mother to fetus in the mouse. γ G1 and γ G2a antibodies detected, respectively, by PCA reactions in mice and guinea pigs appear in the sera of fetuses 6 or more hr after the intravenous injection of the mother with the amounts of antibody used in these experiments. The antibody amount of γ G1 or γ G2a present in the sera of the fetuses is between $\frac{1}{8}$ to $\frac{1}{16}$ of that found in the mothers. Passive lysis with the sera of fetuses was demonstrable within 3 hr after intravenous injection of the mothers and the amount of antibody producing passive lysis is between $\frac{1}{4}$ to $\frac{1}{8}$ of that of the mothers. Passive lysis can, therefore, be detected earlier in the sera of the fetuses and the amount of antibody is slightly greater than the amount of antibodies detected by PCA reactions. This fact could be interpreted as the passage of γ G2b earlier than that of

the γ G2a. Alternate explanations could be that as both γ G2a and γ G2b fix complement, both antibodies are detected by passive lysis and an additive effect occurs, or simply that the sensitivity of the method of passive lysis is greater than that of the PCA reaction method with these antibodies. These experiments were not designed to test small differences in the relative rate of transfer of these antibodies, but show that both antibody classes, γ G1 and γ G2, are transmitted from mother to fetus. These experiments confirm and extend the results of Koch *et al.* (7) who showed that γ G antibodies are transmitted from mother to fetus in mice. However, they used isotope-labeled γ G globulins and did not investigate the different classes of antibodies. In these studies advantage was taken of the possibility of using antibodies and their biological activities to investigate this

TABLE III. Passive Lysis with the Original Ascitic Fluid, the Sera of Mothers and of Fetuses.*

Dilutions	Ascitic fluid	Mothers' sera					Fetuses' sera				
		3	6	18	19	26	3	6	18	19	26
1:5	+	+	+	+	+	+	nd	nd	nd	nd	nd
1:10	+	+	+	+	+	+	+	+	+	+	+
1:20	+	+	+	+	+	+	+	+	+	+	+
1:40	+	+	+	+	+	+	+	+	+	+	+
1:80	+	+	+	+	+	+	—	+	+	+	—
1:160	+	+	+	+	+	+	—	—	—	—	—
1:320	+	—	+	—	—	+	nd	nd	nd	nd	nd
1:640	+	—	—	—	—	—	nd	nd	nd	nd	nd
1:1280	—	—	—	—	—	—	nd	nd	nd	nd	nd

* + = 100% lysis; — = less than 50% or no lysis; and nd = not done.

problem. These results are very similar to what was observed in the guinea pig (10) where it was shown that both the γ G1 and γ G2 antibodies are transmitted from mother to fetus.

Summary. Passively transmitted homologous γ G1 and γ G2 antidinitrophenyl antibodies to pregnant mice near term pass into the circulation of fetuses. With the amounts of antibodies used, γ G1 and γ G2a are detected in the sera of fetuses 6 hr after intravenous injection of the mothers.

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Uric Acid Nephropathy: An Experimental Model (33593)

B. STAVRIC, W. J. JOHNSON, AND H. C. GRICE

Food and Drug Research Laboratories, Department of National Health and Welfare, Ottawa, Canada

In a previous paper¹ it was shown that oxonic acid is a potent *in vivo* inhibitor of uricase. This compound, administered orally or intraperitoneally to rats, gave rise to a marked increase in plasma and urine uric acid levels with a concomitant decrease in urinary allantoin. A greater than twentyfold increase in urinary uric acid with deposition of uric acid in the kidney tubules was observed in rats maintained on a diet containing both oxonic acid and uric acid. The associated nephropathy, which in several respects resembles uric acid nephropathy in the human (1-3), is described in the present paper.

Methods. Male rats (175-200 g) of the Wistar strain were used. The control group

was fed a diet of commercial rat cubes pulverized in a mechanical blender. The second, third, and fourth groups were fed the same diet into which was blended 5% oxonic acid; 1% uric acid; and 5% oxonic acid plus 1% uric acid, respectively. Water was freely available. The rats were on test for varying periods of time up to 28 days.

Histologic sections. The kidneys were exposed under deep ether anesthesia and removed for histologic study. After clamping the right renal artery and vein with a hemostat, the right kidney was removed and placed in 10% formal saline solution. The posterior vena cava was then severed, a needle was inserted into the aorta at the bifurcation of the iliac arteries, and 10% formal saline was used as a perfusate for the left kidney. Formalin-fixed tissues were stained

¹ Johnson, W. J., Stavric, B., and Chartrand, A. P.S.E.B.M. (in press, April 1969, 33791).