PREGNANCY INDUCED HEMAGGLUTININS TO PATERNAL H-2 ANTIGENS IN MULTIPAROUS MICE[1]

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SUMMARY

A breeding colony of C57BL/6J females mated with DBA/2J males was examined to determine the incidence and mechanism of the development in the females of hemagglutinins to paternal antigens. It was demonstrated that pregnancy rather than cohabitation is a necessary condition for the appearance of these antibodies. The hemagglutinin titers for paternal erythrocytes vary in an individual female mouse. Females strongly positive after a given pregnancy may appear as negative after the next and then positive again after another. Hemagglutinin titers were recorded during a single pregnancy in each of four females which were strongly positive immediately prior to pregnancy. The titer "disappeared" during the 3rd week of pregnancy, but reappeared by 1 week postpartum. No evidence of fetal disease or decreased fertility in the female was found as a result of sensitization.

It has previously been demonstrated that C57BL/6J ($H\text{-}2^b$) female mice repeatedly outcrossed to DBA/2 ($H\text{-}2^d$) males develop antibodies which react with the DBA/2 (that is, the paternal) erythrocyte antigens and therefore can agglutinate paternal erythrocytes (*4*). Since no indication was found of the development of hemagglutinins to DBA/2 erythrocytes, except in outcrossed females, it was concluded that this isoimmunization was in some way related to the pregnancy or mating "regime" of these animals.

In studies using the same mouse strains as above, we demonstrated that the immunization of mating females is due to the pregnancy itself, rather than to conditions attendant upon maintenance under mating conditions. We examined the effect on immunization of the number of offspring, the number of pregnancies, and the interval elapsing between pregnancies. We also considered the discontinuity of the immune response in these animals, i.e., a female with a high titer of agglutinins may very well become negative following her next pregnancy and then following subsequent pregnancies be positive again.

MATERIALS AND METHODS

Breeding colony. A breeding colony of 61 cages was established in which two C57BL/6J females were caged with one DBA/2J male continuously throughout

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their breeding life. Serum samples were taken from each female 1 to 3 days postpartum, or at approximately 2 month intervals for sterile animals. Dates of birth of litters and number of offspring per litter were recorded within 36 hours of birth of litter and offspring were then discarded.

Sterile colony. Five DBA/2J males which had been proved fertile were vasectomized and then mated to ten C57BL/6J females. Similarly, ten females with tubal ligation were mated with five known fertile males. Coitus, as evidenced by the appearance of plugs, was known to occur in all cases; however, no pregnancies ensued. Serum samples were taken from each female at 8-week intervals for testing.

Oral ingestion colony. Ten C57BL/6 virgin females were fed newborn (C57-BL/6 × DBA/2) F_1 mice at 3-day intervals over a period of 2 months to test the possibility that oral ingestion of the products of conception might lead to sensitization.

Hemagglutination assay. Sera were collected and stored as previously described (*4*). Erythrocytes were collected into ACD solution containing Merthiolate, washed once at room temperature, and resuspended to 2% in phosphate-buffered saline, pH 6.5 (PBS) (*8*). A modification of the PVP hemagglutination test as previously described (*3*) was introduced during the course of these studies. Incubation of antisera with erythrocytes in dilution of 1/20 to 1/640 was carried out in Owens disposable 10 × 75 test tubes for 1 to 1½ hours. Tubes were then shaken on a guiding flat surface (the light panel of a Thermolyne laboratory light, Model ℵLL5615). A positive reaction was recorded when persistent clumping occurred, a negative when only temporary clumping or a fine cloud appeared.

RESULTS AND DISCUSSION

The data (Tables 1–3), when taken as a whole, indicate that pregnancy rather than existence under breeding conditions is a necessary prerequisite for the development of antibodies to paternal antigens. A total of 104 females were followed for a period of 8 months (Table 1). Of the 19 females with no recorded pregnancies, 17 were negative whenever tested; of the 85 females with one or more pregnancies, only 13 were consistently negative. Thus of the 74 animals who developed antibodies, only 2 were in the group having no recorded pregnancies, and these 2 animals had weak titers. Among those animals with 6 or more recorded pregnancies (Table 2), 20 out of 23 showed strong antibody titers at least once during their breeding lives.

TABLE 1

Hemagglutinin titers in 122 C57BL females with 461 pregnancies

No. females	Females' history	Highest titer recorded		
		0	±	+
18	Died within 3 months with no recorded pregnancies	Not done		
19	After 4 to 8 months of exposure, no recorded pregnancies	17	2	0
85	One or more recorded pregnancy	13	25	47

TABLE 2

Hemagglutinin titers in multiparous females

No. females	No. pregnancies	Highest titer recorded		
		0	±	+
23	6 or more	3	0	20
18	8 or more	2	0	16

Serum samples were drawn from the breeders at about 2 days postpartum. Later experiments have shown that animals who have no detectable hemagglutinins at this time may, within 1 week subsequent to this, test positive. These figures, therefore, represent a minimum estimate of the number of breeders who develop strong positive reactions.

The following experimental "colonies" were then set up to determine whether animals living under the same conditions as the breeding colony, but effectively prevented from becoming pregnant, would become immunized: (1) 10 fertile females were caged with vasectomized males; (2) fertile males were caged with 10 females with ligated fallopian tubes. Although the animals mated regularly, no pregnancies ensued, and no hemagglutinin titers developed.

Finally, as the bearing female usually eats the placenta and fetal membranes and often later cannibalizes some of her litter, we tested for immunization following ingestion of antigen by feeding 12 or more F_1 newborns to each of a group of 10 C57BL/6J virgin females over a period of 1 month. None of these females developed antibodies.

Although a large number of breeders were followed over their entire breeding lives, no pattern of development of response was evident. A given female might become positive early or late in breeding life and, once having been positive, even strongly so, might, following subsequent pregnancies, become negative. Later on she might be positive once again. We attempted to correlate these shifts with number of offspring, interval between pregnancies, and number of pregnancies, but no correlation was readily apparent.

Three strongly positive females were chosen from the breeding colony for more intensive study over a short period of time. At 2 or 3 day intervals small samples of serum were taken and tested for hemagglutinins. In each case, the positive titer lapsed for a short period and then reappeared (Table 3). No litters

TABLE 3

Females with strong antibody titers followed at 2–3 day intervals

Animal	Intervals									Total days[b]
	1	2	3	4	5	6	7	8	9	
35	+++	++	++	+++	+	[a]	[a]	+	++	24
127	++	−	−	−	−	+	+	+	[a]	28
178	++	+	±	−	+	±	+	±	+	21

[a] Recorded as pregnant; no litters recovered.

[b] Days from first bleed to last.

TABLE 4

Hemagglutinin titers during a single pregnancy of previously pregnancy-sensitized females

Female no.	Titer 1–2 wks before pregnancy	Titer during pregnancy			Titer 1 day postpartum	Titer 1 wk postpartum
		1 wk	2 wk	3 wk		
35	+	+	−	−	++	+
127	+	+	+	−	±	++
154	+	±	±	−	±	+
178	+	+	−	−	±	+

were recovered from the females being followed in this way, although two gave indications of pregnancy during the period.

As the repeated warming and handling necessary for taking serum samples may have interfered with successful breeding, these three females (and a fourth who also had a strong titer) were subsequently bled at 2 week intervals until pregnancy occurred. Serum samples were then taken during the first, second and third weeks of pregnancy, at one day postpartum and at one week postpartum. The data in Table 4 show that each female, when pregnant, loses her antibody titer, and this titer is only fully recovered at 1 week postpartum. It may well be that a large part of the variation seen in the survey of the breeding colony (where serum samples were taken at 2 days postpartum) is a reflection of varying abilities to recoup a positive titer after its decimation during pregnancy. Further studies are in progress to determine whether this is the case.

The effects of pregnancy on the immune response were reviewed in 1942 by von Haam and Rosenfeld (*9*). While many of these reports are contradictory, there does appear to be a stimulation of antibody formation by estrogen in many mammals, including the mouse (*7*). Apart from considering the possible endocrine factors of pregnancy, several laboratories have found that the continued breeding of female mice with males of a different strain evokes an immunological response in the female (*1, 4, 6, 7*).

The occasional occurrence of natural isoagglutinins in both male and female C57BL mice has been described by Gorer (*2*). MacDowell and Hubbard (*5*) had previously found no natural isoagglutinins nor did we find any in C57BL males or virgin females caged with DBA animals.

Contrary to what might be expected from analogy with the human, survey of the breeding colony gave no evidence that maternal immunizations to paternal antigens either decreases fertility or leads to fetal disease. No gross signs of erythroblastosis as it occurs in the human, such as newborn jaundice or anemia, were evident in the offspring. Fertile mice, subsequently immunized to paternal antigens either by pregnancy or by spleen injection did not become sterile or grossly less productive.

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