IMMUNOLOGIC STATE OF THE PROGENY OF MICE IMMUNIZED DURING PREGNANCY

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After immunization of mice with sheep's red cells in the last third of pregnancy, the antigen passes through the placenta and is retained in the bone marrow of the fetuses. The passage of antibody-forming cells through the placenta is not observed. Meanwhile, antibodies are found in the sera of newborn mice after disappearance of the antigen. The immunologic response of the progeny of immunized mice is weaker than that of the progeny of normal mice of the same age.

KEY WORDS: Antigen; antibodies; antibody-forming cells; permeability of the placenta.

During pregnancy various antigens as well as antibodies can penetrate into the tissues of the fetus [2-4]. The study of the penetration of antigens and their effect on the subsequent response of the progeny is of great interest in connection with the infectious pathology of childhood [1], and also in the study of the theoretical aspects of the development of immunologic tolerance.

The passage of antigen, antibodies, and antibody-forming cells (AFCs) through the placenta and changes in the response of the progeny to the antigen, through which the mother was immunized, were investigated.

EXPERIMENTAL METHOD

Experiments were carried out on female CBA mice. In the last third of pregnancy (16th-17th day), an intravenous injection of 10^9 sheep's red cells was given, and in some experiments 0.5 mg hyaluronidase (Reanal) was given at the same time. At various times after immunization of the mice their young were exsanguinated and the following investigations were carried out: the number of AFCs by Jerne's method, the antibody titer by hemolysis and hemagglutination tests, and the antigen titer by the inhibition of hemagglutination test. Usually the serum and spleen of the mother mice and the serum, liver, spleen and bone marrow of the progeny were investigated; in each test, organs from three or four fetuses or newborn mice were combined. Altogether 67 females and their progeny were used.

To investigate the immune response of the progeny of immunized mothers, the young mice were tested at the age of 30-45 days by intravenous injection of 10^9 sheep's red cells, On the third day after the test injection they were exsanguinated, the number of AFCs in the spleen was counted, and hemolysins, agglutinins, and erythrocytic antigen were determined in the serum.

In the experiment in which AFCs were transplanted, the pregnant mice in the last third of pregnancy received an intraperitoneal injection of a suspension of spleen cells containing 1050 AFCs obtained from a donor on the third day after immunization. Immediately after transplantation, the animals received an intravenous injection of 0.5 mg hyaluronidase in isotonic solution. The progeny of the mice receiving AFCs were tested at the age of 45 days, just as in the other experiments.

For statistical analysis of the numerical results the geometric means were calculated.

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EXPERIMENTAL RESULTS

The results of investigations to detect AFCs, antigen, and antibodies in the progeny of the immunized mothers are shown in Tables 1, 2, and 3, respectively.

As Table 1 shows, AFCs did not pass from the mother to the fetus and were not formed in newborn mice during the first five days after birth (duration of the experiment), although the peak of AFCs in the mother occurred on the 4th-5th day after immunization, i.e., actually before parturition.

Large quantities of antigen passed through the placenta.

TABLE 1. Number of AFCs in Immunized Females and their Progeny (log $\overline{X}_{geom} \pm \log J_{95}$)

Time after immunization (in days)	Females		Progeny		
	spleen	placenta	liver	spleen	bone-marrow
1	0	0	0	0	0
3	1,4±0,3	0	0	0	0
5	2,0±0,01	—	0	0	0
10	0,2±0,5	—	0	0	0

Legend: Here and in other Tables, number of AFCs is given per 10^6 leukocytes.

As Table 2 shows, 24 h after injection it was found in the mother's spleen, the placenta, and the bone marrow of the fetuses. The fetal serum at this time was not tested because of its small quantity. On the third day the quantity of antigen in the maternal spleen and placenta fell sharply. The titer of antigen in the fetal serum was approximately four levels of dilution higher than in the maternal spleen (multiplicity of dilution 2). The titer of antigen in the fetal bone marrow fell more slowly than in the placenta. On the fifth day, after birth of the young, antigen could not longer be found in the maternal spleen. Its titer in the neonatal serum was six levels of dilution lower and was almost down to zero. It persisted longer in the neonatal bone marrow. On the 10th day after immunization no antigen was found in either the mothers or the progeny.

TABLE 2. Detection of Erythrocytic Antigen in Immunized Mice and and Their Progeny

Time after immunization (in days)	Females		Progeny				
	serum	spleen	placenta	serum	liver	spleen	bone marrow
1	0*	9,6±7,3**	8,3±6,7	—	0	0	7,3±5,5
3	0	3,7±4,4	5,1±4,6	7,8±1,3	1,4±1,2	0	5,7±3,2
5	0	0	—	1,1±3,1	0	0	4,3±2,6
10	0	0	—	0	0	0	0

* Titer of inhibition of hemagglutination test below 10.

† $X \pm S_{\overline{X}}$, calculated from $\log_2$ of titers of inhibition of hemagglutination test; the same in other tables for titers of hemagglutination test.

TABLE 3. Detection of Antibodies in Immunized Females and Their Progeny

Time after immunization (in days)	Antibodies	Females		Progeny			
		serum	spleen	serum	liver	spleen	bone marrow
1	Hemolysins	0	0	0	0	0	0
	Hemagglutinins	0	0	0	0	0	0
3	Hemolysins	1,3±1,3	0	0	0	0	0
	Hemagglutinins	3,3±2,6	0	0	0	0	0
5	Hemolysins	9,3±4,4	0	0	0	0	0
	Hemagglutinins	9,1±4,7	0	0	0	0	0
10	Hemolysins	4,2±3,5	0	2,9±2,4	0	0	0
	Hemagglutinins	10,3±7,3	0,2±1,1	7,7±3,9	0	0	0

Antibodies (Table 3) were found only in the mothers until the fifth day. On the 10th day comparatively high titers of hemagglutinins and lower titers of hemolysins were found in the sera of the young mice. At this time the hemolysin titers of the mother were lower (4.2 ± 3.5) but the hemagglutinin titers were still higher (10.3 ± 7.3). These results show that after birth, when the only connection with the mother was through the milk, the content of antibodies in the young mice continued to rise and neutralization of antigen could possibly be taking place.

TABLE 4. Immunologic Response of Progeny of Immunized and Normal Mice to Test Injection of Antigen

Treatment of mothers	Age of progeny when tested (in days)	Number of AFCs	Hemagglutinins
10^9 sheep's red cells + 0.5 mg hyaluronidase	30	0,3±0,1	4,9±2,3
10^9 sheep's red cells	30	0,4±0,1	2,5±1,2
10^9 sheep's red cells	45	1,2±0,2	0
1050 AFCs + 0.5 mg hyaluronidase	45	1,5±0,1	5,1±3,0
No treatment	30	1,0±0,2	4,2±1,9
" "	45	1,6±0,2	4,6±2,8

The next stage of the work was to study the response of the progeny of immunized and normal females to the antigen with which the mother was immunized (Table 4).

As Table 4 shows, the cellular response of the young of the immunized mothers was significantly lower than the response of the progeny of the normal mice. This was observed when the progeny was tested at the age of both 30 and 45 days. Differences in the antibody titer were less demonstrative, for on the third day after immunization antibodies were only just beginning to appear. No antigen could be found in the serum of the progeny. The response of the progeny of mothers receiving AFCs, tested at the age of 45 days, did not differ significantly from the response of normal young mice of the same age.

The results thus showed that after immunization of mice in the last third of pregnancy the erythrocytic antigen passes through the placenta and is retained in the fetal bone marrow. No AFCs pass through the placenta under these circumstances. Meanwhile, antibodies are found in the serum of newborn mice fed by immunized mothers after disappearance of the antigen. The immunologic response of the progeny of immunized mice is lower than the response of the progeny of normal mice of the same age.

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