IMMUNOLOGIC DISTURBANCES IN MICE BORN AFTER INDUCTION OF A GRAFT VERSUS HOST REACTION IN THE MOTHER

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The immunologic status of mice born after induction of a graft versus host reaction in the mother was studied. Lymphocytopenia, delayed rejection of skin allografts, a decrease in natural resistance to experimental typhoid infection, and a decrease in the number of plaque-forming cells in the spleen after immunization of the mice with sheep's red blood cells and typhoid Vi antigen were found at the age of 1 month. At the age of 2-3 months, the same changes together with a decrease in the number of T-lymphocytes in the spleen and lymph nodes were found only in mice with clinical features of runt disease. In the second year of life depression of the immune response to sheep's red blood cells and enhancement of the response to Vi antigen and a decrease in the number of T-lymphocytes in the spleen and lymph nodes compared with the control were observed in the progeny. An increased concentration of immunoglobulins and transferrins was found in the blood serum and antierythrocytic autoantibodies were detected in some mice.

KEY WORDS: pregnancy; graft versus host reaction; immunologic disturbances.

The causes of development of inborn immunodeficiency states are not yet clear. It has been suggested that these disturbances include in their number the graft versus host reaction (GVHR) arising during pregnancy as a result of exchange of lymphocytes between mother and fetus [8], or the appearance of "forbidden clones," in the mother's immune system, capable of autoaggression [1]. The writers showed previously that most mice born after induction of a GVHR in the mother die during the first 2-3 months or in the second year of life [4-6]. Histological examination of these mice reveals marked changes in various organs of the immune system [5, 6].

Accordingly, in the investigation described below the immunologic status of the progenies born to mothers with the GVHR was studied.

EXPERIMENTAL METHOD

Experiments were carried out on mice obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. CBA ($H\text{-}2^k$) females were crossed with C57BL/6 ($H\text{-}2^b$) males and $(CBA \times C57BL/6)F_1$ females with CBA and C57BL/6 males. A GVHR was induced in the pregnant mice in the third trimester. For this purpose, an intravenous injection of 80 million living spleen cells and the same number of lymph node cells form normal C57BL/6 mice was given to the $(CBA \times C57BL/6)F_1$ mice, and the CBA mice received injections of 180 million cells from C57BL/6 donors, which had been immunized 3 times with spleen cells from CBA mice. Full details of the method of inducing the GVHR were described by the writers previously [4]. To rule out the possibility of death of the progeny from shortage of milk of the experimental mothers, normal lactating females were put in the same cage for fostering. The total leukocyte and lymphocyte count in the blood of the newborn mice were determined periodically; the presence of antierythrocytic autoantibodies was verified by the direct Coombs' test [11]. An electrophoretic investigation of the serum proteins after preliminary treatment of the serum with 1.5% Rivanol solution to remove all other proteins and immunoglobulins and transferrins [3], was carried out by disc electrophoresis in polyacrylamide gel; densitograms from the gels were examined in a microdensitometer (Carl Zeiss, Jena, East Germany). Some mice were killed at different ages and the number of T-lymphocytes counted in their spleen and lymph nodes by the microcytotoxic test [10];

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TABLE 1. Effect of GVHR Induced in Female Mice During Pregnancy on Immunologic Status of Progeny (geometric mean values and 95% confidence intervals)

Group of animals	Age of mice	Total number of leukocytes in 1 mm^3 blood, thousands	Number of lymphocytes in 1 mm^3 blood, %	Number of T-lymphocytes (cytotoxic index, %)		Number of PFC per 10^6 spleen cells after immunization with	
				spleen	lymph nodes	Vi antigen	SRBC
1 (experimental)	25—30 days	4,9* (20) 3,9÷6,2	56* (20) 46÷69	—	—	40* (16) 31÷51	22* (18) 15÷31
2 (control)		6,8 (15) 6,3÷7,2	76 (15) 74÷78	—	—	93 (18) 87÷100	35 (20) 43÷51
3 (experimental)	1½—2 months	3,2+(30) 2,7÷3,9	35+(20) 31÷41	13+(17) 10÷17	45+(17) 41÷49	18+(16) 14÷23	8+(11) 5÷13
4 (experimental)		7,7 (27) 6,9÷8,7	78 (27) 76÷80	20 (18) 16÷26	56 (18) 50÷63	104 (20) 85÷128	100 (20) 83÷120
5 (control)		7,4 (14) 6,6÷8,3	78 (26) 71÷85	24 (14) 22÷27	63 (14) 60÷66	100 (15) 89÷111	83 (16) 68÷102
6 (experimental)	12—18 months	4,0 (20) 2,6÷6,0	45 (20) 35÷57	20 (20) 16÷25	32+(20) 26÷40	98+(20) 93÷102	60+(15) 57÷63
7 (control)		5,6 (20) 4,5÷6,9	56 (20) 52÷60	23 (15) 21÷25	46 (15) 41÷51	57 (18) 56÷59	104 (18) 91÷120

Legend. Statistically significant differences between experiment and control ($P < 0.05$) indicated by asterisk. Number of mice given in parentheses.

anti-θ-serum was obtained from AKR mice immunized with thymus cells from CBA mice [9]. Ability to give an immune response was studied in mice of different ages. For this purpose, some mice were immunized by intraperitoneal injection of 200 million sheep's red blood cells (SRBC) or 10 μg typhoid Vi antigen, and the number of plaque-forming cells (PFC) in the spleen was determined on the 5th day after immunization by the method of local hemolysis in agar [7]. Transplantation immunity was assessed by determining the length of survival of skin allografts from DBA/2 ($H\text{-}2^d$) mice. To study the state of natural antimicrobial immunity, some mice at the age of 1 month were infected intraperitoneally with 100 million typhoid bacteria (strain ty-N 4446), and mice aged 12-15 months were infected with 500 million of the same bacteria.

Similar investigations were carried out on control mice whose mothers had received syngeneic lymphocytes of heat-killed allogeneic lymphocytes during pregnancy. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Mice born after induction of the GVHR in the mothers were outwardly indistinguishable from the controls during the first 3 weeks after birth. Toward the end of the first month, however, some of them began to be retarded in weight compared with the controls. As Table 1 shows, in the experimental mice at this age (group 1) the total leukocyte count and total lymphocyte count in the blood were reduced; after immunization with bacterial and erythrocytic antigens fewer PFC were found in the spleen than in the control (group 2). Skin allografts on the experimental progeny were rejected on average after 14.3 days compared with after 11.9 days in the control ($P < 0.025$).

In the second month of life distinct signs of runt disease (failure to gain in weight, emaciation, diarrhea, ascites) appeared in some of the experimental mice (up to 30%). In most mice developing this disease (group 3) leukopenia and lymphocytopenia, a decrease in the number of T-lymphocytes in the spleen and lymph nodes, and marked atrophy of the spleen (5-38 mg) and thymus (2-17 mg; control values 58-110 and 30-74 mg respectively; in both cases $P < 0.025$) were found. The number of PFC in the spleen after immunization with Vi antigen and SRBC was reduced 6-10-fold compared with the number in the corresponding control (group 5).

In mice with no clinical manifestations of the disease in the second month of life (group 4) the hematologic indices, the number of T-lymphocytes in the spleen and lymph nodes, and also the immune response to the antigens tested were almost indistinguishable from those in the control.

The experimental mice at the age of 1 month were found to be more sensitive to infection with typhoid bacteria: Within 1 month 14 of the 20 animals had died, compared with only three of the 20 in the control group ($P < 0.025$).

A decrease in the number of T-lymphocytes in the lymph nodes and depression of the immune response (a decrease in the number of PFC) to SRBC were found in the experimental mice studied at the age of 12-18 months (group 6), whereas the number of PFC in the spleen of mice immunized with Vi antigen was greater

than in the control. The total blood leukocyte and lymphocyte counts of the experimental mice fluctuated considerably: The leukocyte count of six of the 20 mice was below the minimal values in the control, whereas in seven mice it was higher than the maximal control values. During this period of life no decrease in resistance to experimental typhoid infection was found in the experimental mice. Antierythrocytic autoantibodies were found in eight of the 20 experimental mice and in two of the 20 control mice ($P = 0.025$). In all mice (18) investigated at the age of 8-12 months the serum immunoglobulin (485 ± 8.5 mg %) and transferrin (431 ± 17.3 mg%) levels were higher than those in intact mice of the same age (375 ± 7.0 mg %; $P < 0.01$; 385 ± 9.2 mg %; $P < 0.05$). Amyloidosis of the spleen and liver was found in the same mice on histological examination, and deposition of immunoglobulins was demonstrated in the renal tubules by the Coombs method [6]. A combination of hypergammaglobulinemia with amyloidosis and glomerulonephritis also was observed by other workers when describing certain pathological states of autoimmune nature [12, 13].

The results indicate that the function of the T- and B-systems of immunity is disturbed in the progeny of mothers with an induced GVHR. One of the manifestations of these disturbances, it may be considered, is the depression of the immune response to thymus-dependent erythrocytic antigen and thymus-independet Vi antigen discovered in mice during the first months of life. Meanwhile depression of the response to SRBC and the simultaneous enhancement of the response to Vi antigen in mice in the second year of life are evidence of a disturbance of the helper and suppressor functions of the T-lymphocytes, as a result of which the function of the B-system is potentiated in these mice. Activation of the B-system of immunity in the experimental progeny in the second year of life is also indicated by the raised serum immunoglobulin level. Similar changes in the T- and B-systems of immunity are observed in autoimmune diseases in animals of certain lines [13] and in man during aging [2].

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