IMMUNOLOGIC DISTURBANCES IN MICE FED BY MOTHERS WITH AN INDUCED GRAFT VERSUS HOST REACTION

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Certain forms of inborn immunodeficient states in infants are believed to arise as a result of disturbances in the maternal immune system [1, 10]. Inborn defects of the immune system can be obtained experimentally after induction of a graft versus host reaction (GVHR) in female mice during pregnancy [2-4, 5, 7]. The mechanism of onset of immunologic disturbances in the offspring of mothers with a GVHR is not clear. Attempts to prove that they are due to penetration of lymphocytes from mother to fetus have not been successful, [6]. Since the development of the GVHR is accompanied by the formation of various humoral factors [12] and by activation of oncogenic viruses [14], the possibility cannot be ruled out that these agents may be transmitted from mother to progeny through the placenta and milk and exert a harmful action on the immune system.

The object of this investigation was to study whether immunologic disturbances arise in mice born from normal mothers but fed by mice with an induced GVHR.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL/6)F_1 mice aged 3-4 months. Females were crossed with the same line of males. A GVHR was induced in the pregnant animals 1-2 days before giving birth to their young, by intravenous injection of 60 million living lymphocytes from the spleen and lymph nodes of C57BL/6 mice. Full details of the method of induction of the BVHR were described previously [4]. After the birth of their young, litters of mice born from normal mothers were fed by the mothers with the induced GVHR. To exclude the possibility of death of the offspring from possible lack of milk, each litter was fed by two or three mother mice simultaneously, and after the 14th day, they were returned to normal lactating females. The total number of leukocytes and lymphocytes in the blood was determined in the offspring. Some of the mice were killed and their T-lymphocyte population in the spleen and lymph nodes was determined by the microcytotoxic test [11, 13]. Ability to give an immune response also was studied. For this purpose the mice were immunized intraperitoneally with 200 million sheep's erythrocytes (thymus-dependent antigen) or with 10 mg typhoid VI-antigen (thymus-independent), and on the 5th day after immunization the number of plaque-forming cells (PFC) was determined in the spleen by the local hemolysis in agar method [8]. The state of transplantation immunity was determined from the duration of survival of skin allografts from DBA/2 mice. Some mice dying or killed in a poor condition were autopsied. Tissues were fixed in Carnoy's fluid and embedded in paraffin wax; sections were stained with hematoxylin-eosin, Congo red, by Brachet's method, and with Schiff's reagent by Hotchkiss' method. Mice born and fed by normal mothers served as the control. The significance of differences between experimental and control results was determined by Fisher's method for a fourfold table.

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TABLE 1. Immunologic Indices for Mice Born from Normal Mothers but Fed by Mice with Induced GVHR (arithmetic mean and limits of variations)

Group of animals	Age of mice	Total number of leukocytes, thousands/μl blood	Number of lymphocytes in 1 μl blood, %	Number of T-lymphocytes (cytotoxic index in %)		Number of PFU per 10^6 spleen cells after immunization with	
				spleen	lymph nodes	Vi-antigen	sheep's erythrocytes
1 (experiment)	30—40 days	3,2 (1,2—7,8)* $n=20$	34 (18—49)* $n=20$	17 (8—22)* $n=8$	38 (30—61)* $n=8$	31 (4—42)* $n=10$	42 (10—56)* $n=10$
2 (experiment)		7,7 (2,3—10,2) $n=20$	81 (60—88) $n=20$	24 (12—41) $n=10$	61 (38—72) $n=10$	106 (55—185) $n=10$	96 (52—162) $n=10$
3 (control)		7,8 (5,2—9,8) $n=15$	72 (60—82) $n=15$	27 (20—36) $n=10$	59 (48—68) $n=10$	128 (68—170) $n=10$	130 (44—175) $n=10$
4 (experiment)	12—15 months	4,8 (2,0—22,4) $n=20$	42 (12—88)* $n=20$	16 (11—38)* $n=10$	32 (13—42)* $n=10$	112 (52—166)* $n=15$	115 (38—145) $n=15$
5 (control)		6,8 (3,8—11,2) $n=20$	62 (38—70) $n=20$	26 (22—38) $n=10$	48 (40—66) $n=10$	62 (49—82) $n=15$	128 (85—177) $n=15$

*Differences between experiment and control statistically significant ($P < 0.025$).

EXPERIMENTAL RESULTS

Young mice fed by foster mothers with an induced GVHR developed normally for the first 3-4 weeks after birth and were outwardly indistinguishable from the control. In the second month of life some of them began to be retarded in body weight, and 15 of the 92 mice (16.2%) died at the age of 1.5-3 months. An increased mortality also was observed in the second year of life, as a result of which only 23 of the offspring (24.8%) survived until the age of 2 years. In the control, 29 of 35 mice (82.8%) survived to this age.

A study of the immunologic status of the mice at the age of 30-40 days showed that most animals retarded in weight (by 20% or more) (Table 1, group 1) had leukopenia and lymphocytopenia, as well as a reduced number of T-lymphocytes in the spleen and lymph nodes; the number of PFC in the spleen was reduced after immunization with Vi-antigen and sheep's erythrocytes compared with the control (Table 1, group 3). Skin allografts were rejected by the experimental mice on average after 15.2 days compared with 11.7 days in the controls ($P < 0.025$). In mice with no clinical signs of disease in the second month of life (Table 1, group 2) no significant disturbances of immunologic status were found. In the experimental mice investigated at the age of 12-15 months (Table 1, group 4) a statistically significant decrease in the number of lymphocytes in the blood and a decrease in the number of T-lymphocytes in the spleen and lymph nodes were found. The immune response to Vi-antigen was considerably increased compared with the control (Table 1, group 5).

In all the experimental mice which died at the age of 2-3 months severe atrophy of the spleen (12-42 mg) and the thymus (2-23 mg) was found (51-118 and 35-80 mg respectively in the control). Histologically, a decrease in the number of small lymphocytes was discovered in the thymus-dependent zones of the spleen and lymph nodes; the predominant cells were medium-sized and large lymphocytes, reticulum-cells, and plasma cells. Marked hypoplasia was found in the thymus with absence of subdivision into cortex and medulla. In 4 of 9 mice amyloidosis was visible in the liver and spleen. Islands of atypical reticulum cells, infiltrating the capsule and trabeculae (Fig. 1a), were found in two mice in the spleen and lymph nodes, and in one of them they were found in the liver and lungs at the same time. These changes were assessed as a manifestation of malignant transformation and reticulum-cell tumors were diagnosed (Dunn's classiciation [9]). The tumor cells were large, pale cells of irregular shape with a vesicular nucleus.

Amyloidosis (Fig. 1b), often accompanied by disappearance of cells from the lymphoid tissue, was present in the liver and spleen of 11 of the 17 mice which died in the second year of life. Among the cells which still remained most were pyroninophilic blast cells, megakaryocytes, and histiocytes. Foci of infiltration lymphocytes and histiocytes, containing numerous plasma cells, appeared in the liver, kidneys, and lungs. In four mice the liver, spleen, and lymph nodes were greatly enlarged; histologically lymphocytic neoplasma were diagnosed in these young animals (Fig. 1c). The tumors discovered were transplanted into adult (CBA × C57BL/6)F_1 mice. Neither amyloidosis or tumors could be found in the control mice.

It can be concluded from these results, together with those of previous investigations [2, 4, 5], that pathological processes of the GVHR type in the maternal immune system are accompanied by the formation

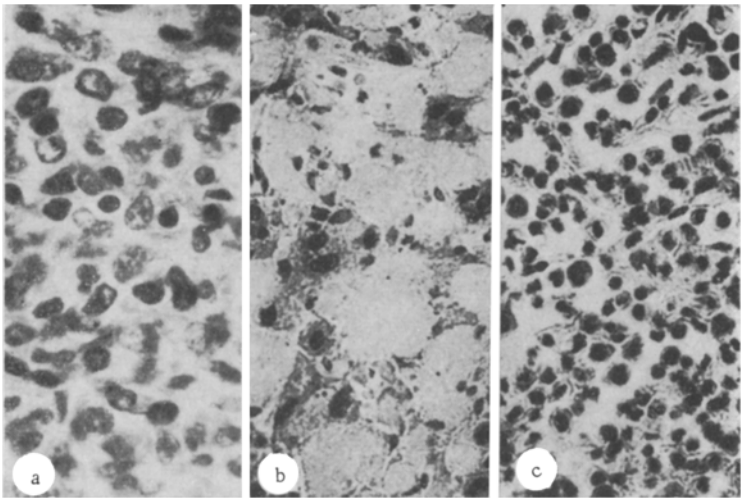

Fig. 1. Photomicrograph. Histological changes in mice fed by foster mothers with induced GVHR: a) Reticulum-cell tumor in liver, 630×; b) amyloidosis of liver, 280×; c) lymphocytic neoplasm in liver, 280×. Hematoxylin-eosin.

of factors which can contribute to the onset of immunologic disturbances in the offspring, being transmitted to them via the placenta and the mother's milk.

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