MORPHOLOGICAL CHANGES IN THE LATE PERIODS OF LIFE IN MICE BORN AFTER INDUCTION OF THE GRAFT VERSUS HOST REACTION IN THEIR MOTHERS

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Various organs of mice born after induction of a graft versus host reaction in their mothers during pregnancy were investigated histologically in the second year of life. Atrophic changes predominated in the lymphoid tissue and amyloidosis was observed in the spleen and liver of many (61.2%) mice. Infiltration with lymphocytes was found in the liver, kidneys, lungs, and heart; some mice developed glomerulonephritis, vasculitis, and degeneration of the liver. Tumors of the lymphoid tissue were found in 17.7% of cases, compared with only 4.1% of cases in the control group of mice of the same age. Some of the tumors were transplanted into adult F_1 mice. The cell-free extract, if injected into newborn mice, did not induce the development of tumors during observation for 14 months.

KEY WORDS: inborn immunological disturbances; mother–fetus system; graft versus host reaction; histology.

Since certain organs and tissues of the fetus are formed under the influence of the corresponding maternal organs [2], it has been suggested that there is a direct connection between disturbances in the immune system of the mother and the formation of pathological changes in the immune system of the progeny [1, 3, 5, 7, 13]. In experiments undertaken to verify this hypothesis experimentally, immunologic disturbances were induced in pregnant animals by the graft versus host reaction (GVHR), for this reaction can be regarded as a model of autoimmune disturbances and of immunologic conflict between mother and fetus [12].

Previous experiments by the writers showed that marked functional and morphological disturbances of the immune system arise in mice born from mothers with an induced GVHR, dying during the first month of life [8-11]. In the remaining progeny, dying mainly in the second year of life, the character of lesions in the organs has not yet been sufficiently clearly established. For that reason, in this investigation the various organs of these animals were studied histologically.

EXPERIMENTAL METHOD

Female CBA mice were crossed with C57BL/6 males, and (CBA × C57BL/6)F_1 females with C57BL/6 and CBA males. The GVHR was induced in pregnant females during the third trimester. For this purpose, 80 million living spleen and lymph node cells from normal C57BL/6 mice were injected intravenously into (CBA × C57BL/6)F_1 mice, and 180 million cells from C57BL/6 donors, immunized three times with spleen cells of CBA mice, were injected into CBA mice. Full details of the method of inducing the GVHR were described by the writers previously [9]. A histological investigation was made of the thymus, spleen, lymph nodes, liver, kidneys, lungs, heart, and intestine of mice dying or killed, in a poor condition, in the second year of life. The tissues were fixed in Carnoy's fluid and 12% formalin and embedded in paraffin wax; sections were stained with heamtoxylin-eosin, Congo red, by Brachet's method, and with Schiff's reagent by Hotchkiss' method. Mice of the same age, from normal mothers or mothers receiving heat-killed lymphocytes from C57BL/6 mice, served as the control.

In cases when a sharp increase in size of the lymphoid organs and liver, interpreted as a possible manifestation of tumor growth, was discovered at autopsy a 40% cell suspension was prepared from the tissues of these enlarged organs, and for the purpose of tumor transplantation it was injected intraperitoneally or subcutaneously into adult (CBA × C57BL/6)F_1 mice.

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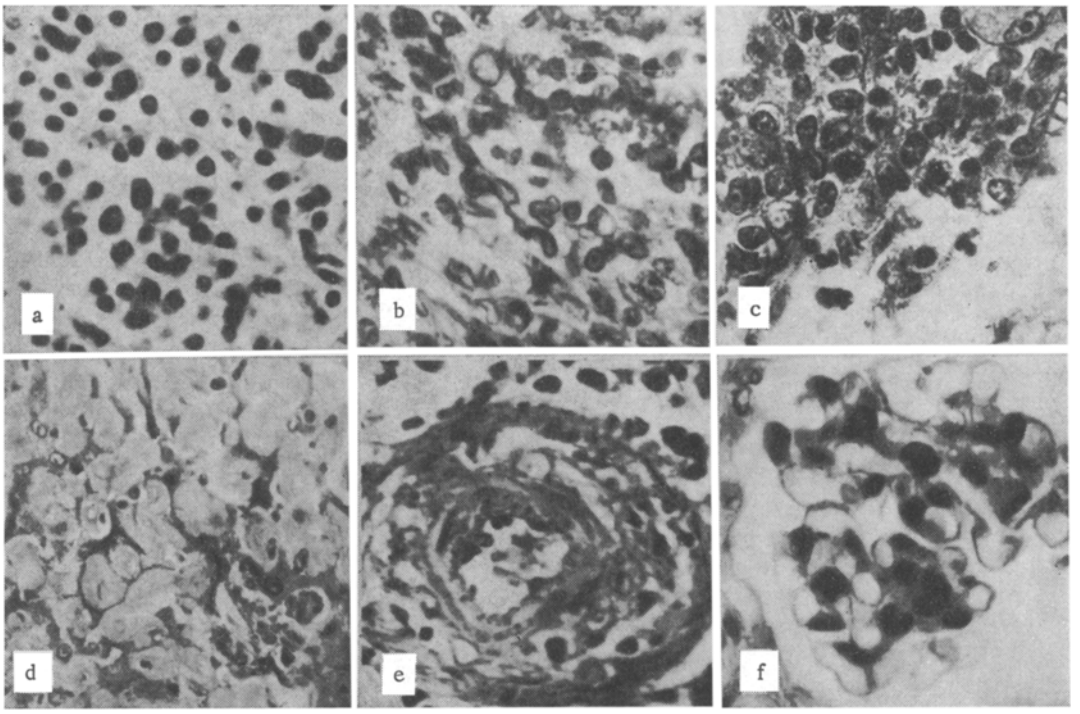

Fig. 1. Microscopic changes in organs of mice 12-18 months after birth from mothers with induced GVHR: a) lymphocytic tumor of mesenteric lymph node; b) type A reticulocytic tumor in spleen; c) infiltrative growth of thymic epithelium; d) amyloidosis of the liver; e) changes in central artery of splenic follicle of "onion" sclerosis type; f) thickening of basement membranes of renal glomerulus with the formation of structures of the "wire loop" type. Hematoxylin-eosin, magnification: a-c, e, f) 630×, d) 280×.

A cell-free extract prepared from the same suspensions by freezing (in liquid nitrogen) and thawing three times was injected intraperitoneally into newborn (CBA × C57BL/6) mice during the first 18 h after birth.

EXPERIMENTAL RESULTS

The histological structure of certain organs in 18 of the 62 experimental mice developing the disease in the second year of life was changed as a result of proliferation of cells which, from their morphology and character of growth, could be regarded as reflecting malignant transformation of lymphoid and reticular tissue. Neoplasms were classified according to Dunn [14]. The tumors in 11 cases had the structure of lymphocytic neoplasms, in five cases of type A reticulocytic tumors, and in two cases of type B reticulocytic tumors. Tumor development in eight mice was accompanied by marked enlargement of the spleen (up to 2-3 g), lymph nodes, and liver; the thymus in three mice was enlarged to 90 mg, whereas in the control its weight was 20-30 mg. Besides lymphoid organs, tumor cells were found simultaneously in the liver, kidneys, lungs, and intestine.

Cells of the lymphocytic tumors were very similar to mature lymphocytes and immature lymphocytes of the lymphoblast type and they contained numerous mitoses (Fig. 1a). The presence of these cells simultaneously in the lymphoid organs, kidenys, periportal spaces and sinusoids of the liver, and the intestine and also the infiltrative type of growth were characteristic of lymphatic leukemia.

Tumor cells of the type A reticulocytic neoplasms consisted of large pale irregularly shaped cells with a vesicular nucleus containing several nucleoli (Fig. 1b). Small reticular cells with irregularly shaped nuclei and with clumps of chromatin in the nuclei were also seen here. Pathological mitoses were frequently found.

Reticulocytic tumors of type B were represented as clearly demarcated regions in the spleen and lymph nodes in which atypical cells were arranced diffusely among the small lymphocytes and plasma cells.

Marked anaplasia of the thymic epithelium and its infiltrative growth into the surrounding tissue were observed in the thymus in three cases (Fig. 1c), whereas the peripheral lymphoid organis preserved their normal histological structure with predominance of pyroninophilic blast cells and medium-sized lymphocytes.

The tumors detected in six of the nine cases were successfully transplanted into adult F_1 mice. After injection of cell-free extract into the newborn mice no tumors developed in the course of 14 months of observation.

In 38 mice, including six with tumors, amyloidosis of the liver (Fig. 1d) and spleen was observed, often in conjunction with depopulation of the lymphoid tissue of its cells. However, in the eight cases in which amyloidosis was mild or the 11 cases in which it was absent altogether, hyperplasia of the follicles was observed in the spleen and lymph nodes on account of proliferation of numerous pyroninophilic blast cells and histiocytes, which supplanted small lymphocytes in the thymus-dependent zones. Foci of lymphohistiocytic infiltration, containing many plasma cells, were discovered in the liver, kidneys, lungs, and heart. In the central arteries of the splenic follicles the picture observed in eight cases was similar to that of "onion" sclerosis (Fig. 1e). Foci of necrosis were found in the liver, with "balloon" degeneration and pathological mitoses in the hepatocyte nuclei. Damage to the glomeruli accompanied by thickening of the basement membranes, with the appearance of structures resembling "wire loops" (Fig. 1f; five cases), the formation of hyaline casts (two cases), or the development of productive obliterative arteritis (three cases), was observed in the kidneys. The character of the morphological changes was independent of the genotype of the progeny tested.

In the control group of 24 mice a type A reticulocytic tumor was found in only one at the age of 21 months, in the mesenteric lymph nodes. Amyloidosis of the organs was not found in the control mice.

The results described above, together with those of previous investigations [4, 9-11], suggest that pathological immune processes arising in the mothers shortly before or during pregnancy, and following a course similar to that of GVHR, can give rise to the development of disturbances in the immune system of the progeny. These disturbances are very similar to the manifestations of autoimmune disease arising in animals of certain lines under the influence of latent viruses [17].

The mechanism of origin of the disturbances in the progeny is not clear. It may be connected with penetration of the donor's cells into the fetus, with the development of a GVHR in it [15]. Another cause that has been suggested is the participation of humoral factors or activation of oncogenic viruses in the mothers as a result of GVHR and their transmission to the progeny through the placenta and milk.

The results of these experiments suggest that the onset of certain forms of immunodeficiency states and also of autoimmune disorders and malignant tumors of lymphoid tissue in man, the highest frequency of which occurs in the early and late periods of life [6], is linked to some degree with disturbances in the immune system of the mother during pregnancy.

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