MORPHOLOGICAL CHANGES IN UNIRRADIATED HYBRIDS WITH TRANSPLANTATION SICKNESS

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In the early stage of the graft versus host reaction developing in hybrids (CBA × C57B1/6j) F_1 as a result of transplantation of parent spleen cells from C57B1/6j mice into them, increased proliferation of lymphoid tissue is observed. This is followed by progressive lymphoid atrophy and fibrosis. In the late stage of transplantation sickness due to the graft versus host reaction, besides the lymphoid atrophy degenerative and destructive changes are found in the liver and spleen. In the large intestine in the late stage of the disease, gross changes develop, including inflammation and ulceration of the mucosa and submucosa, thrombosis and hemorrhages in the small vessels, and total necrosis of the intestine wall.

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It is now undisputed that the leading role in the pathogenesis of transplantation sickness arising as a result of the graft versus host reaction (GVHR) is played by the immunologic reaction of the donor's immunologically competent cells against the foreign antigens of the recipient [6, 13]. However, the pathomorphological changes arising in the recipient during the GVHR and their role in the development of transplantation sickness have not yet been adequately studied. The difficulty in the study of this problem is that in some pathological states arising during the GVHR changes in the host may take place through the action of various external factors. In particular, in the secondary sickness of radiation chimeras, the main cause of which is considered by most investigators to be the GVHR [1, 3, 10], some changes in the body arise as the result of irradiation.

To study the changes developing in the body of the recipient during the GVHR it is preferable to use a technically "pure" system excluding any possible radiation effect. This demand is satisfied by a system in which the GVHR is produced by injecting spleen cells from the parents into the F_1 hybrid. In such a system the hybrid is tolerant to cells of the parent line for genetic reasons, but the parent cells can react against antigens of one of the parents contained in the hybrid's body.

The object of these experiments was to study pathological changes taking place in hybrids during the GVHR at different phases of its development.

EXPERIMENTAL METHOD

Experiments were carried out on 175 F_1 hybrids (CBA × C57B1/6j) weighing 16-18 g. Spleen cells from mice of the parent line C57B1/6j were injected intravenously into 150 hybrids in order to evoke a GVHR. A suspension of spleen cells in Hanks' solution was prepared by mincing the organs in a glass homogenizer and passing the mass several times through a Kapron filter. All procedures were carried out under aseptic conditions. To count dead cells eosin was used in a dilution of 1:2000. The suspension was kept on ice and injected into the retroorbital venous sinus of the hybrids in a dose of 110-120 × 10^6 living cells in a volume of 0.25-0.3 ml between 30 and 60 min after preparation. Heparin was added to the suspension before injection in a dose of 5-10 units per mouse. None of the animals died in these cases from thrombo-embolism even when the suspension was injected very quickly.

The animals were sacrificed 15-25 at a time at different times after transplantation of the spleen cells: 5, 10, 15, 20, 25, 30, and 40 days. Twenty-five hybrids were left alive after transplantation to remain under observation. Another 25 hybrids receiving no injections of spleen cells also were sacrificed.

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At autopsy morphological changes were found in the intestine, liver, spleen, mesenteric lymph glands, and thymus. Sections stained with hematoxylin–eosin were used for histological investigation.

EXPERIMENTAL RESULTS

The control mice developed signs of transplantation sickness 15-20 days after injection of the spleen cells (loss of weight, reduced mobility, untidiness of the hair, exhaustion, diarrhea), causing death of most of these animals within 30-40 days after transplantation.

Enlargement of the spleen and lymph glands was observed macroscopically in the mice sacrificed after 5-15 days. Enlargement of these organs reached a maximum 10-15 days after transplantation. Later a progressive atrophy of the spleen and lymph glands developed, and this reached a maximum in the late stage of the GVHR. The thymus was not enlarged in the early stage of the GVHR and it started to atrophy 10 days after transplantation. Thickening of the mucous membrane with the formation of ulcers and hemorrhages on its surface were observed in the large intestine of some of the mice killed in the late stage of the GVHR (20-40 days after transplantation).

The lymphoid follicles of the spleen and lymph glands were grossly enlarged 5 days after transplantation and they showed evidence of hyperplasia, chiefly because of proliferation of the lymphoid elements. Ten days after transplantation the lymphoid follicles began to atrophy and the number of histiocytes was appreciably reduced. The lymphoid atrophy was particularly marked in the late periods of the disease. At the same time foci of myeloid hematopoiesis were sometimes observed in the sinuses of the spleen, and fibrosis of the pulp of the spleen and lymph glands was intensified. In the thymus a decrease in the number of lymphoid cells was noted during the development of transplantation sickness, and was followed by replacement by connective tissue.

Hyperplasia of the Kupffer cells appeared in the liver 5 days after transplantation, and was followed by the onset of cloudy swelling and fatty degeneration of the hepatocytes, accompanied by regeneration. In the late stage of the GVHR amyloidosis was sometimes present.

Enlargement of the lymphoid follicles was found in the intestine 5-10 days after transplantation, especially in the Peyer's patches and solitary follicles, and inflammatory changes occurred in the mucosa and submucosa. On the following days the number of lymphocytes in the lymphoid tissue of the intestine was reduced, and the number of histiocytes was correspondingly increased. Gross destructive changes were found in the large intestine of 17% of the mice sacrificed in the late stage of the GVHR: necrosis of the mucosa and submucosa, focal ulcers and hemorrhages, the formation of bacterial thrombi or thrombi on a background of stasis in the small blood vessels of the intestine.

No changes were found at autopsy and during histological examination in the organs of hybrids not receiving injections of parent cells.

The results mainly confirm data previously published, indicating that during the development of the GVHR the increased proliferation of lymphoid tissue observed in the early stage of the reaction is replaced by increasing signs of atrophy and fibrosis.

As well as changes in the lymphoid system of the mice in the late stage of transplantation sickness, gross degenerative and destructive changes were found in the other organs, including the large intestine. Concurrently with our own report [2], a description of these changes in the intestine and other organs of adult unirradiated hybrids with transplantation sickness was given by Cornelius and co-workers [9]. Other workers [11, 12] observed less marked changes in the intestine under the same conditions.

The results show that during the GVHR the earliest changes are observed in the lymphoid tissue, whereas the intestine becomes affected at a comparatively late stage of the disease.

Some workers consider that the changes in the intestine are due to the cytotoxic action of the immunologically competent cells of the graft [9, 14, 15]. Others [8] see the cause of these disturbances in the action of bacteria present in the intestine, for in bacteria-free radiation chimeras with secondary disease no such changes develop in the intestine.

The results of the present investigation suggest that as a result of the cytotoxic action of the donor's lymphocytes on the intestinal mucosa, and also as a result of atrophy of the lymphoid apparatus of the intestine, the latter becomes sensitive to the action of its own bacterial flora, which may cause the development

of extensive destructive changes therein. Injury to the intestinal barrier, and the considerable changes in the lymphoid system as a whole of the recipients may, in turn, facilitate the penetration of microorganisms capable of influencing the clinical course and outcome of the transplantation sickness from the intestine into the blood stream. These hypotheses are confirmed by the results of earlier experiments [4, 5, 7]; these showed that in transplantation sickness produced in unirradiated hybrids by transplantation of spleen cells from the parents, an autoinfection regularly develops in the majority of the mice in the late stage of the disease and exerts a significant influence on the outcome of the transplantation sickness.

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