REACTIONS OF LYMPHOID AND HEMATOPOIETIC TISSUES TO GROWTH OF A SYNGENEIC TRANSPLANTABLE HEMANGIOPERICYTOMA IN MICE

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One aspect of the problem of relations between tumor and host is the leukemoid reaction [1-8, 10-14], the causes of which are not yet clear although several hypotheses have been put forward [3, 7, 9]. Research workers have found that in the course of development of the leukemoid reaction all branches of hematopoiesis are affected, although correlation has not been found between changes in the myeloid and lymphoid branches of hematopoiesis [8]. No reference can be found in the literature to morphometric studies of lymphoid organs during the development of the leukemoid reaction, although such a method could yield quantitative data on spatial relations between the various branches of hematopoiesis. Such information, together with the peripheral blood and bone marrow picture, could shed light on the mechanisms of the leukemoid reaction.

The object of this investigation was a morphometric analysis of the spleen and cytologic study of the bone marrow and peripheral blood of mice with a transplantable syngeneic hemangiopericytoma (HAPC) [4, 5].

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male (CBA × C57BL/6j)F_1 mice weighing 24.0 ± 2.0 g, bred at the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. The animals were divided into two groups: A syngeneic HAPC was transplanted into the mice of group 1 in a dose of 5×10^6 cells subcutaneously in the dorsal region, and a suspension of spleen cells of syngeneic animals was injected subcutaneously into the mice of group 2 (control) in the same dose. The number of leukocytes and erythrocytes in 1 µl peripheral blood was counted and the leukocyte formula calculated 1, 2, 3, 7, 10, 14, and 17 days after transplantation of the HAPC. The animals were killed (5 mice at each time, altogether 70) and the spleen and femoral bone marrow were removed. The spleen was weighed, fixed in Bouin's fluid and embedded in paraffin wax; histological and morphometric investigations were carried out on sections 4 µ thick, stained with azure II-eosin, using Avtandilov's grid. The areas occupied by white and red pulp and the germinal centers were determined and the ratio between areas occupied by the different cells of the granulocytic and erythroid series of hematopoiesis, plasma cells, blast cells, and megakaryocytes in the red pulp was calculated. Blood and bone marrow films were stained with azure-eosin and a differential count made of 200 cells in blood films and 1000 cells in marrow film. The results were subjected to statistical analysis by Student's t-test at the $P \leqslant 0.05$ level of significance of difference between means.

EXPERIMENTAL RESULTS

Data showing the principal changes in the mouse spleen associated with growth of the HAPC are given in Fig. 2. During growth of the tumor (Fig. 2B) an increase in weight of the spleen was observed (Fig. 2A). The area occupied by the red pulp increased progressively, and by the 10th day after transplantation of the HAPC it was twice its initial value, whereas the relative area occupied by the white pulp decreased (Fig. 2C). Meanwhile changes also took place in the cell composition of the red pulp. Starting with the 3rd day after trans-

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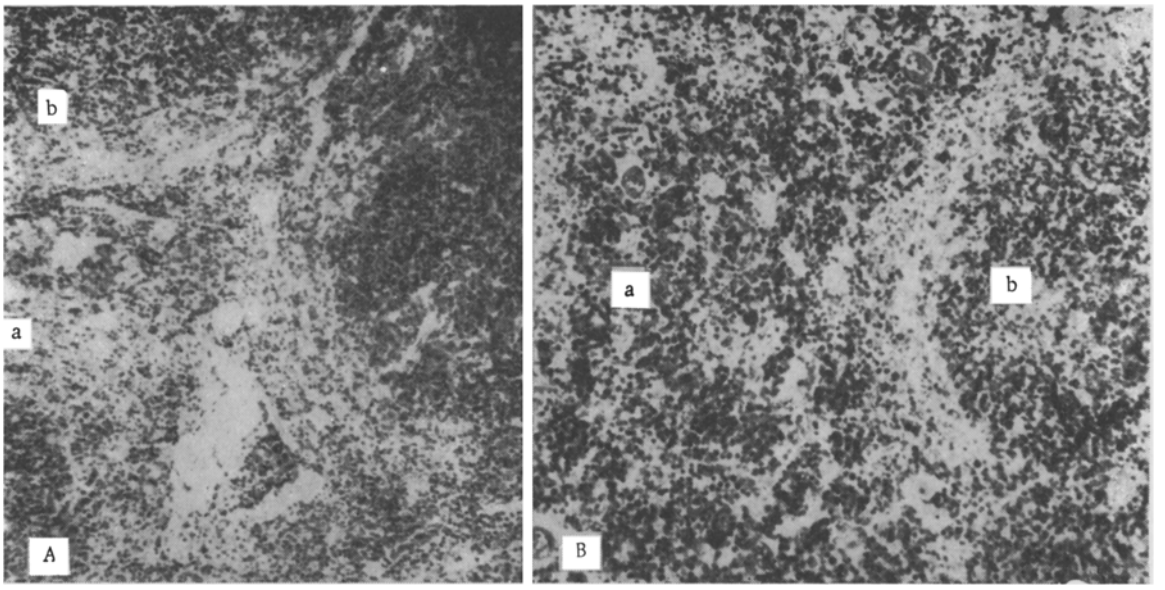

Fig. 1. Changes in spleen associated with growth of hemangiopericytoma. A) Red (a) and white (b) pulp in spleen of a control mouse; B) erythropoiesis in red pulp (a) 14 days after transplantation of tumor: white pulp (b). Ocular 10, objective 10, azure II-eosin.

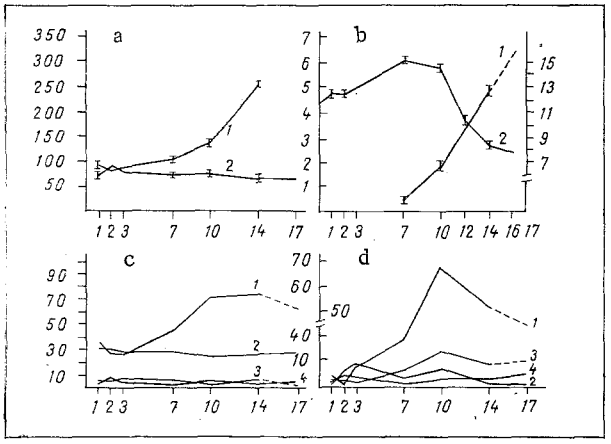

Fig. 2. Changes in spleen and blood associated with growth of hemangiopericytoma. Abscissa, days after transplantation of HAPC; ordinate: a) weight of spleen (in mg); b) weight of tumor (in g) and erythrocyte count (in millions); c) areas occupied by red pulp (in percent) in experiments (1) and control (2) and by germinal centers in experiments (3) and control (4); d) areas (in percent) occupied by erythroblasts in experiments (1) and control (2), and by blast cells in experiments (3) and control (4).

plantation of the HAPC the area occupied by cells of the erythroid series at different stages of maturity increased, to reach 50-70% of the total area of the red pulp by the 10th-14th day. There was a parallel increase in the area occupied by blast cells — to 14% of the area of the red pulp by the 10th day (Fig. 2D). The areas occupied by cells of the granulocytic series and megakaryocytes increased until the 14th-17th day, but areas occupied by plasma cells were unchanged. Starting with the 10th day after transplantation of HAPC the red pulp was filled with large foci of erythroblasts and other blast cells, and also with hypersegmented neutrophils (Fig. 1B). Immature cells of the granulocytic series and eosinophils were arranged diffusely or formed small foci in the red pulp, chiefly beneath the capsule. The composition of the white pulp differed significantly from the control. The trend of changes in the peripheral blood was as follows: increasing leukocytosis on account of polymorphs and monocytes, with fluctuating changes in the lymphocyte count; the trend of the bone marrow indices showed a decrease in the number of stab cells, polymorphs, and lymphocytes and some decrease in the number of cells of the erythroid series, whereas there was no

change in the number of myeloblasts, promyelocytes, and myelocytes. These results are in agreement with those obtained previously [4, 5]. Marked activation of the erythroid series in the spleen showed the necessity of counting the number of erythrocytes in 1 μl peripheral blood. An additional group of ten animals with transplanted HAPC erythrocytes were counted at the same times as the other indices. On the 7th-9th day erythrocytosis developed, and this was followed by a fall in the erythrocyte count with the appearance of anemia on the 14th-17th day.

The results are evidence that the leukemoid reaction to HAPC is characterized, not only by the properties already described, but also by the appearance of marked erythroid hematopoiesis in the red pulp of the spleen starting with the third day after transplantation of HAPC, and the appearance of erythrocytosis in the blood on the 7th-9th day, followed by the development of anemia. Activation of erythropoiesis and, to a lesser degree, of granulopoiesis in the red pulp of the spleen is evidence of involvement of the myeloid branch of hematopoiesis in the reaction. Admittedly, it is not yet possible to judge the functional normality of the proliferating cells, because positive correlation was not found between the number of cells of the erythroid series in the spleen and the number of erythrocytes in the peripheral blood. The lymphoid branch of hematopoiesis in the spleen underwent no significant changes during development of the HAPC. The state of the lymphoid system can be assessed more fully after analysis of the structure of the sinus and lymph nodes.

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