CHANGES IN THE LYMPH GLANDS OF MICE WITH TRANSPLANTABLE LEUKEMIA

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In diseases of the blood system and, in particular, in the leukemias structural changes are almost constantly observed in the lymph glands, corresponding to the form of leukemia. It has been shown that the hemopoietic elements do not enter the gland from outside, but are formed from the reticulum cells of the gland itself. It is not clear, however, which reticulum cells in fact undergo metaplasia: the sinus [1,2] or adventitial [3] cells, the lymphoreticulum cells of the follicles [7], or immature cells of the reticular stroma [4-6].

The object of the present investigation was to study the process of leukemic change in the lymph glands in mice with transplantable leukemia, starting with the early periods after transplantation. The character of the initial and subsequent changes in the lymph glands, especially the regional lymph glands, was investigated in order to discover whether metastasization of leukemic cells takes place from the transplanted material or whether they arise autochtonously from the elements of the lymph gland. In the latter case, it was important to determine which cells give rise to leukemic metaplasia.

EXPERIMENTAL METHOD

The lymph glands were taken from 100 mice of the Afb line with a transplanted hemocytoblastic leukemia at various times from the 1st day of transplantation to the moment of death (the 14th day). On the 1st-3rd day of the experiment, 45 animals were taken, on the 4th day 20, on the 5th-7th day 20, and on the 8th-14th day 15 animals.

The lymph glands were removed together with the surrounding cellular tissue for histological investigation: in all cases, those regional in regard to the site of transplantation, and in some cases distant glands: mediastinal, subcutaneous, and mesenteric. The tissue was embedded in celloidin-paraffin wax, fixed with Bouin's fluid, and stained with hematoxylin-eosin, by Van Gieson's method, with azure-eosin, and with iron-hematoxylin by Heidenhain's method; in isolated cases, Foot's silver impregnation method was used. For control purposes, the lymph glands were taken from 10 mice of the same line.

EXPERIMENTAL RESULTS

The lymph glands of the control animals were light gray in color, they measured 0.1 × 0.1 cm, and the structure of the cortical and medullary layers was clearly distinguishable. A loose network of settled reticulum cells could be seen in the sinuses. The gland was rich in lymphocytes. No features peculiar to the species were observed.

In the first 2 h after transplantation, the regional lymph glands increased in size very slightly (0.1 × 0.2 cm). A conspicuous feature was the stretched sinuses, in which neutrophilic leukocytes, erythrocytes, macrophages with hemosiderin, and individual cells possessing a cytoplasm densely packed with basophilic granules could be seen in the loops of the reticular stroma. Among the numerous lymphocytes of the cortical layer were individual large reticulum cells with a light nucleus and a delicately blue tinged rim of cytoplasm. Well defined germinal centers were present in the follicles. The medullary cords were poor in lymphocytes and consisted mainly of reticulum cells, increased in volume and lying in close contact with one another.

At the beginning of the 3rd day of the experiment, a marked decrease in the number of lymphocytes was observed not only in the medullary cords, but also in the cortical layer of the gland, exposing the reticular stroma. Cells with a densely basophilic cytoplasm began to appear singly or in pairs in the medullary cords. Sometimes they were united into a common syncytium, but most of them appeared to be separate; the nuclei of the cells were enlarged and so also were the nucleoli, while around the nuclei could be seen a narrow, densely basophilic rim of cytoplasm (Fig. 1). In their morphological properties, the cells resembled hemocytoblasts. The marginal sinuses

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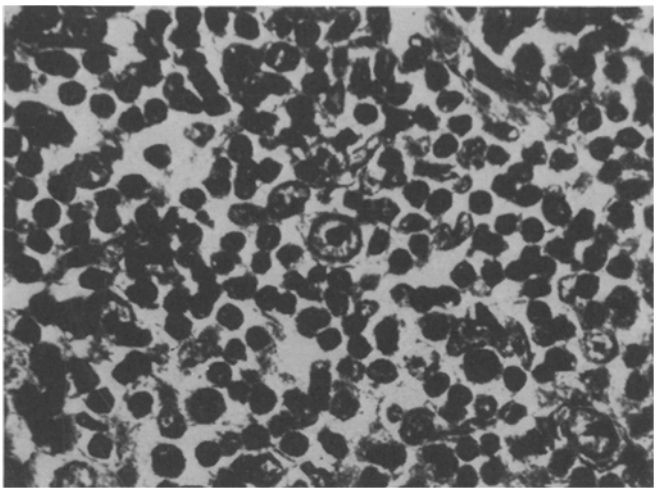

Fig. 1. Enlarged reticulum cells and an occasional hemocytoblast in the medullary layer of a lymph gland. Stained with azure-eosin. Immersion.

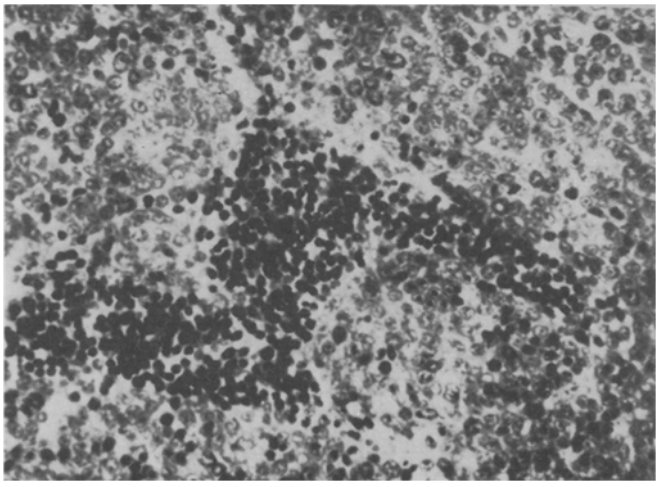

Fig. 2. Replacement of the tissue of a lymph gland by leukemic cells. The sinus is stretched and filled with lymphocytes. Stained with azure-eosin. Objective 40, ocular 7.

of the gland as before were stretched and neutrophilic leukocytes were absent. Phagocytosed erythrocytes and nuclear fragments could be seen in the cytoplasm of the sinus cells. The sinuses of the medullary cords were filled with lymphocytes. In the follicles of the cortical layer clearly defined germinal centers were seen. In the center of the follicles and in the perifollicular zone single, large cells resembling hemocytoblasts were observed, in appearance like the cap of a mushroom. Sometimes they were arranged in small clusters or half-moons at the border between the cortical and medullary layers. At these periods of the experiment, no mitoses were found.

Later, during the 4th day, the lymphocytes disappeared almost completely from the tissue of the gland; they persisted only beneath the capsule as clusters which sometimes resembled follicles without germinal centers. In the medullary cords, hemocytoblasts were predominant, and in the cortical layer of the gland, the hemocytoblasts lay in small groups among the enlarged reticulum cells. At this period, mitoses (1-2 per field of vision) were found in the hemocytoblasts. Solitary free cells similar to hemocytoblasts were found in the sinuses among the lymphocytes and macrophages; their cytoplasm was densely basophilic and sometimes they possessed short projecting processes, so that they appeared to be connected to the wall of the sinus.

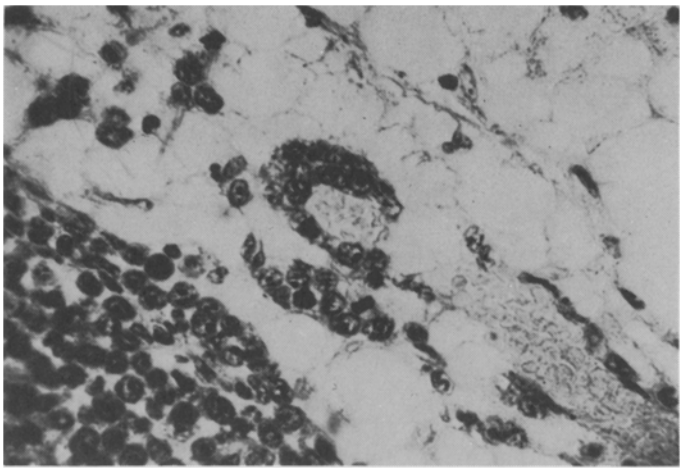

Fig. 3. Cuffs of hemocytoblasts around the capillaries of the fatty areolar tissue. Mitoses. Stained by Van Gieson's method. Objective 40, ocular 15.

On the 5th-6th day of transplanted leukemia, the size of the lymph gland had increased to 0.2 × 0.3 cm and its tissue consisted of light reticulum cells, among which were hemocytoblasts, many in a state of mitosis, arranged in groups and large foci. The medullary cords were almost completely replaced by hemocytoblasts, whereas in the cortical layer under the capsule they were arranged in small clusters. Mature lymphocytes were seen only under the capsule in the form of small groups. Individual hemocytoblasts, lymphocytes, and fragments of nuclear material were present in the sinuses.

In the later stages after transplantation, starting with the 7th-8th day, the lymph gland consisted of a continuous mass of hemocytoblasts, among which many mitoses could be seen. The sinuses of the gland were compressed or stretched, and filled with lymphocytes (Fig. 2), hemocytoblasts, and individual macrophages. The sinus cells could not be detected and the wall of the sinus was composed of leukemic cells.

In many cases, on the 10th-11th day after transplantation, when the tissue of the lymph gland was entirely replaced by hemocytoblasts, these cells began to appear outside the capsule of the gland, in the fatty areolar tissue around the capillaries and nerves, in the form of cuffs and cords (Fig. 3).

In the distant lymph glands, the transformation of the tissue and the involvement of the glands in the leukemic process took place simultaneously with the transformation of the regional gland or 1-2 days later. The peripheral lymph glands increased in size to 0.5 × 0.5 × 0.7 mm, and lay in bunches, adherent neither to one another nor to the skin. The visceral glands, especially the mesenteric, were sometimes very large (2 × 4 cm). Their capsule was always distinct, and they were never adherent to the neighboring organs. The mesenteric lymph glands, together with the thymus, which was enlarged and involved in the leukemic process, compressed the respiratory passages of the mouse. However, these glands likewise were not conglomerated into one mass, but they remained discrete.

In contrast to the regional gland, during the first days after transplantation no leukocytes were detected in the sinuses of the distant glands, but later, especially in the abdominal glands, large amounts of hemosiderin were seen in the cells both of the medullary cords and of the sinuses. The pattern of the appearance of the hemocytoblasts in these glands was the same as in the regional gland.

Usually at the moment of death of the mice, destruction of leukemic cells was observed neither in the lymph glands nor in the organs. In three animals, however, on the 11th and 12th days after transplantation, tiny foci of necrosis of the newly formed cells and numerous nuclear fragments were found in the abdominal glands, lying either in the zone of necrosis or diffusely throughout the pulp of the gland.

In the vessels and sinuses, among the mass of erythrocytes, and also in the tissue of the gland clusters of normoblasts with a dense, dark nucleus and a pink rim of cytoplasm were observed. In the gland tissue, among the hemocytoblasts, collections of cells resembling proerythroblasts in appearance were seen. Very probably extramedullary erythropoiesis was taking place here. The bone marrow was completely replaced by hemocytoblasts, and among them only the magakaryocytes remained.

Hence, parallel with the development of leukemic changes in the focus of transplantation, the structure of the lymph glands was transformed and they were involved in the leukemic process. The transformation began with hyperplasia of the reticulum cells of the medullary and cortical layers of the gland, accompanied by a reduction in the number of lymphocytes in the tissue.

Differentiation of the individual reticulum cells into hemocytoblasts was observed first in the medulla and later in the cortex of the gland. The increase in the number of hemocytoblasts took place both on account of the transformation of local cells and as a result of mitoses, the number of which increased with an increase in the time after transplantation.

The sinus cells retained their phagocytic activity for a long time. Hemocytoblasts appeared much later in the sinuses than in the pulp of the gland, so that the suggestion that they arose from the transplanted material by metastasization can be ruled out.

The results of these investigations agree with data published in the literature [2,8] demonstrating the importance of reticulum-cell hyperplasia as a state preceding the formation of leukemic cells. The authors cited consider that hyperplasia and leukemic transformation affect only the medullary cords (without the active participation of the lymphoid follicles of the gland).

According to the present findings, leukemic cells arise in all subdivisions of the gland, primarily in the medullary cords and in the stroma of the medullary and cortical layers, and later in the follicles. The source of formation of the leukemic hemocytoblasts is the pluripotent cells of the reticular stroma of the lymph gland.

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