ROLE OF THE KUPFFER CELLS OF THE LIVER IN PRIMARY LEUKEMIA

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In the course of development of leukemia foci of hemopoiesis are always formed in the liver, both in the capillaries and in the periportal zones. The participation of the liver in extramedullary hemopoiesis in various pathological states has frequently been described, but different opinions are held on the source of the myeloid cells. Most authors consider that this source in the Kupffer cells – the only one of the local cells capable of giving complete hemopoiesis [1, 5, 7, 10, 18].

Investigators who have studied experimental leukemia also consider that the source of formation of the undifferentiated leukemic cells is the reticular cells of the organs, including the Kupffer cells of the liver [6, 16]. A gradual conversion of the reticular cell via a series of intermediate forms into a hemocytoblast has been observed in these conditions.

Some workers altogether deny the possibility that the Kupffer cells can be converted into a hemocytoblast with myeloid activity. The suggestion has been made that hemopoiesis in the liver is associated with special mesenchymal cells quite unrelated to the Kupffer cells [9]. Other authors [14, 17] consider, however, that the source of formation of the hemopoietic cells in the liver and other organs is wandering hemopoietic elements.

The object of the present investigation was to study the changes in the Kupffer cells in mice with transplanted leukemia in order to discover the role of these cells in the leukemic foci in the liver.

EXPERIMENTAL METHOD

The experimental animals were 100 mice of line Afb, highly susceptible to leukemia, aged from 2 to 3.5 months, inoculated with a transplantable hemocytoblastic leukemia. The experiments were carried out from the 1st until the 12th day of the disease. Every day 7-8 mice were taken for investigation. The controls were 10 inbred mice of the same age groups as the experimental series. The liver was weighed at different times after transplantation. Bouin's fluid was used for fixation and the sections were stained with hematoxylin-eosin, eosin with azure, with iron hematoxylin by Heidenhain's method, and also by Van Gieson's method. A histochemical investigation of the nucleic acid activity was made, staining with gallocyanin and by Feulgen's method for DNA and by Brachet's method for RNA. Two parallel sections were used. One was treated with ribonuclease, and both were then stained with methyl green–pyronine. The results of the investigation were interpreted by the intensity of staining and a 3-point system (+, ++, and +++) was used. The Kupffer cells and the free cells were counted in a particular square of the field of vision, marked out by a micrometer scale and constant for all periods of the investigation, and the numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

In the control series the liver of the mice weighed from 1.3 to 1.5 g. Its surface was smooth, its tissue was dark chestnut in color, and its pattern was normal. The periportal connective-tissue zones contained a few fixed stromal cells and solitary lymphocytes. The Kupffer cells were elongated, round, or irregularly triangular in shape, with dark flattened and oval nuclei; as many as 110 (±13) were counted in 10 fields of vision. The Kupffer cells contained little RNA (+) and the intensity of their reaction for DNA varied: the cells with flattened nuclei, like the endothelium of the veins, stained a bright red color (+++), and the cells with a thin, oval nucleus were less brightly stained (++).

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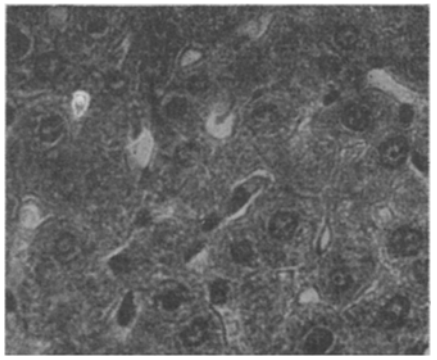

Fig. 1. Hyperplasia of the Kupffer cells. Hematoxylin-eosin. Objective 40×, ocular 15×.

In the first 2 days after transplantation the weight of the liver was unchanged, but in some cases edema and very slight loosening of the fibers were observed in the periportal zones. The number of Kupffer cells in 10 field of vision increased to 160 (±12). On the 3rd day the mean weight of the liver was 1.5-1.55 g and the number of Kupffer cells in 10 fields of vision was 266 (±18). Although remaining fixed, they increased in size, became round, and projected into the lumen of the capillaries, while the cytoplasm devloped a delicate basophilia and the nucleus was hyperchromic (Fig. 1). Besides erythrocytes, solitary lymphocytes and macrophages were seen in the lumen of the hepatic capillaries. On the 4th-5th day the weight of the liver increase to 1.6 g. The number of Kupffer cells in 10 fields of vision increased to 300 (±34). By this time their appearance varied: elongated, oval, or round cells were seen, with a dark, hyperchromic or a light nucleus containing a nucleolus, and lying either singly or in small groups. The Kupffer cells with their light nuclei projected into the lumen of the capillaries, joined to their wall by an almost invisible pedicle. The cytoplasm of the cells became basophilic in color and RNA had accumulated, staining a deeper red color by Brachet's method (++). Two or three nucleoli appeared in the nucleus, solitary mitoses were observed, and the nuclei of the cells stained clearly for DNA (+++).

The number of free cells in the lumen of the capillaries had increased. They included lymphocytes, macrophages, and large cells with basophilic cytoplasm and a light oval or lenticular nucleus, containing small angular or large particles of chromatin and nucleoli. In their morphological picture these cells corresponded to hemocytoblasts. They lay freely as solitary cells in the lumen of the capillaries, sometimes in close relationship with the enlarged Kupffer cells. In the periportal zones a slight loosening of the tissue fibers was observed and the large, light reticular cells were more numerous and connected by processes. Solitary lymphocytes were present.

On the 6th-8th day after transplantation the weight of the liver varied from 1.65 to 1.7 g. Clusters and chains of hemocytoblasts could be seen in the capillaries. Kupffer cells, mainly large and round, projected into the lumen of the capillaries and were isolated from the vessel wall; some of the cells were almost unchanged in structure—they were elongated and contained a compact, hyperchromic nucleus. The number of cells in 10 fields of vision was increased at this period to 550-700. Few mitoses were seen (5-7 in 10 fields of vision) in both the fixed and the free cells. Intracapillary clusters of hemocytoblasts were uniformly distributed throughout the lobule of the liver, and sometimes they accumulated near the central veins. Lymphocytes had disappeared from the periportal connective-tissue zones, the cells of the stroma were free from processes, their cytoplasm had become basophilic, and the cells were arranged in the manner of a sleeve around the vessels and bile ducts.

At the height of the disease (10th-12th day after transplantation) the weight of the liver was 2 g. The liver tissue had lost its normal structure, the trabeculae were thinner, and the liver cells were atrophied. The intralobular capillaries were dilated and goblet-shaped protrusions of their lumen were observed, containing large collections of hemocytoblasts (Fig. 2), including many undergoing mitosis. In 10 fields of vision the number of cells was 980 (±37), mostly lying freely; the commonest cells were hemocytoblasts, but sometimes proerythroblasts and solitary erythroblasts and megakaryocytes were seen (Fig. 3). Fixed Kupffer cells with a thin basophilic rim of cytoplasm were observed either singly or in small groups, and they differed from leukemic hemocytoblasts in their more compact nucleus and the clearly visible connection with the capillary wall. In the periportal zones around the vessels and bile ducts wide sleeves of cells were formed, consisting of hemocytoblasts with mitoses among them (2-3 per field of vision). Most of the leukemic hemocytoblasts contained only a little DNA and RNA (+ or ++). On staining by Feulgen's method their nuclei appeared pale pink and the outline of the cytoplasm was visible as a narrow pink border. The nucleolus always gave a bright staining reaction for RNA. In a few nucleotides the nucleotide content was increased in both the nucleus and the cytoplasm (+++). This applied mainly to cells in a state of mitotic division.

In the liver of mice with transplanted leukemia the changes taking place during the first 2 days after transplantation were therefore very slight: only some loosening of the fibers and edema of the stroma

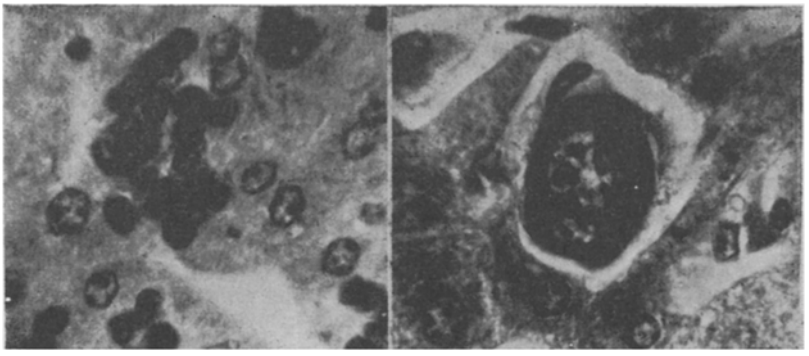

Fig. 2. Isolated foci of hemopoiesis in the capillaries. Van Gieson. Objective 40×, ocular 15×.

Fig. 3. Megakaryocyte in the capillaries of the liver. Van Gieson. Immersion.

were observed in the periportal zones. On the 3rd day hyperplasia of the Kupffer cells and hyperchromatosis of their nuclei were observed. Counting showed a successive increase in the number of Kupffer cells from 160 in the first 2 days to 980 on the 10th-12th day of the disease. Initially the cells became rounded and their volume increased; the nucleus became paler, nucleoli appeared in it, and the cytoplasm developed a basophilic hue. A few mitoses were observed. Some Kupffer cells lost their connection with the capillary wall and became free-lying. These free cells had a round nucleus with neucleoli and a narrow rim of basophilic cytoplasm, i.e., they resembled hemocytoblasts in appearance.

Most of the newly formed hemocytoblasts were undifferentiated; as they proliferated they formed small foci of cells, either evently distributed throughout the lobule or concentrated near the central veins. However, the hemocytoblasts evidently retained their ability to differentiate, for in some cases cells of the erythroid series, of various stages of maturity, and megakaryocytes were found in the hepatic capillaries even in the later stages after transplantation.

In the period of leukemia, when many immature leukemic cells appeared in the peripheral blood, the number of leukemic foci in the liver increased very rapidly. This was evidently due to retention of leukemic hemocytoblasts circulating in the blood in the hepatic capillaries, where they proliferated to form new leukemic foci. Three periods could thus be distinguished in the development of the changes in the liver in transplanted leukemia: a period of nonspecific hyperplasia of the Kupffer cells, a period of differentiation and specific proliferation of the Kupffer cells with their isolation from the vessel wall, and a period of leukemic hyperplasia with colonization of circulating leukemic cells flooding the capillaries of the liver.

The study of the nucleic acid content in the mouse liver preparations by qualitative histochemical methods was of a pilot nature in many respects and it cannot be claimed to have given a complete picture. However, if the results are compared with the results of the histochemical investigation of the blood cells in normal and leukemic conditions resported in the literature [1, 3, 4, 8, 12, 15], it may be concluded that the isolated and free-lying Kupffer cells contained more RNA then the fixed cells, or in other words, their proliferative potential was greater.

Statistical analysis confirmed that the increase in the number of Kupffer cells in the liver during the development of the disease was significant.

There are thus two sources of origin of the foci of leukemic infiltration in the liver: Kupffer cells becoming isolated and converted into leukemic cells in the early periods after transplantation, and colonization of circulating cells in the leukemic period.

The foci of leukemic infiltration arising in the liver were mainly intravascular. Perivascular foci of leukemic infiltration were seen in the region of the periportal zones as a result of specific proliferation and differentiation of the stromal cells.

The nucleic acid metabolism was depressed in most of the hemocytoblasts, and they stopped proliferating and died. Some hemocytoblasts contained large amounts of nucleotides, actively dividing by mitosis, and these were responsible for the quantitative growth of the leukemic cells.

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