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MORPHOLOGICAL CYTOCHEMICAL CHARACTERISTICS OF SPONTANEOUS HEMOBLASTOSIS OF AKR MICE

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UDC 616.155.392:576.31

KEY WORDS: AKR mice; lymphosarcoma; cytological and cytochemical investigation.

Spontaneous hemoblastoses, causing death of 70-90% of AKR mice in the first year of life, have been widely used in various branches of experimental oncology and hematology. However, available information on the morphological characteristics of hemoblastosis arising in mice of this strain is highly contradictory. For example, one investigator has described all the known morphological variants of hemoblastosis in mice of this strain [5], whereas others [1] have described AKR leukemia as hemocytoblastosis and myelosis, and a third group [2] have stated that the myeloid form of leukemia is observed most frequently in the animals which they studied. Finally, in more recent investigations the view has been expressed that hemoblastoses in AKR mice are lymphoid in genesis [4] and some workers consider them to be generalized lymphosarcomas, whereas others are inclined to regard them as lymphatic leukemias [3, 6]. The contradictory nature of these data to some degree reflects present difficulties in the differential diagnosis of tumor-like leukemias and generalized lymphosarcomas, for there are as yet no morphological criteria for differentiation of these two processes. In view of the facts described above it was decided to study the morphological characteristics of hemoblastoses in AKR mice, using modern methods of cytological and cytochemical analysis.

EXPERIMENTAL METHOD

Experiments were carried out on 500 AKR mice of both sexes obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, at the age of 2 months. The animals were kept on a standard diet. Of the total number of mice 400 were kept under observation until natural death; from the remaining group of 100 animals ten were killed for investigation every month. All animals which died or were killed were autopsied. Peripheral and abdominal lymph nodes, the thymus, spleen, liver, lungs, and kidneys were taken for histological analysis. The organs were fixed in 10% formalin and embedded in paraffin wax; sections were stained with hematoxylin and eosin. Squash preparations of internal organs and bone marrow films stained by Leishman's method were used for cytological analysis. Parallel with the cytological investigation, in 36 of 400 mice with hemoblastosis diagnoses on the basis of inspection and hematologic investigation a cytochemical study was made of squash preparations of lymph nodes, spleen, liver, lungs, and kidneys, including the PAS reaction and determination of activity of the following enzymes: α -naphthyl acetate esterase, peroxidase, acid and alkaline phosphatases. Parallel tests were carried out with sodium fluoride, an inhibitor of α -naphthyl acetate esterase, and with potassium-sodium tartrate, an inhibitor of acid phosphatase.

EXPERIMENTAL RESULTS

Among the 400 animals kept under observation until natural death, hemoblastoses were found in 77.5% of mice. Clinically these mice showed marked dyspnea and a barrel chest, due to the presence of an enormous tumor of the thymus. Analysis of the peripheral blood picture of most of these mice revealed wide variation in the numbers of leukocytes and blast cells. At autopsy

Department of Experimental Animals and Department of Endogenous Carcinogenesis, All-Union Oncologic Scientific Center, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 98, No. 7, pp. 70-73, July, 1984. Original article submitted July 4, 1983.

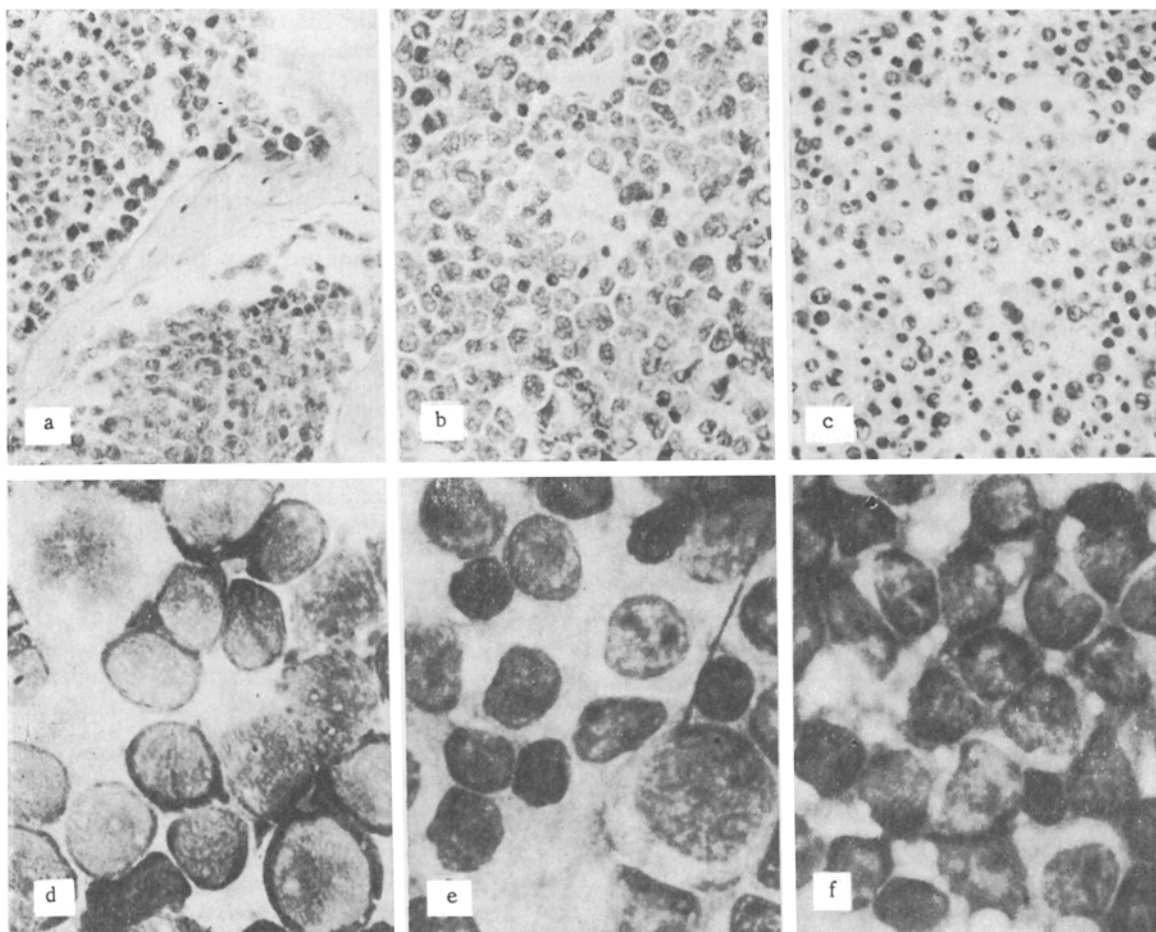


Fig. 1. Morphological characteristics of hemoblastoses in AKR mice. a) Bone marrow. Foci of proliferation of lymphoblasts. Hematoxylin and eosin, 250 \times ; b) thymus. Total proliferation of lymphoblasts. Hematoxylin and eosin, 400 \times ; c) spleen. Focal concentrations of lymphoblasts. Hematoxylin and eosin, 250 \times ; d) concentrations of lymphoblasts in squash preparations of thymus (lymphosarcoma of blast type). Azure and eosin, 630 \times ; e) lymphosarcoma of mixed type. Concentrations of lymphoblasts, lymphocytes, and prolymphocytes. Leishman's stain, 630 \times ; f) lymphosarcoma of lympho-prolymphocyte type. Concentrations of lymphocytes and prolymphocytes. Leishman's stain, 630 \times .

of all the animals the picture was similar: an enormous tumor of the thymus, grayish-white in color, tumor-like lesions of the peripheral and abdominal lymph nodes, frequently adherent to each other to form a confluent tumor conglomerate, and hyperplasia of the liver and spleen. Multiple tumor nodules could be observed along the course of the intestine. Microscopic investigation of sections of the spleen, lymph nodes, and thymus showed complete obliteration of the pattern, total proliferation of leukemic tissue, and invasion of the surrounding cellular tissue (Fig. 1: a, b, c). Together with diffuse infiltration, huge foci of infiltration were found in the liver, lungs, kidneys, muscles, subcutaneous cellular tissue, and cellular tissue of the internal organs. Investigation of cytological preparations revealed very large concentrations of various cells of the lymphoid series in squash preparations and films of the lymph nodes and internal organs.

The lymphoid genesis of these cells was confirmed by cytochemical investigation of squash preparations, which demonstrated absence of peroxidase activity in every case, whereas acid phosphatase was discovered in the form of large or small granules, and it was completely inhibited by potassium-sodium tartrate.

Most animals (88.5%) thus showed the picture of generalized hemoblastosis. However, it was impossible to tell from the results of autopsy whether this was a generalized form of lymphosarcoma or leukemia with tumor growth. A firm diagnosis was made by investigation of the

bone marrow, which was involved in the process in only 62% of animals with hemoblastosis. Absence of leukemic changes with the presence of extensive involvement of internal organs and peripheral lymph nodes was an important argument in support of the diagnosis of generalized lymphosarcoma. The correctness of this diagnosis was confirmed indirectly by the discovery of tumors of the thymus only or of the thymus and one peripheral lymph node in 11.5% of mice with hemoblastoses, with no bone marrow involvement. Further supporting evidence was given by the results of a study of 100 animals killed between the ages of 2 and 12 months, in which hemoblastosis began in every case with a lesion of the thymus, and the picture of generalized lymphosarcoma, accompanied in some cases by secondary leukemic changes, was observed in only some of these mice killed in the latter stages.

In the course of cytological analysis different types of hemoblastoses could be distinguished depending on the predominant neoplastic cells in the squash preparations.

Lymphosarcoma of blast type (40.8%) was distinguished by predominance of lymphoid blast cells (Fig. 1d), among which small, medium-sized, and large blast cells with an oval, round, or irregular nucleus, were most frequently encountered, so that these cases could be described as a polymorphonuclear variant. In the macroblast variants large cells with a large round nucleus, very delicate chromatin granules, and the presence of one to three large nucleoli were distinctly predominant, whereas in the microblast variant the commonest cells were relatively small monomorphic blast cells with a round nucleus occupying the greater part of the cell, a narrow rim of cytoplasm, delicately granular chromatin, and small monomorphic nucleoli. Lymphosarcoma of blast type was characterized cytochemically by the presence of cells with both low and high activity of the enzymes studied, plus a weakly positive granular reaction for glycogen. In some hematopoietic cells the reaction for glycogen was absent. In five animals, investigation of enzyme activity revealed a strong reaction for alkaline phosphatase.

Lymphosarcoma of mixed type (19.7%) was characterized by predominance of blast cells, lymphocytes, and prolymphocytes in squash preparations (Fig. 1e).

Lymphocytes and prolymphocytes (Fig. 1f) predominated in squash preparations of lymphosarcoma of lympho-prolymphocyte type (39.5%). In the atypical lympho-prolymphocyte variant the commonest cells in squash preparations were small, medium-sized, and large cells which morphologically resembled mature lymphocytes and prolymphocytes, but differed from them in marked fragmentation of the nucleus: The chromatin was divided into numerous large and small granules, separated from each other by multiple grooves. This type of lymphosarcoma was characterized cytochemically by moderate activity of all three enzymes studied, but in three cases the reaction for alkaline phosphatase was strong.

Analysis of the results of the cytochemical investigation showed that activity of one of the enzymes tested was increased in both localized and generalized forms of lymphosarcoma, whereas activity of two or more enzymes was increased only in the generalized form. At the same time, cytochemical investigation revealed increased activity of one enzyme (acid or alkaline phosphatase or nonspecific esterase) in the leukemic cells of most mice. This indicated that many lymphoid cells lost the features characteristic of lymphocytes of healthy animals during blast transformation.

This investigation thus demonstrated that spontaneous hemoblastoses in AKR mice are as a rule generalized forms of lymphosarcoma, accompanied in some cases by secondary involvement of bone marrow, and distinguished by a high degree of cytochemical and cytological heterogeneity, which must be taken into consideration when they are used as an experimental model.

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