INVESTIGATION OF LYSOSOMAL HYDROLASE ACTIVITY DURING THE DEVELOPMENT OF EXPERIMENTAL LEUKEMIA

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KEY WORDS: mouse leukemia; lysosomal enzymes; protective reaction of the body.

In the light of modern views on the role of viruses as an etiological factor in the development of cancer and leukemia in man and animals [13] the study of the relations between oncogenic viruses and the host organism is of considerable interest. An important role in these relations is played by cells of the mononuclear phagocytic system and, in particular, the Kupffer cells of the liver [4, 5], which have a well developed lysosomal apparatus. Among the lysosomal hydrolases an important place in this context is occupied by nucleases which, according to data in the literature, can not only perform protective functions in the cell, by hydrolyzing virus nucleic acid, but can also participate in cell pathology [2, 6]. Meanwhile, the question of the role of lysosomal enzymes as a protective factor in cells exposed to transformation and penetration by viruses still remains open. According to data in the literature [10], virus particles in these cells are "stripped" in the lysosomes, and their nucleic acid preserves its polymerization and infectivity during the first few hours after entering the lysosomes.

In the present investigation a comparative study was made of the activity of certain lysosomal enzymes during the development of experimental mouse leukemia induced by Friend's virus. This disease is an erythroblastic leukemia with proliferation of both erythroid and reticulum cells of the spleen [7, 9]. The liver cells are not exposed to the direct action of the virus in this disease and their functional role in the body is preserved. Activity of acid DNase (DNase II), acid RNase, and acid phosphatase was investigated in the spleen and liver tissue of mice during the development of Friend's virus leukemia.

EXPERIMENTAL METHOD

Experiments were carried out on male DBA/2 mice weighing 18-20 g. Supernatant (15,000g, 30 min) of a 10% splenic homogenate from mice with Friend's leukemia was used as the virus-containing material. The virus-containing material was injected intraperitoneally in a dose of 0.3 ml. Animals of the control group received an injection of 0.3 ml of 0.14 M NaCl. The mice were killed by decapitation. The liver and spleen were perfused in situ with cold 0.14 M NaCl by the writers' own method using a special combined stand and needle holder (made with the assistance of engineer K. V. Lysenko). The organs were removed and homogenized in a glass homogenizer with a glass pestle at 4000 rpm for 3.5 min. The dilution of the tissues (w/v) before addition to the incubation medium was as follows: for determination of hepatic DNase II, by 15 times, splenic, by 100 times; for determination of hepatic acid RNase, by 200 times, splenic, by 10 times; for determination of hepatic and splenic acid phosphatase, by 1000 times. Activity of these hydrolases was determined by the microexpress method of Pokrovskii et al. [3]. Total protein in the tissue homogenates was determined by Lowry's method [8].

EXPERIMENTAL RESULTS

Two groups of animals were used: control and experimental. The mice were killed on the 3rd, 5th, 7th, 10th, 12th, 15th, 17th, and 20th days after injection of the virus-containing material. The mean results of two experiments are illustrated in Fig. 1. Each point on the curves corresponds to mean results obtained during the investigation of 10 animals. Activity of DNase II and acid RNase was expressed in micromoles of nucleoside monophosphates, and activity of acid phosphatase in micromoles p-nitrophenol per milligram protein. For greater clarity the results of the tests on animals of the experimental group are shown in Fig. 1 as

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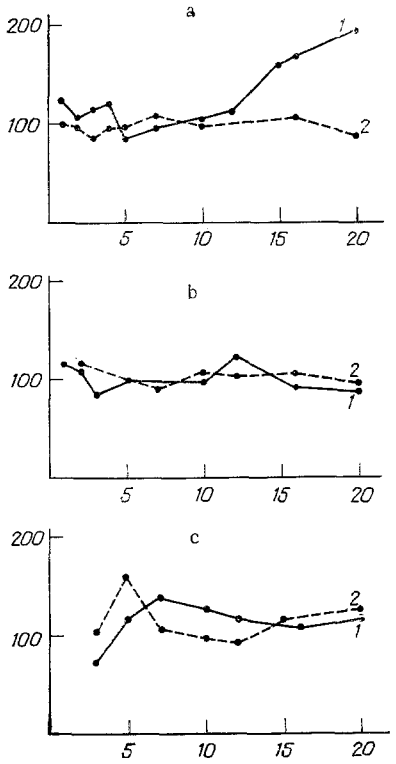

Fig. 1. Activity of DNase II, acid RNase, and acid phosphatase in the liver (1) and spleen (2) of mice during the development of Friend's virus leukemia. a) DNase II, b) acid RNase, c) acid phosphatase. Abscissa, days after injection of virus-containing material; ordinate, enzyme activity (in % of control).

percentages of the control. It will be clear from Fig. 1 that activity of DNase II and acid RNase in the splenic tissue of the experimental animals was indistinguishable from the activity of these enzymes in the control. Acid phosphatase activity in the spleen of the infected animals was increased only during the first 5 days after inoculation with Friend leukemia virus. Later, the activity of this enzyme was indistinguishable from the control. A second small increase in its activity was observed only toward the end of the experiment. Investigation of the activity of these enzymes in the liver showed that the sharpest changes affected DNase II. DNase activity was increased as early as on the 12th day after inoculation with the virus, it increased progressively, and toward the 20th day of the experiment it reached 200% of the control level.

According to data in the literature, virus synthesis in erythroblastic leukemia begins on the 2nd-4th day after entry of the virus into the host animal and is accompanied by cellular changes leading to splenomegaly and leukemia [11]. In the initial period of the disease the liver appears normal, and not until later does infiltration of the sinusoids by reticulum and blast cells take place. This reaction of DNase II, as the writers showed previously [1], is connected with enhancement of the protective functions of the organ and not with pathological changes, which appear only in the last stages of the disease. The increase in DNase II activity in the liver took place on account of activation in the lysosomal apparatus of the Kupffer and endothelial cells, in which a marked increase in the number of primary lysosomes was observed. This behavior of lysosomal DNase probably points to its protective function, directed against the nuclear material of the blast cells and of the intermediate virus-specific DNA, which possesses infectivity [12]. Activity of acid RNase in the liver was unchanged compared with the control. The level of acid phosphatase activity in the liver on the 5th-12th day of the experiment was 20-40% higher than the control. On the following days activity fell, and by the 20th day it exceeded that of the control by only 8-10%.

Friend's leukemia virus is known to possess affinity for cells of the hematopoietic organs. In the course of maturation, the largest number of virions is found in the spleen, where they reproduce mainly in leukemic

cells and megakaryocytes, and they are found only rarely in the liver cells [14]. It can be tentatively suggested that the absence of a response of DNase II in the spleen cells is connected with the fact that the virus acts in a certain manner on the lysosomal apparatus of the infected cells. An alternative possibility cannot be ruled out, namely that Friend virus, which has many antigens common with the cytoplasmic membrane of cells sensitive to the virus on its surface, is not recognized as "foreign" and, consequently, escapes the action of lysosomal DNase.

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CONTACT INTERACTION BETWEEN ASCITES HEPATOMA 22a CELLS AND SOLID SUBSTRATE

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Under certain conditions, widely different types of cells can adhere to the surface of any solid substrate such as glass, certain polymers, metals, and so on. Adhesion is the initial phase of a complex reaction of contact interaction between cell and solid substrate. The study of the particular features of this reaction in tumor cells is not only of general biological, but also of medical, interest: Processes such as metastasization and invasive growth may be based on a disturbance of contact interaction with the substrate [1]. Recent investigations have shown that the character of contact interaction with the substrate is considerably modified in cells subjected to tumor transformation, compared with their normal analogs [2-6].

Cells which evidently stand at the highest level of tumor progression, namely ascites tumor cells, are of great interest. According to some features these cells possess the highest degree of transformation. It was considered important to discover to what extent the reaction of contact interaction with a solid substrate is disturbed in ascites cells.

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