# POSSIBLE ROLE OF DN-ASE I IN THE DEVELOPMENT OF EXPERIMENTAL LEUKEMIA

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Increased activity of serum DNase I and of splenic inhibitor of DNase I was found during the development of Friend's virus leukemia. Increased activity of inhibitor in the spleen also was discovered after intravenous injection of exogenous DNase I into mice. The possible role of serum DNase I in the development of experimental leukemia is discussed.

KEY WORDS: mouse leukemia; DNase I; DNase I inhibitor; cell proliferation.

The DNases, enzymes which specifically hydrolyze DNA, are present in all animal tissues [7, 8]. Despite intensive research in many laboratories of the world during the last two decades, no general agreement has yet been reached regarding the functional role of these enzymes in animals and man. In early publications the DNases were regarded as one cause of damage to nuclear DNA in pathology. In later studies a protective role has been ascribed to them and they have been linked with other systems of defense, namely interferon formation and the immune system [3, 5, 9].

In the investigation described below the functional role of serum DNase I was studied on a model of experimental mouse leukemia.

## EXPERIMENTAL METHOD

Experiments were carried out on male DBA/2 and BALB/c mice weighing 18-20 g. The virus-containing material consisted of supernatant (15,000 g, 30 min) of a 10% homogenate of the spleen of mice with Friend's leukemia in 0.14M NaCl, which was injected intraperitoneally in a dose of 0.3 ml. Animals of the control group were given an intraperitoneal injection of 0.3 ml of 0.14M NaCl. Blood was taken from the tip of the tail at different times after infection. In some experiments blood taken from the animals after sacrifice also was used.

To determine activity of intracellular DNase I inhibitor the animals were killed, the spleen was perfused in situ with cold 0.14M NaCl, and was then homogenized "gently" in a Potter–Elvehjem glass homogenizer with Teflon pestle for 120 sec. The splenic homogenates from the control and experimental mice thus obtained (w/v = 10) were centrifuged at 150,000 g for 30 min in a titanium angle rotor of the MSE Superspeed 65 ultracentrifuge. The supernatants (soluble fraction of the cell extract) were used for determination of activity of the inhibitor. This was judged from the action of the supernatants on bovine DNase I (chromatographically purified preparation, Novosibirsk). Activity of the inhibitor was determined by the following scheme: bovine DNase I + $Mg^{++}$ + supernatant – inhibition for 30 min at 37°C in buffered medium, pH 7.0-7.2, followed by addition of DNA substrate. The subsequent course of the analysis was described previously [2]. The difference between the original and residual bovine DNase I activity indicated the activity of the inhibitor. To determine DNase activity a microexpress method [2] was used. Total protein in the samples was determined by Lowry's method in Pokrovskii's micromodification [6, 13]. DNA isolated from calf thymus by the methods of Georgiev [1] and Kirby [11] was used as substrate for determination of DNase activity (and activity of the inhibitor).

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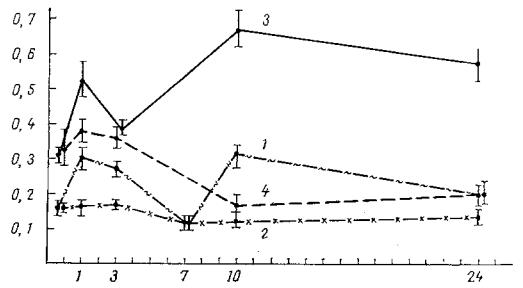


Fig. 1. Changes in serum DNase I activity of DBA/2 and BALB/c mice during development of Friend's virus leukemia. Abscissa, time (in days) after injection of virus-containing material; ordinate, DNase I activity (in $\mu$moles nucleoside monophosphates). Control: 2) DBA/2, 4) BALB/c; leukemia: 1) DBA/2, 3) BALB/c.

## EXPERIMENTAL RESULTS

In the experiments of series I the dynamics of serum DNase I activity was studied in mice during the development of Friend's virus leukemia. Collection of blood for determination of serum DNase I activity was carried out individually on each animal before infection with the virus (background) and later at various times after injection of the virus-containing material (4 and 18 h, and 3, 7, 10, 20, and 23 days). The mean results of seven experiments on DBA/2 mice and three experiments on BALB/c mice are illustrated in Fig. 1 . Two groups of mice, with 5-7 animals in each group, took part in each experiment.

It will be clear from Fig. 1 that the serum DNase I activity of the infected animals (experimental group) rose above the control level after 4 h and reached a maximum 18 h after injection of the virus containing matrial. Serum DNase I activity of the DBA/2 mice remained high for 3 days, then fell to its original level by the 7th day of the experiment, and rose again until the 10th day. By the end of the experiment activity of the enzyme had fallen a little. Similar results were obtained with BALB/c mice. The degree of development of the leukemia was judged from the increase in weight of the spleen in mice examined in each experiment. The weight of the spleen began to increase on the 7th day after inoculation of the virus, and by the end of the experiment (20-25 days) its mean weight was 2 g, 20 times greater than the weight of the normal spleen (0.1 g).

To determine intracellular activity of DNase I inhibitor the spleens were taken from BALB/c mice on the 1st and 23rd days after injection of virus-containing material, i.e., at times when the serum DNase I activity was still high. Activity of the inhibitor was determined as described in "Experimental Method." The results are shown in Fig. 2, in which the original bovine DNase I activity in samples without the addition of inhibitor was taken as 100%. As Fig. 2 shows, activity of the inhibitor was higher in the spleen of the animals with leukemia than in the control.

The increase in activity of the inhibitor in the spleen and the simultaneous increase in serum DNase I activity could indicate a definite functional connection between these proteins in the body. According to data in the literature, DNase I inhibitor, contained in the serum and also in cells of lymphoid organs, forms a stable, highly specific complex with DNase I [12]. If inhibitor protein is injected into an animal, it sharply stimulates hematopoiesis [4]. Recently published data show that DNase I inhibitor belongs to the class of actin proteins [12], which are known to participate in various intracellular processes, such as cytokinesis, phagocytosis, and so on [15]. The possibility cannot be ruled out that an increase in serum DNase I activity lowers the activity of the inhibitor circulating in the blood stream of leukemic mice. This change in the ratio between activities of DNase and inhibitor is a special form of trigger mechanism for initiating synthesis of the intracellular inhibitor. A direct connection between serum DNase I and intracellular inhibitor was demonstrated by the writers in model experiments in which exogenous DNase I was injected into mice. It was shown previously that murine and bovine DNases I have a definite degree of homology [2]. On these grounds it was decided that bovine DNase I could be administered to mice. Bovine DNase I was injected into the caudal vein over a period of 4 days: 7 mg on each of the first 2 days, 10 mg on each of the last 2 days. Animals of the control and experimental groups were sacrificed 1 h after the final injection of enzyme and the levels of splenic inhibitor and serum DNase I

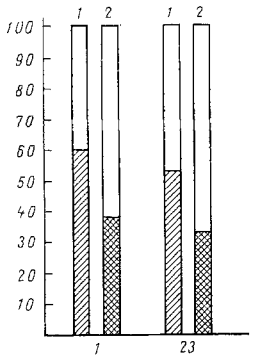


Fig. 2

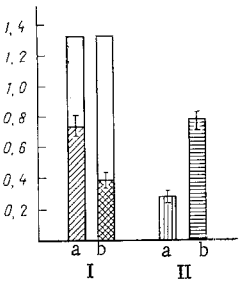


Fig. 3

Fig. 2. Inhibition of bovine DNase I activity by supernatant of spleens of normal (1) and leukemic (2) mice. Abscissa, time (in days) after injection of virus-containing material into mice; ordinate, residual bovine DNase I activity (in % of original activity).

Fig. 3. Changes in DNase I activity (in $\mu$moles nucleoside monophosphates) in blood serum and activity of DNase I inhibitor in spleen of BALB/c mice after injection of bovine DNase I. a) Control; b) experiment. I) Inhibited (top part of column) and residual (bottom part) bovine DNase I activity after incubation with splenic supernatants; II) DNase I activity in mouse blood serum.

were determined as described in "Experimental Method." The results of these experiments are given in Fig. 3; they show that activity of the inhibitor in the experimental animals was significantly higher than in the control. The serum DNase I activity of the experimental animals was twice as high as in the control. The increase in DNase I activity in the blood stream of animals after injection of exogenous DNase is thus probably the trigger mechanism for synthesis of intracellular inhibitor.

The following mechanism of action of the DNase I–inhibitor system can thus be postulated on the basis of the results of the present experiments and data in the literature. Injection of virus antigens into mice leads to an increase in serum DNase I activity and to a corresponding decrease in the activity of free inhibitor. This, in turn, acts as a signal for the initiation of synthesis of intracellular DNase I inhibitor in the spleen and also, perhaps, in other lymphoid organs. The possibility cannot be ruled out that accumulation of the inhibitor, an active protein, in the cells of the lymphoid system is one link in a complex chain of events leading ultimately to cellular proliferation in the spleen and, in this case, to proliferation of reticular cells, the presence of which is characteristic of Friend's leukemia [14]. Stimulation of cellular proliferation may perhaps be connected with changes in the shape of the cells under the influence of the actin inhibitory protein accumulating in the spleen cells. There are indications in the literature that the shape of the cells may affect the rate of cell division [10].

An increase in serum DNase I activity in mice, although to a lesser degree than in the experiments described above, also was found after intraperitoneal injection of other antigens, including extracts of rat spleen, rabbit $\gamma$-globulins, and so on. It can accordingly be postulated that serum DNase I plays the role of a unique "signal" or triggering mechanism for proliferation, and also, perhaps, for cloning of cells in lymphoid organs, and thus behaves as a nonspecific stimulator of the immune system. In this sense, proliferation of spleen cells in mice with leukemia can be regarded as a special case of abnormal cellular proliferation, in which the same common mechanism of regulation of cell division (DNase I–inhibitor) acts. This hypothesis on the mechanism of cell proliferation unquestionably calls for further confirmation.

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# MODIFICATION OF THE TOXIC AND ANTITUMOR PROPERTIES OF FTORAFUR BY ACTION AIMED AT NONSPECIFIC MICROSOMAL OXIDASES


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Phenobarbital and methylcholanthrene, inducers of nonspecific microsomal oxidases, inhibit the development of neurotoxic shock produced in mice by large doses of ftorafur, but they increase the mortality among the animals on the 4th-8th days after administration of the compound. Inhibitor SKF525 A has the opposite action on both types of toxic manifestations. The antitumor effect of ftorafur, determined on the basis of the decrease in weight of the spleen in mice with Rauscher leukemia, is greatly enhanced by preliminary administration of phenobarbital, alone or together with methylcholanthrene, to mice.



KEY WORDS: ftorafur; nonspecific microsomal oxidases.


The antitumor agent ftorafur has achieved widespread popularity because of its lower toxicity, compared with other fluoropyrimidines, toward the epithelium of the small intestine and hematopoietic organs [1, 2, 8].

It is considered that this property of ftorafur is most probably due to its slow breakdown and conversion into the active principle, 5-fluorouracil [5, 6].

This character of its metabolism, ensuring the long circulation of low concentrations of 5-fluorouracil in the body, also gives ftorafur lower antitumor activity than 5-fluorouracil in relation to several experimental tumors [3, 7]. Attempts to enhance its antitumor activity by the use of massive doses of the compound have proved it possible, for in large doses ftorafur gives rise to neurotoxic complications, the severity of which is proportional to the sessional dose of the drug [4].

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