probably connected with their ability to form complexes with metals and to reduce their prooxidant properties. This property of the polyamines is of great importance for correction of the trigger mechanisms of development of oxygen poisoning.

The effectiveness of arginine as a protective agent against hyperoxia is thus determined by the broad spectrum of its normalizing action.

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# STUDY OF DEOXYRIBONUCLEASE I ACTIVITY IN A DEOXYRIBONUCLEASE I—INHIBITOR SYSTEM


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Despite intensive research in various laboratories of the world the functional role of deoxyribonuclease (DNase) I in eukaryotes has not been unequivocally established [11]. One reason for this is evidently that DNase I in the course of evolution has acquired another (regulatory) function in addition to its catalytic (hydrolytic) function. Functional heterogeneity, due both to the chemical properties of the molecules and participation of the same enzyme in different processes (repair, recombination, replication, restriction) is a characteristic feature of the nucleases [4, 9].

Besides nucleases, their inhibitors also are found in prokaryotes and eukaryotes. In animals inhibitors of both DNase I and DNase II have been found [8, 13].

A study of the inhibitor of DNase I in warm-blooded animals showed that it is present not only in the serum, but also inside the cells and forms a specific inhibitor—enzyme complex [14]. It is stated in the literature that the inhibitor of DNase I belongs to the class of actin proteins [12]. It has accordingly been suggested that it may play a role in the proliferation of spleen cells [3].

The object of this investigation was to study the relations between serum DNase I and its intracellular inhibitor *in vivo*.


KEY WORDS: exogenous deoxyribonuclease I; splenic inhibitor.



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TABLE 1. Activity of DNase I Inhibitor in Splenic Supernatants and of DNase I in Mouse Serum after 4 Injections of Exogenous DNase I, Bovine Albumin, and 0.14 M NaCl (M ± m)

| Line of mice | Activity of inhibitor | | | | | Activity of DNase I | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.14 M NaCl | DNase I | P | albumin | P | 0.14 M NaCl | DNase I | P | albumin | P |
| BALB/c | 0,493±0,047 | 0,720±0,080 | <0,05 | 0,522±0,049 | >0,5 | 0,080±0,017 | 0,142±0,021 | <0,05 | 0,061±0,009 | >0,25 |
| DBA/2 | 0,414±0,034 | 0,644±0,056 | <0,05 | 0,450±0,065 | >0,5 | 0,044±0,006 | 0,190±0,011 | <0,001 | 0,070±0,009 | >0,05 |
| CBA | 0,790±0,053 | 0,800±0,060 | >0,5 | 0,700±0,064 | >0,25 | 0,113±0,006 | 0,206±0,022 | <0,05 | 0,114±0,008 | >0,5 |
| C57BL/6 | 0,313±0,036 | 0,384±0,052 | >0,5 | 0,384±0,052 | >0,25 | 0,164±0,014 | 0,252±0,016 | <0,02 | 0,202±0,004 | >0,05 |

Legend. Activity of DNase I expressed in units of optical density of samples at 260 nm; values of P calculated by comparing DNase activity with that found after addition of 0.14 M NaCl.

## EXPERIMENTAL METHOD

Experiments were carried out on male DBA/2, BALB/c, CBA, and C57BL/6 mice weighing 12-30 g. A chromatographically purified preparation of bovine DNase I and bovine serum albumin were used for intravenous injections. Normal rabbit γ-globulins, isolated by Cohn's method [7], high-polymer DNA isolated from calf thymus by the methods of Georgiev [1] and Kirby [10], virus-containing material consisting of the supernatant (15,000g, 30 min) of a 10% homogenate of the spleen of mice with Friend's leukemia, made up in 0.14 M NaCl, and supernatant of rat spleen homogenate were used for intraperitoneal injection as antigens. The antigens were injected once only: DNA in a dose of 2 mg, γ-globulins and splenic supernatant in concentrations of 15-20 mg total protein. The animals were killed at different times after injection of the above antigens and the organs were perfused and homogenized gently. The homogenates were centrifuged at 150,000g for 30 min and activity of DNase I inhibitor was determined in the resulting supernatant. Activity of the inhibitor was determined by the method described previously [3]. Activity of DNase I in the animals' sera and in samples with inhibitor was determined by the method [2] in the authors' modification. Total protein was determined by Lowry's method [15].

## EXPERIMENTAL RESULTS

To determine causative relations between DNase I and the intracellular inhibitor, an increase in DNase I activity in mouse spleen was produced experimentally by injection of exogenous DNase I. Bovine DNase I, which shares common immunologic features with mouse DNase I [2], was used for this purpose. The DNase was injected into mice of different lines, differing in their sensitivity to leukemia virus and possessing different haplotypes.

The DNase I preparation was injected into the caudal vein, the number of injections varying from 2 to 9 (one injection of 10 mg of the preparation in the course of one day). Bovine serum albumin and 0.14 M NaCl respectively were injected into mice of the two control groups. Each group consisted of 5 animals and the experiments were repeated at least three times. The animals were killed 1.5 and 18 h after the last injection of antigen and of 0.14 M NaCl and activity of the inhibitor was determined in the liver, thymus, and spleen. The results of determination of activity of the inhibitor in the spleen and of DNase activity in the serum of the mice after four injections of bovine DNase I are given in Table 1.

Bovine DNase I activity, inhibited as a result of the addition of splenic supernatant containing inhibitor to the incubation mixture, served as indicator of activity of the DNase I inhibitor. Activity of the inhibitor was directed proportional to the inhibited enzyme activity. Activation of the inhibitor in the spleen, incidentally, did not depend on the number of injections but was observed only when the level of DNase activity in the serum was increased, i.e., during the first few hours after the injection.

It will be clear from Table 1 that in DBA/2 and BALB/c mice (haplotype H-$2^d$), susceptible to leukemia virus, activity of the inhibitor in the spleen cells was increased. Injection of 0.14 M NaCl and of bovine serum albumin had no such action. In mice of lines with low susceptibility to leukemia virus — C57BL/6 and CBA (haplotype H-$2^b$ and H-$2^k$, respectively) — injection of exogenous DNase caused no significant activation of the intracellular inhibitor, although its activity in the serum was considerably increased. Activation of the inhibitor, according to the observations made, did not take place in the liver and thymus of the animals. Parallel with the above investigations, the spleen of the mice of the above-mentioned groups was weighed. After four injections of DNase I the weight of the spleen in DBA/2 and BALB/c mice increased much more rapidly than in CBA and C57BL/6 mice.

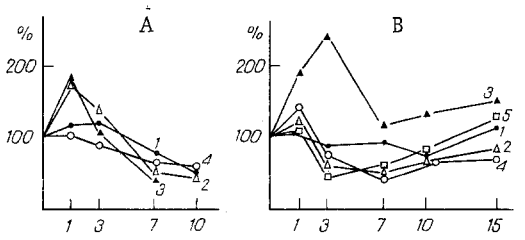


Fig. 1. Activity of DNase I in serum of mice after injection of various antigens: A) C57BL/6; B) DBA/2 mice. 1) 0.14 M NaCl; 2) supernatant of rat spleen; 3) virus-containing material; 4) γ-globulins; 5) DNA. Abscissa, time of investigation (in days); ordinate, DNase I activity (in percent of background level).

On the basis of analysis of the results it can be suggested that a causative connection exists between serum DNase I and the intracellular inhibitor (actin protein), as a result of which an increase in DNase activity in the serum initiates activation of the intracellular inhibitor in the spleen. Meanwhile the weight of the spleen rises more rapidly. This effect was observed in mice with the $H\text{-}2^d$ haplotype [5], whose genetic susceptibility to Friend's leukemia virus is partly controlled by genes located in the main histocompatibility H-2 complex [6]. The possibility cannot be ruled out that the DNase–inhibitor system performs an important functional role in proliferation of spleen cells, and so participates in the immune response of the animal. Data obtained after injection of various antigens into mice may be evidence to some extent in support of this hypothesis (Fig. 1). As Fig. 1 shows, serum DNase I activity increased on the first day (18 h) after intraperitoneal injection of certain antigens. The increase in activity of the inhibitor in the spleen revealed by the method used in the present experiments was possibly due to its activation in B lymphocytes. Meanwhile thymus cells (T lymphocytes) evidently do not react to an increase in DNase I in the serum. The absence of any such effect also was found in liver cells (hepatocytes and Kupffer cells). To obtain a final answer to the question of the place occupied by the DNase I–inhibitor system in immunity, further investigations are required at the cellular and subcellular levels.

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