EFFECT OF Clostridium perfringens TYPE A TOXIN AND METABOLIC PRODUCTS OF Clostridium butyricum ON LIVER LYSOSOMES in vitro

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During incubation of the lysosomal fraction of the albino mouse liver with Clostridium perfringens type A toxin and also with the toxin and filtrate of a broth culture of Clostridium butyricum, and increase in the specific acid phosphatase activity was observed. The action of C. perfringens toxin on the lysosomal membrane was potentiated under the influence of metabolic products of C. butyricum. Potentiation of the action of C. perfringens toxin on the lysosomes was due to thermostable substances in the C. butyricum filtrate.

KEY WORDS: lysosomes; Clostridium perfringens; Clostridium butyricum; acid phosphatase.

The authors showed previously that under the influence of metabolic products of Clostridium butyricum the hemolytic and lethal effects of Clostridium perfringens toxin are potentiated [9] and disturbances of cerebral cortical function [11] and changes in the terminal blood stream [10] are aggravated.

The object of this investigation was to study changes in acid phosphatase activity of the liver lysosomes under the influence of C. perfringens toxin and metabolic products of C. butyricum as a possible microbial association in anaerobic gas gangrene.

EXPERIMENTAL METHODS

Lysosomes were isolated from the liver of albino mice by the method of De Duve et al. [14]. The livers from three or four mice killed by total exsanguination were homogenized in 0.25 M sucrose solution and 0.001 M EDTA solution in a Dounce homogenizer. Differential centrifugation was carried out on a K-24 centrifuge (East Germany) at 2°C. Lysosomes suspended in 5 ml of sucrose and EDTA solution were used in the experiments.

Acid phosphatase was determined by the method of Bessey et al. [13] in the modification of Levitskii et al. [5]. To 0.4 ml of p-nitrophenyl phosphate in citrate buffer, pH 4.8, 0.1 ml of a suspension of lysosomes was added and the mixture was incubated for 30 min at 30°C. To stop the reaction, 5 ml of a 0.05 M solution of NaOH was added. Extinction was measured on the Spekol spectrophotocolorimeter (East Germany) at 410 nm.

Specific activity of acid phosphatase was assessed in milliunits/mg protein (mu/mg), 1 mu being taken to be the quantity of enzyme hydrolyzing 1 nmole of substrate during incubation for 1 min.

Protein in the lysosome suspension was determined by the method of Lowry et al. [16] in Levitskii's modification [4]. To 0.2 ml of lysosomal fraction in 0.85% NaCl solution 2 ml of solution C was added; solution C was made from solution A and B in the ratio of 50:1 and 0.2 ml of Folin's reagent, diluted 1:1 with distilled water. Extinction was measured at 750 nm after 30 min.

Dry toxin of C. perfringens type A, batch 29, produced by the Khar'kov Scientific-Research Institute of Vaccines and Sera, and a filtrate of a broth culture of C. butyricum strain No. 237 were used in the experiments.

In the experiments to study the effect of C. perfringens toxin on acid phosphatase activity in the lysosomes, successively smaller fractions of the quantity of toxin were used, mixed with an equal volume of

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TABLE 1. Specific Acid Phosphatase Activity (in mu/mg protein) of Liver Lysosomes under the Influence of C. perfringens Type A Toxin and Filtrate of a Culture of C. butyricum

Series of experiments	Ingredients	Dose of toxin, μg					Control (C)
		200	100	50	25	12,5	
I	Toxin	5,879±0,058	5,996±0,126	6,217±0,145	5,896±0,055	6,014±0,057	5,322±0,040
	P_{C-I}	<0,001	<0,001	<0,001	<0,001	<0,001	
II	Toxin with native filtrate	6,572±0,066	6,555±0,058	6,808±0,100	6,859±0,191	6,690±0,039	—
	P_{I-II}	<0,001	<0,001	<0,001	<0,001	<0,001	
III	Toxin with heated filtrate	6,521±0,058	6,732±0,072	6,994±0,080	7,232±0,113	6,892±0,122	—
	P_{I-II}	<0,001	<0,001	<0,001	<0,001	<0,001	
	P_{II-III}	>0,05	>0,05	>0,05	>0,05	>0,05	

lysosome suspension, and incubated at 30°C for 15 min. Next, substrate for acid phosphatase was added to 0.1 ml of this mixture and incubation continued for a further 30 min at 30°C. After addition of NaOH solution to stop the reaction, extinction was measured and the specific acid phosphatase activity calculated. In the experiments of series II the dose of toxin was successively reduced by half and mixed with filtrate of the C. butyricum culture in equal volumes, after which acid phosphatase was determined as described above. In the experiments of series III the toxin was mixed with filtrate of a culture of C. butyricum previously heated on a water bath at 100°C for 1 h.

EXPERIMENTAL RESULTS

The experiments of series I showed that under the influence of C. perfringens toxin the specific acid phosphatase activity of the lysosomes was increased (Table 1). It will be noted that a considerable increase in the specific activity of the enzyme took place whether comparatively large or small doses of toxin were added to the medium. Liberation of acid phosphatase of mouse liver lysosomes under the influence of C. perfringens type A toxin may be evidence that the toxin acts on the lysosome membranes. Bernheimer and Schwartz [12] observed correlation between the hemolytic properties of certain bacterial toxins and their ability to induce destruction of lysosomes, and they suggested that erythrocyte and lysosome membranes are similar. Since C. perfringens toxin possesses hemolytic properties and, as the results obtained above show, liberates acid phosphatase from liver lysosomes, this may confirm the similarity between the erythrocyte and lysosome membranes.

The experiments of series II showed that the specific acid phosphatase activity after combined treatment with toxin and filtrate of the broth culture of C. butyricum was increased compared with that under the influence of the toxin alone (Table 1). Incubation of the lysosomal fraction with the C. butyricum filtrate did not lead to an increase in specific acid phosphatase activity. Consequently, metabolic products of C. butyricum potentiate the action of C. perfringens type A toxin on lysosome membranes, as shown by an increase in the specific activity of the enzyme.

The writers showed previously that the toxic effects of C. perfringens toxin are potentiated by a thermostable component of the filtrate of a culture of C. butyricum [9]. It follows from the results of the experiments of series III that the action of the toxin on the lysosomal fraction of mouse liver was potentiated by the action of C. butyricum filtrate even when heated on a water bath to 100°C for 1 h (Table 1). It can thus be concluded that potentiation of the action of C. perfringens toxin on the liver lysosomes was due to thermostable substances in the C. butyricum filtrate.

Statistical analysis of the results showed that the difference between the specific activity of the experimental and control series and also differences in the results of the tests with toxin and with a mixture of toxin and filtrate (native and heated) were significant in all cases ($P < 0.001$).

It can be concluded from the results of these investigations that the action of C. perfringens toxin is associated with labilization of the liver lysosomes of the mice followed by liberation of acid phosphatase. Several workers [1-3, 6-8] claim that lysosomal enzymes (cathepsins, DNase, RNase, phosphatases, β-glucuronidase, etc.) liberated by bacterial toxins, may cause severe damage to the infected animal as a result of their action on tissue substrates.

One of the factors which may be responsible for the more severe course of anaerobic gas gangrene if microbial associations are present in wounds is potentiation of the action of C. perfringens toxin on the lysosomal membranes under the influence of metabolic products of C. butyricum.

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ANTIBODY-STIMULATING FACTOR SECRETED BY SPLEEN CELLS in vitro

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The intensity of antibody formation in irradiated syngeneic recipients was studied in relation to its restoration by various cell suspensions. A lymphocyte suspension free of macrophages restored the ability of the irradiated recipients to respond to sheep's red blood cells only weakly. Addition of peritoneal or splenic macrophages to the lymphocytes led to a sharp increase in plaque formation. The same response was produced by injecting a suspension of spleen cells incubated beforehand in vitro for 3 h at 37°C into the recipients. After incubation of the spleen cell suspension in this way in the absence of antigen, a factor capable of sharply increasing antibody formation in irradiated recipients on restoration of their immune response by injection of donors' lymphocytes was secreted into the medium. The antibody-stimulating factor was not produced after incubation of lymphocytes for the same times. It is suggested that the antibody-stimulating factor appearing in the medium during culture of spleen cells in vitro in the absence of antigen is formed by cells of the mononuclear phagocytic system.

KEY WORDS: macrophages; antibody-stimulating factor; lymphocytes.

The role of macrophages in the realization and regulation of the immune response is undisputed. Numerous investigations have shown that macrophages and their products can both stimulate and depress the immune response. Stimulation of lymphocytes in vitro by antigens or mitogens does not take place in the absence of macrophages or of the factor secreted by them which activates lymphocytes [3]. However, under certain conditions macrophages may depress the response of lymphocytes to antigens and mitogens [2]. Most of these investigations were undertaken in vitro. It has not yet been established what role in the effects exhibited by macrophages is played by their number and functional activity or whether this depends on the presence or absence of a particular subclass of cells.

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