STIMULATION OF NONSPECIFIC IMMUNITY BY SOME BACTERIAL POLYSACCHARIDES

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Recently the study of substances which are capable of activating nonspecific defense mechanisms of the host within a short time has been rapidly developing. Of these substances the most prominent have been the polysaccharide components which compose the bacterial cell wall. The majority of known biologically active bacterial lipopolysaccharides were isolated from Gram-negative pathogenic bacilli. In the Laboratory of New Antibiotics of the Department of Microbiology at the Central Institute of Postgraduate Medicine, under the leadership of Z. V. Ermoleva, there has been isolated from the nonpathogenic organism Acetobacter xylinum a polysaccharide which is capable of sharply raising the resistance of the host to a number of harmful effects; this substance has been tentatively named ciine (acetoxin). In previous publications [2,3], the properties of ciine as regards the increased resistance of the host to staphylococcal infections and to the action of ionizing irradiation were described. Inasmuch as ciine has no antibacterial properties in vitro, the suggestion was made that the effect was concerned with the host reaction.

Nonetheless, a number of details of the host reaction after the introduction of this polysaccharide remain unexplained. The range of this reaction has not been established, i.e., whether it concerns several infections or just those mentioned and it has not been established with what factors in immunity the effect of ciine is concerned or if the dependence of the effect of ciine is on the mode of introduction or the nature of the material. The results presented below are an attempt to obtain an answer to these questions.

METHODS AND RESULTS

The effect of ciine on the development of infections evoked by pathogenic strains of staphylococci, Escherichia coli, Pseudomonas aeruginosa (Pseudomanas pyocyanea) and Proteus vulgaris were studied in white mice weighing 15-18g (more than 2000 mice) which received 200 ug of ciine in saline 18-24 hr before the administration of the lethal dose of bacteria. The control animals were given a corresponding amount of saline intraperitoneally. The infected animals were observed for 10 days. The results were statistically analyzed. A portion of them are presented in Fig. 1, in which the figures on the ordinate represent the index of survival of the mice (the ratio of number of surviving mice to the number of mice in the experiment).

These experiments showed that with all the infections studied the administration of ciine sharply reduced the development of sepsis and increased the survival of the mice. It can be considered that ciine stimulates nonspecific natural immunity of the animal, especially to the bacterial polysaccharides isolated by other authors from pathogenic bacteria [5,7]. On this basis, it was permissible to assume that the introduction of ciine is effective independent of the character of infection. However, this proposition was not supported by experiments. Upon infecting the mice with Friedländer's bacillus, a virulent strain of Brucella melitensis or vaccinating strains of Pasteurella tularensis (Bacterium tularense), ciine did not increase the resistance of the host to the infection and the mice receiving ciine died at the same rate as the control group. This bears witness to the fact that the defense mechanism stimulated by ciine does not protect the mouse from the development of these infections.

Attempts were made to establish what components of immunity are concerned in the defense reaction which raises resistance of the mice to infection. To clarify the role of the local nervous system of the peritoneum, the effect of a novocain block of the peritoneum on the reaction evoked by ciine was determined. For this purpose, one group of mice was given ciine intrapertioneally 24 hours before infection, and 10 min after the intraperitoneal infection

0.5-0.25 ml of novocain was given. The control group of mice received ciine in physiological saline and no novocain. It was established that ciine protected the animals from death both in the presence and absence of novocain, whereas nearly all the animals of the control group died. Thus, the conclusion was drawn that the action of ciine has no connection with the local nervous system of the peritoneum.

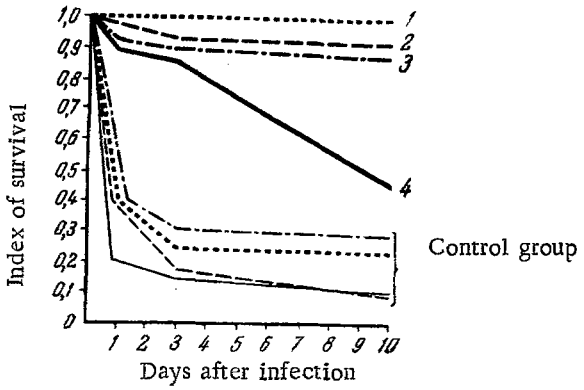

Fig. 1. Effect of ciine on different infections. 1) Proteus vulgaris; 2) Escherichia coli; 3) Pseudomonas aeruginosa (Pseudomonas pyocyanea; 4) Staphylococcus aureus.

Additional data on the defense mechanisms stimulated by ciine were obtained in experiments in which the effect of local tissue immunity of the peritoneum on the infectious agent was excluded. In these experiments ciine was introduced intraperitoneally, as usual, but the culture of E. coli strain 145 was given intravenously. The development of infection after the administration of ciine was markedly inhibited. The index of survival on the 10th day was 0.85 in the experimental group and 0.2 in the control group; the index of survival of the mice in the reverse situation (ciine given intravenously and the infection given intraperitoneally) was 0.68 in the experimental group and 0.2 in the control group on the 10th day. In both instances the administration of 200 μg of ciine clearly increased the survival of the mice in comparison with the control group, indicating that the defense effect was connected not only with the peritoneal but also with other systems of the host.

In experiments using E. coli sepsis, it was shown that the effect of ciine, as well as some other polysaccharides (zymosan and preparation K-12*), was greater with intraperitoneal than with intravenous administration. This difference was particularly clear cut with staphylococcal sepsis. According to the literature data [2,7], neither preparation K-12 nor zymosan increases the resistance of mice to staphylococcal sepsis. In our experiments with intravenous administration of these preparations, the same results were obtained. On the other hand, with intraperitoneal administration, both zymosan and K-12 showed the same action as ciine in markedly suppressing the development of staphylococcal sepsis.

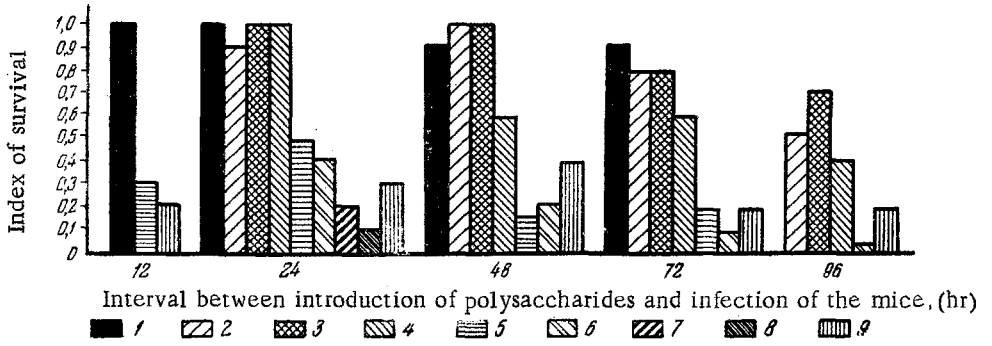

Fig. 2. Activity of different preparations of polysaccharides on E. coli sepsis. 1) Ciine A; 2) ciine B; 3) preparation K-12; 4) zymosan; 5) agar; 6) polyglycine; 7) grisean; 8) pyrogenal; 9) physiological saline.

The difference in the effect of different modes of administration of this preparation permits the conclusion that the local reaction of the peritoneum plays a definite role during the intraperitoneal introduction of the preparation and intraperitoneal infection of the animal; however it does not completely explain the action of ciine on the reaction of the peritoneum.

* Preparation K-12 (a polysaccharide isolated from E. coli strain K-12) was kindly supplied by Dr. D. Rowley (England).

In analyzing the general reaction of increased resistance of the host to infection, the possibility of ciine influencing antibacterial activity of the serum of the animals occurred to us. We measured the antibacterial activity of the serum of rats before and after intraperitoneal injection of ciine in a dose of 10 mg per kg with regard to E. coli strain 145, which was used in vivo experiments on the mice. It turned out that the bactericidal activity of the serum of rats as regards E. coli strain 145 was not very great, but it was increased somewhat after the administration of ciine. A detailed analysis of this phenomenon will be published separately.

We ascertained the length of the effect observed with a single parenteral injection of ciine. With this aim experiments were done in which mice were infected at different periods of time after the injection of the ciine. It was established that in a short period (5 hr) after the introduction of ciine the survival of the mice was not significantly increased; however, increasing this interval the difference between the controls and the experimental groups increased, reaching a maximum at 18-24 hr after administration of ciine and gradually lowering until it was no longer noticeable between 72 and 96 hr. In Fig. 2 the indexes of survival of mice in relation to the interval of time between injection of different polysaccharide preparations and infection of the animal are presented (the index of survival on the 3rd day after infection is shown in each instance, since this is sufficiently characteristic).

The experimental data obtained permit an approach to the solution of the problem stated above on the mechanism of stimulation by ciine and other biologically active polysaccharides. It should be considered without doubt that the ability of ciine to increase the survival of the infected host to lethal doses of bacteria or sublethal irradiation with x-rays is connected, not with the direct antibacterial properties of the preparation, but with the reaction of the host. As far as this is concerned, it has been demonstrated many times that ciine has no antibacterial activity in experiments in vitro and also that to achieve a maximal effect a definite period of time is needed (from 12-24 hr). It has also been shown that the condition of increased resistance to a number of pathogenic effects elicited by ciine can be maintained for a long time (up to 96 hr). A comparison of different polysaccharides from the point of view of the level and duration of their anti-infective action (see Fig. 2) permits separating them into 2 groups, of which one turns out to have a high and prolonged effect (ciine K-12, zymosan) and the other only an unstable and temporary effect (agar, polyglycine, pyrogenal, grisean). In comparing the effect of the latter group with the previously noted role of the local immune apparatus of the peritoneum, which is even stimulated by a sufficent quantity of saline, it can be supposed that the small and transient anti-infective action is connected with mobilization of local mechanisms of the peritoneum.

In regard to the data presented, it would be expedient to examine the dependence of the effect in relation to the period of administration and the dose of the preparation which has been established by other authors [6]. In view of the defence action evoked in our experiments in the peritoneal cavity by the introduction of saline, one might doubt the specific nature of the temporary increases in resistance to infection noted by these authors, who used small doses of preparation. As can be seen, a more or less reliable effect can be observed 18-24 hr following the injection of ciine. This supposition also supports the fact that in all instances the intraperitoneal injection of the preparation gives higher and more resistant effects than subcutaneous or even intraveneous administration with which the effect of the reaction of the peritoneum on the outcome of the infection is markedly reduced. The greater effectiveness of intraperitoneal injection of the first group of polysaccharides apparently can be explained by the fact that in this instance the local and generalized effects of the preparation on the host are summated.

Actually, our experiments revealed both a decrease in the sifting of bacteria from the blood of the majority of the experimental animals [1], who had previously received a single injection of ciine, and an increase of the bactericidal activity of the blood of the rat. If the decrease in the sifting of bacteria can be partially explained by the stimulation of phagocytosis, the increase of the bactericidal activity of the blood can be related to some reaction of a general form. The unusual nature of this phenomenon may be inferred from the fact that it develops at a rate immeasurably greater than the rate of formation of specific antibody.

Some are of the opinion that the increase of bactericidal activity of the blood and the resistance of the organism to infection after the introduction of bacterial polysaccharides is connected with an increase in the titer of properdin [5,6]. The results obtained by us can be explained by the increase in the titer of properdin only with difficulty, for the numerous attempts to reveal an increase of bactericidal activity in the blood of mice after administration of ciine were unsuccessful. The curve of the properdin titer relationship to the interval of time after the administration of zymosan or ciine to the animals does not correspond to the curve of the increase in survival [5].

Evidently, the general defense reaction of the organism after the administration of polysaccharides is not exhausted by the intensification of any single system. From this point of view the data presented are of decided interest

[1], inasmuch as ciine stimulates the hypophyseal-adreno-cortical system, which plays a large role in the defense of the organism in infection [4].

Besides this, an experiment which has been carried out in our laboratory has given more detailed information of the characteristic effect of ciine on the RES. It was found that ciine actually evokes characteristic changes in the RES which can be qualitatively measured. However, the increased activity of the macrophages noted in these experiments becomes evident in a shorter period of time (4 hr) than the biological effect of ciine and ends earlier than the defensive reaction of ciine (72 hr after following the administration of ciine, the activity of the macrophages has returned to the original level).

On the basis of all the observations, we came to the conclusion that ciine and similar biologically active polysaccharides have a polyvalent action on the organism which consists of activation of the local tissue reaction and of the total phagocytic activity of the RES, and an increase in the bactericidal activity in the blood. However, in a quantitative comparison of the anti-infective effect, the role of yet other defense factors requiring further study can be supposed.

This is indicated, in particular, by defense against ionizing radiation observed with the introduction of ciine because it is difficult to assume that the action of ciine is exhausted by its anti-infective effect. Data on the effects of ciine on aseptic inflammation and regeneration tissues also attest to the polyvalence of the reaction of the host to the introduction of ciine.

The material presented most assuredly indicates the importance of investigating different aspects of the biological activity of polysaccharides of bacterial origin.

SUMMARY

A substance with polysaccharide component was isolated from Acxylinum, which was called ciine. This substance sharply increases the resistance of white mice to sepsis caused by staphylococcus, E. coli, Pr. vulgaris and B. pyocyaneum. The action of ciine is connected with the participation of a number of body protective systems. Experiments demonstrated the activation of the local peritoneum apparatus, stimulation of the phagocytic activity of the reticulo-endothelial system as a whole and a rise of the blood bactericidal activity. Comparison of the effect produced by ciine with the action of certain other substances of the polysaccharides has made it possible to divide all the substances tested into 2 groups. Ciine, zymosan and polysaccharide obtained from the K-12 E. coli, belonged to one group whereas agar, pyrogenal, polyglycine, grysean-to another one. Substances of the first group provoke a protective effect if administered 12 to 96 hours prior to infection of the mice. The protective action of the substances belonging to the second group is weaker, inconstant and is manifested only when administered 12 to 24 hours prior to infection.

LITERATURE CITED

1. A. I. Brande, et al., Antibiotiki, No. 3, p. 23 (1959).
2. Z. V. Ermol'eva, et al., Antibiotiki, No. 6, p. 46 (1958).
3. Z. V. Ermol'eva, et. al., Antibiotiki, No. 4, p. 37 (1960).
4. E. Kass, Bact. Rev. 24, (1960) p. 177.
5. M. Landy and L. Pillemer, J. exp. Med. 104, (1956), p. 383.
6. L. Pillemer, et al., Science, 120, (1954), p. 279.
7. D. Rowley, Brit. J. exp. Path., 37, (1956), p. 223.

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