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 (Pseudomonas 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 Friedlander'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 intraperitoneally 24 hours before infection, and 10 min after the intraperitoneal infection
0.5-0.26 ml of novocain was given. The control group of mice received cliine in physiological saline and no novocain. It was established that cliine 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 cliine has no connection with the local nervous system of the peritoneum.

Additional data on the defense mechanisms stimulated by cliine were obtained in experiments in which the effect of local tissue immunity of the peritoneum on the infectious agent was excluded. In these experiments cliine was introduced intraperitoneally, as usual, but the culture of E. coli strain 145 was given intravenously. The development of infection after the administration of cliine 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 (cliine 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 cliine 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 cliine, 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 cliine in markedly suppressing the development of staphylococcal sepsis.

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 cliine 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).