EXPERIMENTAL STUDY OF NONSPECIFIC PROTECTIVE RESPONSES TO INFECTION WITH CONDITIONALLY PATHOGENIC MICROORGANISMS

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The effect of nonpathogenic and pathogenic strains of Escherichia coli, staphylococci, and streptococci on mechanisms of nonspecific immunity was studied. Pathogenic strains inhibited the activity and transformation of the lymphocytes and disturbed the balance between the ingestive and enzymic functions of the various cells, especially macrophages, which could be prevented or diminished by administration of 4-methyluracil. Nonpathogenic strains, expecially the nonpathogenic staphylococcus, both activated and inhibited protective responses depending on the conditions of infection, as a result of which the sensitivity of the animal to infection by other microorganisms of the conditionally pathogenic group could be increased and the manifestation of their pathogenic properties facilitated.

The wide distribution of conditionally pathogenic microorganisms in nature and the increase in their role in human pathology [1-3, 7] make it necessary to study their effects on the nonspecific protective reactions of the host; this was the motivation behind the present investigation.

EXPERIMENTAL METHOD

Experiments were carried out on 9 groups of albino mice infected intraperitoneally. The first 6 groups were infected with one of the following species of microorganism: Escherichia coli – enteropathogenic strain O111 (EPEC), nonpathogenic (NPEC) strain No. 173 [4], staphylococci – nonpathogenic (NS) and pathogenic (PS), and streptococci – hemolytic (HS) and nonhemolytic (NHS); the 7th group was infected with NPEC on which EPEC endotoxin was adsorbed; group 8 received NPEC 7 days after triple injection of equal doses of NS; group 9 received EPEC or PS after 5 injections of 0.25 ml 1% 4-methyluracil (MU) solution. The peritoneal exudate of the animals was studied 1-72 h after infection – bacteriologically (the frequency of positive cultures and the intensity of growth [4, 6]), psychologically [5], and, in some animals, histochemically – by staining with Feulgen's and Brachet's methods and by Gomori's method for acid and alkaline phosphatase [8].

EXPERIMENTAL RESULTS

Many lymphocytes, transitional forms, and blast cells with microorganisms adsorbed on them were observed in the exudate 1-3 h after infection with NPEC, NS, and NHS; clusters of bacteria, poorly stained, were seen around some lymphocytes. Intensive phagocytosis by young and mature macrophages was replaced after 6-24 h by destruction of microorganisms. Neutrophils appeared not before 3-6 h, but their phagocytic activity was low, especially after infection with NPEC. The DNA content in the cells of these animals was changed only slightly, but their RNA content was increased after 24 h, mainly in the lymphoblasts. Acid phosphatase activity was relatively high on the first few days in the lymphocytes, and in the other cells it increased after 48-72 h ($P < 0.01$). Alkaline phosphatase activity was increased in the lympho-

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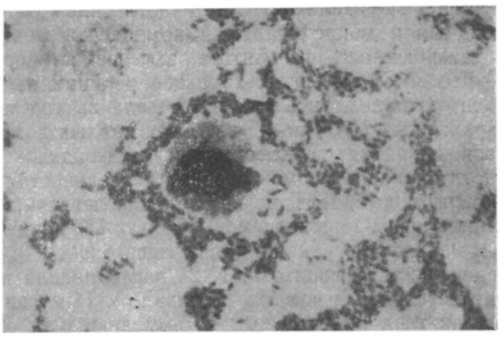

Fig. 1

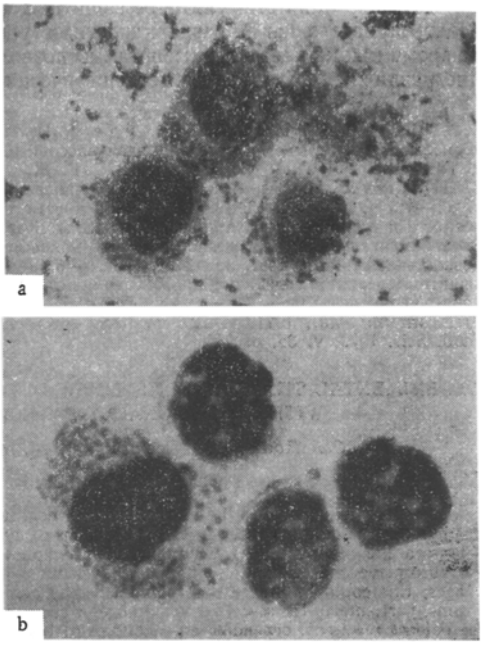

Fig. 2

Fig. 1. Absence of phagocytosis by macrophages after infection with enteropathogenic strains of E. coli, 1350×.

Fig. 2. Activation of cell reaction in animals treated with MU and infected with enteropathogenic strains of E. coli (a) and pathogenic staphylococci (b); phagocytosis by macrophages and blast-transformation of lymphocytes, 1350×.

cytes, blast cells, and macrophages after 24-48 h ($P < 0.001$). The proportion of positive cultures fell significantly after 3-6 h. No positive cultures were obtained from the exudate of most animals after 48-72 h.

After infection with EPEC, PS, and HS transitional forms of lymphocytes rarely appeared in the exudate and blast forms more rarely still, especially during the first few days. Neutrophils were constantly present in large numbers and their phagocytic activity was greater than that of the macrophages. The phagocytosis was incomplete in character and sometimes the phagocytes were destroyed. The phagocytic activity of the macrophages was strongest after infection with EPEC (Fig. 1). The DNA content in the exudate cells in this group was considerably modified, and their RNA content was smaller. Alkaline phosphatase activity was lower than after infection with NPEC, while acid phosphatase activity was found only during the first hours, after which it fell sharply. The incidence of positive cultures of microorganisms continued high in the overwhelming majority of animals until 48-72 h.

After infection with NPEC+EPEC endotoxin the lymphoid and macrophagal components of the response were noticeably inhibited, the activity and degree of completion of phagocytosis were reduced, and the incidence of positive cultures remained high for 24 h. After the second day the character of the response changed, phagocytosis was activated, blast cells appeared and the incidence of positive cultures fell. In animals infected with NPEC after injection of equal doses of NS the migration and transformation of the lymphocytes were inhibited more sharply still, and the ingestive and enzymic activity of the macrophages was depressed. The animals died from doses of NPEC which were not lethal in the control. Administration of MU before infection with EPEC or PS activated the lymphocytes and macrophages, blast cells appeared sooner (Fig. 2a, b), and phagocytosis was more complete. The incidence of positive cultures fell after 24 h. After infection with a lethal dose of PS 50 ± 13.5% of the animals survived until the 14th day whereas all the control animals died by the 5th day.

The effect of conditionally pathogenic microorganisms on the protective responses of the host animal was thus determined by the properties of the strain and the conditions of infection. Pathogenic strains suppressed the lymphoid component and disturbed the course of some intracellular processes, including the balance between the ingestive and enzymic functions. MU increased the effectiveness of the protective responses. Nonpathogenic strains as a rule activated lymphoid cells and macrophages. However, with a change

in the conditions of infection some of them (NS) sharply inhibited the protective responses, thus increasing the sensitivity of the host to other conditionally pathogenic microorganisms (NPEC). This fact must be taken into account when the role of individual strains of conditionally pathogenic microorganisms is assessed in pathology.

In order to study stimulators of protective mechanisms, it is advisable to use pathogenic strains of conditionally pathogenic microorganisms, whereas their nonpathogenic strains can be used to detect the inhibitory effect of various factors on nonspecific immunity.

LITERATURE CITED

1. A. I. Braude and Z. V. Ermol'eva, in: Proceedings of the 15th All-Union Congress of Epidemiologists and Microbiologists [in Russian] (1971), p. 80.
2. L. A. Genchikov and V. G. Sokolovskii, Zh. Mikrobiol., No. 5, 80 (1971).
3. V. I. Ioffe, in: Clinical and Epidemiological Immunology [in Russian], Leningrad (1968), p. 8.
4. E. A. Oleinikova, Characteristics of Antibacterial Defense in Childhood and the Pathogenesis of Enterocolitis, Doctoral Dissertation, Khar'kov (1965).
5. V. V. Rogovin, An Experimental Cytological Study of Macrophages with Differences of Functional Activity, Author's Abstract of Candidate's Dissertation, Moscow (1969).
6. V. D. Rozenberg, Nonspecific Protective Responses of the Organism to Experimental Staphylococcal Infection, Candidate's Dissertation, Khar'kov (1970).
7. E. Goldstein, W. S. Tyler, and P. D. Hoeprich, Arch. Intern. Med., 127, 1099 (1971).
8. S. Hussian and M. K. Hamdy, J. Food Sci., 35, 585 (1970).