ROLE OF CYCLIC AMP IN THE DEVELOPMENT OF EXPERIMENTAL SALMONELLA INFECTION

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In salmonellosis the macrophages play a leading role in defense of the body against infection [5]. The writers showed previously in experiments with macrophage culture in vitro that the outeome of interaction between a culture of macrophages and salmonellas is determined by the cyclic 3,'5'-adenosine monophosphate (cAMP) level: an endogenous and exogenous rise of the cAMP level in the macrophages leads to intensive multiplication of salmonellas and death of the macrophages [2, 4]. Meanwhile, in salmonellosis the formation of the nonhumoral defense mechanism takes place during persistence of salmonellas in the body [5], and mainly the spleen is involved in this process [6]. There is also evidence that the regulating action of splenic macrophages on proliferative activity of lymphocytes is reduced in salmonellosis [8]. With these considerations in mind it was decided to study changes in the cAMP level in the splenic macrophages of mice with salmonella infection and also to study the course of salmonellosis in mice with a lowered cAMP level in their macrophages.

EXPERIMENTAL METHOD

A virulent strain of *Salmonella dublin* C-96 and strain C-44 with weak virulence [1] were used.

The cAMP level in the splenic macrophages was determined in CBA mice weighing 13-15 g. The mice were infected with the bacterial strains intraperitoneally in a dose of 10^4 cells. At certain time intervals after infection the number of salmonellas in the spleen was determined in three of a group of six mice, and the cAMP level in the splenic macrophages was determined in the other three mice. Splenic macrophages were isolated by adhesion to plastic, by the method [8]. For this purpose the spleen was homogenized in 5 ml of medium 199, cooled to 4°C, with the addition of 5% fetal calf serum; the homogenate was filtered through several layers of gauze and distributed on the surface of a plastic Petri dish 100 mm in diameter, so that the thickness of the layer did not exceed 2-3 mm; the dish was then incubated for 90 min at 37°C. Nonadherent cells were removed, and the adherent cells (macrophages) were washed several times with medium 199, and then harvested from the surface of the dish with a rubber pestle. Samples containing 10^7 cells were taken, and dry acid extracts prepared from them [2] for determination of their cAMP level. Quantitative cAMP assay was carried out by means of a c-AMP Assay Kit (Amersham International, England). The results were expressed as the number of picomoles cAMP per 10^7 cells. The cAMP level in splenic macrophages of intact (uninfected) mice served as the control.

Propranolol (Obsidan, from East Germany) was used as a drug which lowers the cAMP level [9]. The effect of propranolol on the course of salmonellosis was studied in noninbred male albino rats weighing 18-20 g. The mice were infected perorally with *S. dublin* C-96 in a dose of 10^6 cells. On the 3rd day after infection, against the background of a developing infectious process, the number of salmonellas was determined in the spleen of the mice. The experimental group, consisting of 50 mice, received propranolol intraperitoneally in a dose of 10 mg/kg [3], and continued to receive the same dose on the next 3 days. The control group (30 mice) were injected with physiological saline. The animals were kept under observation for 60 days.

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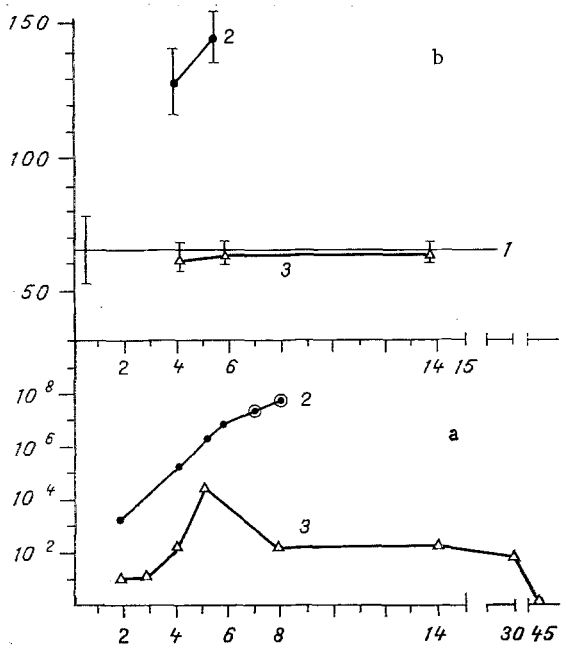

Fig. 1. Changes in cAMP level in splenic macrophages of mice in the course of salmonella infection. a) Multiplication of salmonellas in mouse spleen. Abscissa, time after infection (in days); ordinate, number of CFU per organ; b) change in cAMP level in splenic macrophages of mice. Abscissa, time after infection (in days); ordinate, number of picomoles cAMP/10^7 cells. 1) Uninfected mice (control); 2) mice infected with *S. dublin* C-96; 3) mice infected with *S. dublin* C-44.

To assess the effect of propranolol on the cAMP level of peritoneal macrophages and splenic macrophages, propranolol was injected into nine noninfected mice in accordance with the scheme mentioned above, after which the cAMP level in the splenic macrophages was determined as described above, and the cAMP level in the peritoneal macrophages was determined as described in [2]. Uninfected mice receiving physiological saline served as the control.

The number of salmonellas in the spleen was determined as described in [1]. Seeding was carried out on Kaufman agar (Merck). The results were expressed as the number of colony-forming units (CFU) per organ.

EXPERIMENTAL RESULTS

The experiments showed that strain C-96, after intraperitoneal injection into mice, multiplied intensively in the spleen, and the animals died when the 10^7 CFU level was reached (Fig. 1a). Strain C-44, with low virulence, also multiplied well in the mouse spleen, to reach 10^4 CFU by the 5th day, but later, however, its bacterial titer fell to 10^2 CFU, and remained at that level for 30 days. None of the mice died after infection with strain C-44.

Measurement of cAMP in the splenic macrophages showed that its level in uninfected (control) mice varied from 50 to 75 pmoles/10^7 cells, on average 65 ± 4.5 pmoles/10^7 cells. In the case of infection by the virulent strain C-96 a lasting increase in the cAMP level in the splenic macrophages was observed (Fig. 1b, 2). In mice infected with strain C-44, with low virulence, the cAMP level in the splenic macrophages did not exceed the control values (Fig. 1b, 3), despite persistence of the microorganism in the organ. Consequently, the cAMP level in the splenic macrophages changed in the course of salmonella infection depending on the virulence of the pathogenic agent. Its increase correlated with the intensity of multiplication of the virulent strain, thus predetermining the lethal outcome of the infection. Considering data to show that the functional activity of certain organs [9] is determined by the ratio between their cyclic nucleotide levels, it can be tentatively suggested that the increase in cAMP concentration in the splenic macrophages during infection with the virulent strain leads to dysfunction of these cells. As pointed out in [8], this may adversely affect lymphoid cells and the formation of resistance. In the case of infection by the strain with

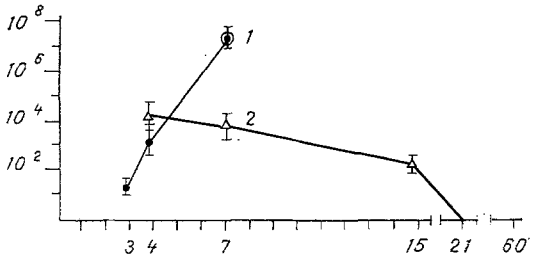

Fig. 2. Effect of propranolol on persistence of salmonellas in mouse spleen. Abscissa, time after infection (in days); ordinate, number of CFU per organ. 1) Mice infected with *S. dublin* C-96 (control); 2) mice infected with *S. dublin* C-96 and treated with propranolol. Circled symbol signifies seeding from dying mice.

low virulence, no increase in the cAMP level in the splenic macrophages was observed; there was no decrease in their functional activity and no adverse effect on the formation of resistance, and as a result, generalization of the infection and death of the animals did not take place.

The cAMP level of the splenic macrophages, like that of peritoneal macrophages [2], thus determined the course of salmonellosis. It was therefore interesting to study the effect of substances lowering the intracellular cAMP level on the course of salmonellosis. Preliminary experiments showed that injection of propranolol lowered the cAMP level in the splenic macrophages to 34 ± 4.3 pmoles/10^7 cells, and in the peritoneal macrophages to 36 ± 1.15 pmoles/10^7 cells compared with 62 ± 6 pmoles/10^7 cells in the control.

As the experimental model with which to study the effect of propranolol on the course of salmonellosis we chose the infection caused in mice by peroral administration of the agent, as the most natural route of infection with salmonellas [7]. The experiments showed that after peroral infection of mice with *S. dublin* C-96 in a dose of 10^6 cells 100% of the animals died in the control group. In the group of mice treated with propranolol only 38% died. Investigation of persistence of the agent in the spleen showed (Fig. 2): in the control group of mice strain C-96 multiplied intensively in the spleen, to reach 10^7 CFU by the 7th day, when it caused death of the animals. In the group of mice treated with propranolol the number of salmonellas in the spleen did not exceed 10^4 CFU, and no salmonellas were seeded from the spleen after the 21st day after infection. When surviving animals of the experimental group were killed on the 6th day and seedings taken from the spleen, liver, and gall bladder, the absence of a salmonella-carrier state was confirmed.

The cAMP level of the macrophages thus regulates the course of salmonella infection. In the writers' view, research aimed at choosing preparations which will increase macrophage activity through the cyclic nucleotide system, and will thus help to prevent the generalization of the infectious process in salmonellosis, can yield promising results.

LITERATURE CITED

1. M. N. Boichenko, Zh. Mikrobiol. Épidemiol., Immunobiol., No. 2, 106 (1986).
2. M. N. Biochenko, and I. L. Zhilina, Zh. Mikrobiol. Épidemiol., Immunobiol., No. 10, 60 (1983).
3. O. M. Glazman, É. K. Orlova, and L. A. Zhmurenko, Khim.-Farm. Zh., No. 7, 791 (1983).
4. I. L. Zhilina, S. I. Elkina, and M. N. Boichenko, Zh. Mikrobiol. Epidemiol., Immunobiol., No. 6, 99 (1984).
5. F. Collins, Vet. Immunol. Immunopathol., 3, No. 5, 66 (1982).
6. F. Galdiero, N. Benedetto, and N. Quarto, Infect. Immun., 21, No. 2, 349 (1978).
7. A. Hohmann, G. Schmidt, and D. Rowley, Infect. Immun., 22, No. 3, 763 (1978).
8. T. Saito-Taki, H. Asou, and M. Nakano, Microbiol. Immunol., 24, 439 (1980).
9. D. Scevda, G. Barbarini, P. Marone, et al., Boll. Ist. Sieroter. Milan, 59, No. 6, 655 (1980).