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EFFECT OF α-INTERFERON ON ENZYME LEVELS OF ADENOSINE METABOLISM AND MACROPHAGAL BACTERICIDAL ACTIVITY IN STAPHYLOCOCCAL INFECTION

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UDC 578.245:576.38.679

KEY WORDS: interferon; mouse macrophages; bactericidal activity; adenosine deaminase; 5'-nucleotidase

An important role in the pathogenesis of staphylococcal diseases is played by congenital and acquired pathologies of the phagocytic system [1, 5]. The writers previously studied the protective action of α-interferon (IFN) in experimental staphylococcal infection, due to activation of macrophages [6-8]. Meanwhile the mechanisms of stimulation of phagocytic cells by IFN in staphylococcal infection have still largely not been identified: changes in activity of the enzyme systems, the state of the intracellular metabolic processes, and the link with specific factors of immunity have not yet been adequately studied. The solution of these problems is an urgent task, for it is essential to an understanding of the mechanisms of formation of the antibacterial immune response in staphylococcal diseases, and also of its goal-directed regulation by immunomodulators. The functional activity of immunocompetent cells can be disturbed as a result of a change in activity of the enzymes of adenosine metabolism, namely 5'-nucleotidase and adenosine deaminase [9, 10]. The intracellular adenosine level determines the degree of maturation of receptors on the cells, and if present in excess, it causes inhibition of the differentiating and proliferative activity of the T and B lymphocytes, and also of macrophages [9-12].

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 0007-4888/92/0007-0994$12.50

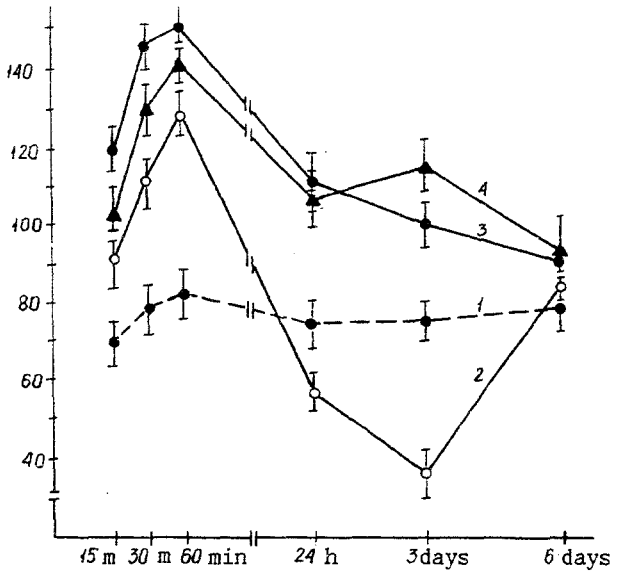

Fig. 1. Changes in bactericidal activity of macrophages of mice infected with staphylococcus and intact mice, under the influence of IFN. Abscissa, time of observation; ordinate, bactericidal activity (in conventional units). 1) Intact, treated with placebo; 2) infected, treated with placebo; 3) intact, treated with IFN; 4) infected, treated with IFN.

The aim of the present investigation was to study activity of enzymes controlling adenosine metabolism, namely adenosine deaminase and 5′-nucleotidase, in phagocytic cells in staphylococcal infection and the modulating action of α-IFN.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice weighing 18-20 g. A culture of *Staphylococcus aureus,* strain Wood 46 was used. Mice were infected by intraperitoneal injection of a living 24-h culture of the staphylococcus (LD_{30}). Peritoneal cells were obtained by the method in [2]. The peritoneal cells were washed out and suspended in culture medium RPMI-1640, containing 10% fetal calf serum, and layered on plastic Petri dishes. After incubation at 37°C for 60 min the adherent cells (macrophages) were washed off twice with Hanks' solution and then removed mechanically. A preparation of homologous α-IFN, obtained on cells of transplantable murine line L-929 of fibroblasts by induction with Newcastle disease virus (NDV), as described previously [7], was used. The supernatant medium of cells cultured under similar conditions but without addition of NDV was used as a placebo. The antiviral activity of IFN was determined by microtitration in a culture of transplantable L-929 cells against 100 TCD_{50} of vesicular stomatitis virus. IFN was injected intraperitoneally into the animals in a dose of $1 \cdot 10^3$ U/mouse simultaneously with infection with the staphylococcus. The oxygen-dependent bactericidal activity of the macrophages was studied in the nitro-BT test by Gracheva's method (1984). The level of adenosine deaminase and 5′-nucleoidase activity in the macrophages was determined by the method described previously [6] and expressed in nmoles/min/10^6 cells. The results were subjected to statistical analysis and differences between the mean values were evaluated by Student's test with a level of probability of error of not more than 5%.

TABLE 1. Changes in Adenosine Deaminase Activity in Macrophages of Mice Infected with Staphylococcus and Intact Mice Under Influence of IFN

Group of animals	Activity of enzyme in nmoles/min/10^6 cells					
	time of observation					
	15 min	30 min	60 min	24 h	3 days	6 days
Infected + placebo	21,3±2,3	17,8±2,0	16,4±1,3*	10,5±1,4*	14,4±2,0*	18,8±1,8
Infected + IFN	22,5±1,9	21,3±2,4	20,9±1,8	30,3±2,7*	32,2±2,5*	24,8±2,5
Intact + IFN	23,7±2,0	27,2±2,6	29,1±2,1*	49,6±3,8*	41,6±2,5*	30,9±2,7*
Intact + placebo	23,5±2,8	24,1±1,3	23,9±1,9	22,5±3,4	24,9±2,5	21,0±1,0

Legend. Here and in Table 2: *$p < 0.05$ relative to enzyme activity in macrophages of intact mice treated with placebo.

TABLE 2. Effect of IFN on 5′-Nucleotidase Activity in Macrophages of Mice Infected with Staphylococcus and Intact Mice

Group of animals	Activity of enxyme in nmoles/min/10^6 cells					
	time of observation					
	15 min	30 min	60 min	24 h	3 days	6 days
Infected + placebo	24,1±1,9	32,0±3,1*	43,9±2,9*	54,3±3,0*	46,4±4,0*	27,0±2,1
Infected + IFN	23,6±2,9	21,6±2,4	25,3±2,4	11,3±0,9*	10,1±0,9*	21,3±2,4
Intact + IFN	23,1±2,2	16,3±1,0	11,2±1,1*	6,0±0,2*	7,1±0,4*	13,8±1,0*
Intact + placebo	22,7±1.9	23,1±2,5	21,0±2,6	22,9±3,4	20,5±1,7	23,6±2,1

EXPERIMENTAL RESULTS

In staphylococcal infection the oxygen-dependent bactericidal activity of peritoneal macrophages was found to be changed (according to the results of the nitro-BT test), and the changes were phasic in character. It will be clear from Fig. 1 that during the first hour after infection of the mice with the staphylococcus the bactericidal activity of the phagocytic cells rose sharply compared with activity of macrophages in intact animals (control). However, after 24 h and on the 3rd day of the experiment activity of the phagocytes of the infected mice was observed to fall below its level in the controls. Normalization of the bactericidal activity of the macrophages took place on the 6th day after injection of the staphylococcus into the animals (Fig. 1).

Infection of mice with the staphylococcus also caused a rapid increase in 5′-nucleotidase (Table 1) and decrease in adenosine deaminase (Table 2) activity of the macrophages. Moreover, the greatest change in enzyme activity of the phagocytic cells occurred in the period between 24 h and 3 days after infection. The level of 5′-nucleotidase and adenosine deaminase activity in the macrophages of the infected animals was restored to the control value on the 6th day of the experiment.

It was later shown that injection of IFN into mice infected with the staphylococcus and intact mice activates oxygen-dependent bactericidal activity of the macrophages and changes the levels of activity of adenosine deaminase and 5′-nucleotidase. For instance, under the influence of IFN the bactericidal activity of the phagocytic cells of the infected mice remained high 24 h and 3 days after the beginning of the experiment, and subsequently fell toward the level of macrophagal activity of the intact mice treated with the placebo (control) on the 6th day. As Fig. 1 shows, after injection of IFN into intact mice the bactericidal activity of the macrophages increased to the highest level during the first hour of the experiment. Between 24 h and 3 days after injection of IFN activity of the phagocytic cells decreased, to reach the control level on the 6th day of the experiment.

Table 1 contains data on the effect of IFN on adenosine deaminase activity in macrophages of mice infected with the staphylococcus and intact mice. As Table 1 shows, the adenosine deaminase activity of macrophages of infected mice returned to normal within the first hour after injection of IFN. Later, in the period between 24 h and 3 days of the experiment, the enzyme activity of the phagocytes reached its highest level compared with the control values and also compared with adenosine deaminase activity in the macrophages of infected animals treated with the placebo. Activity of the enzyme in macrophages of the infected mice fell to the control level on the 6th day after in-

jection of IFN (Table 1). Similar results also were obtained when the effect of IFN was studied on adenosine deaminase activity of the macrophages of intact mice (Table 1). Incidentally, activity of the enzyme in the macrophages of intact animals treated with IFN remained at a high level compared with the control until the 6th day of the experiment (Table 1).

Meanwhile, 5′-nucleotidase activity in the macrophages decreased under the influence of IFN (Table 2). For instance, during the first hour after injection of IFN into infected mice the 5′-nucleotidase activity of the phagocytes returned to the control level. After 24 h and on the 3rd day, activity of the enzyme in the macrophages of the infected animals fell, under the influence of IFN, compared with the control values, and thereafter returned to normal on the 6th day of the experiment. As the results in Table 2 show, the level of 5′-nucleotidase activity in macrophages of intact mice fell compared with the control values 60 min after injection of IFN.

In staphylococcal infection the oxygen-dependent bactericidal activity of phagocytic cells is thus inhibited, and their 5′-nucleotidase and adenosine deaminase activity also is modified. Incidentally, an increase in 5′-nucleotidase activity and a decrease in adenosine deaminase activity may lead to the accumulation of intracellular adenosine, which is an inhibitor of the functional activity of immunocompetent cells [9, 11]. Homologous α-IFN in a dose of $1 \cdot 10^3$ U/animal, potentiates oxygen-dependent bactericidal activity of macrophages of mice infected with the staphylococcus and intact mice, but also, as we showed previously, it activates phagocytosis and stimulates the ability of phagocytes to spread [6-8]. On the basis of analysis of the experimental results it can be concluded that the modulating action of IFN on functional activity of phagocytic cells in staphylococcal infection and also under normal physiological conditions, is linked with a change in the intracellular metabolism of purine compounds and, in particular, of adenosine, resulting from increased activity of adenosine deaminase in the phagocytes and a decrease in their 5′-nucleotidase activity. Venous blood was obtained from healthy donors and mixed in the ratio 9:1 since changes in the level of purine metabolism in immunocompetent cells play an important role in the genesis of certain immunodeficiency diseases [9-12], the effect of α-IFN on activity of the enzymes of adenosine metabolism in the macrophages can evidently be regarded as one of the most important mechanisms of its immunomodulating action in staphylococcal diseases.

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