Effect of Fibronectin on the Generation of Superoxide Anion by Neutrophils in Alimentary Toxicoinfections and Experimental Salmonella Endotoxemia

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Production of O_2^- by neutrophils and the effect of fibronectin on it are studied in patients with alimentary toxicoinfections and in rats with salmonella endotoxemia. Fibronectin is found to affect the generation of superoxide anion by neutrophils, the nature of its effect depending on the number of neutrophils and background free radical activity. Fibronectin stimulates the suppressed and lowers the enhanced generation of O_2^- by neutrophils.

Key Words: *superoxide anion; fibronectin; alimentary toxicoinfections; salmonella endotoxemia*

Disturbances in the antiinfection resistance of organisms may be related not only to a drop in the number of phagocytes, but also to disturbances in their regulation due to changes in the properties of the corresponding ligands (immunoglobulins, complement, etc.) and phagocyte receptors [2]. Fibronectin (FN), a molecule necessary for adhesion, binding, and elimination of microorganisms and decomposition products, is counted among these regulators. There is no consensus on the role of FN in the modulation of the mechanisms of phagocyte killer activity, specifically the free radical mechanism.

The aim of the present study was to investigate the effect of plasma FN on free radical processes in neutrophils in patients with alimentary toxicoinfections (ATI) and in rats with experimental salmonella endotoxemia.

MATERIALS AND METHODS

A total of 50 patients with ATI (gastroenterological variant of moderate severity according to the usual clinical criteria) were observed in the course of the infectious process from days 1 through 9 inclusive. Salmonellosis was bacteriologically verified in 44% of cases. No reliable differences were found between patients with salmonellosis and ATI of unknown etiology. Experimental salmonella endotoxemia in rats was reproduced by injecting lipopolysaccharide from *Salmonella typhimurium* strain N 415 in a dose of 1 mg/kg (LD_{50}) in 1 ml physiological saline. The experiments were carried out on 40 male Wistar rats weighing 160-200 g. The animals were kept on standard vivarium chow and were given water in adequate amounts. Physiological mobility, appearance of thirst and diarrhea, and rectal temperature were evaluated after 0.5, 1, 3, 5, and 24 h of the infectious process. The controls were injected with 1 ml sterile physiological saline. The animals were decapitated under ether narcosis.

Blood concentration and heparin-binding activity of FN were measured by enzyme-linked immunoassay [3].

The state of FN receptors in the neutrophil cytolemma in the control and experimental group was studied as described elsewhere [6] in our modification. Neutrophils were isolated from fresh blood as described previously [5]. Neutrophil suspensions containing 10^6 cells/ml in the incubation buffer were used in the experiments. The purity of the neutrophil

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population was controlled by measuring lipids as described elsewhere [4]. The concentration of superoxide was measured using superoxide dismutase-inhibited reduction of cytochrome C. The neutrophil suspension (100 μl) was added to 1 ml incubation medium containing 128 mM NaCl, 4 mM $CaCl_2$, 5.2 mM KCl, 5.6 mM D-glucose, 10 mM HEPES, and 50 μM cytochrome C (the pH was adjusted to 7.40 with 1 M KOH). Superoxide dismutase (16 μg/sample) and incubation buffer (20 μl) were added to the control samples. FN in final concentrations of 150 and 300 μg/ml was used as the activator for the samples containing neutrophils from patients, while for samples from donors we also used N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) in a concentration of 4×10^{-5} M, lipopolysaccharide (final concentrations 5 and 10 μg/ml), and an FMLP+FN mixture (final concentrations 4×10^{-5} M and 150 μg/ml, respectively). The level of O_2^- was expressed in nmol/10^6 cells. The data were compared with the analogous parameters of 44 donors.

RESULTS

It was found that under the same conditions the level of O_2^- in the incubation mixture containing neutrophils from donors depends on the number of these cells. Lowering the number of neutrophils in the incubation medium from 3.75×10^6 to 0.025×10^6 markedly increases the concentration of O_2^- per 10^6 cells. This is apparently due to the fact that above a certain limit of neutrophil concentration in the incubation medium inductors of O_2^- generation, in particular FN, lipopolysaccharide, and FMLP, act as membrane superactivators.

The blood concentration of FN in patients during the acute stage of ATI was reliably lower than in the control group. During the course of detoxication therapy this index somewhat increased but even in late convalescence it remained below the normal level ($p<0.05$). The activity of plasma FN was evaluated by its ability to bind with heparin and to form a heparin precipitate in the cold. In patients with ATI the heparin-binding activity of FN was sharply decreased: it was minimal during the acute stage of the disease and progressively rose toward convalescence, remaining, however, below normal. Previous experiments on animals revealed the same dynamics of blood concentration and activity of FN in salmonella endotoxemia [2].

The dynamics of the baseline O_2^- production in patients with ATI is presented in Fig. 1, *a*. Marked activation of O_2^- production during early convalescence was followed by its sharp suppression (more than 3-fold) on days 4-5 of the disease. The maximal baseline O_2^- production was observed during late convalescence. Both doses of FN induced activation of O_2^- production at the early stage of the disease and during late convalescence, the concentration of O_2^- at the height of clinical manifestations and during early convalescence being within the limits of the control values (Fig. 1, *b*, *c*).

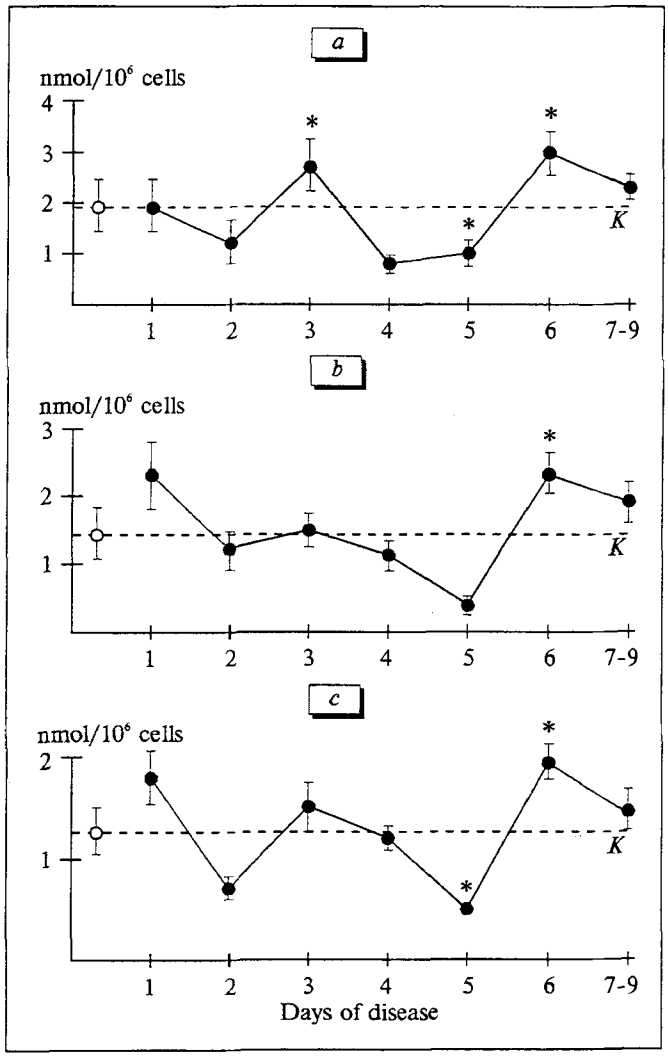

Fig. 1. Dynamics of O_2^- concentration in incubation medium containing neutrophils from ATI patients. Baseline level (*a*) and stimulation with 150 μg/ml (*b*) and 300 μg/ml (c) FN. *$p<0.05$ in comparison with the preceding day. Here and in Fig. 2: *C* denotes control.

The dynamics of the O_2^- content in the incubation medium containing neutrophils from rats with salmonella endotoxemia is presented in Fig. 2. The baseline level of O_2^- sharply increases just 0.5 h after the injection of lipopolysaccharide but progressively drops during the acute period and returns to normal only after 24 hours. When the inductor FN was added to the same incubation system, we observed a gradual rise of the O_2^- level, which peaked at 3 hours and returned to normal after 24 hours.

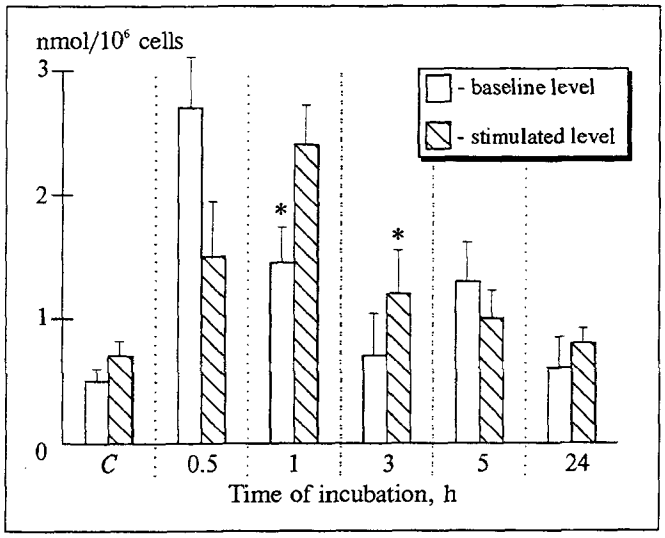

Fig. 2. Dynamics of O_2^- concentration in the incubation medium containing neutrophils from rats with salmonella endotoxemia. *$p<0.05$ in comparison with the preceding value.

Thus, FN markedly affects O_2^- generation by neutrophils in both patients with ATI and animals with salmonella endotoxemia. A key pathogenetic role in the disturbances of adaptive processes in the organism is played by lipopolysaccharide endotoxin, which changes the properties of phagocytizing cells, primarily neutrophils. The activity of these cells may change due to variations in the concentration and state of FN sites, but apart from this, modulation of neutrophil reactivity with respect to FN may also be of importance. For instance, we observe a pronounced neutrophil-stimulating effect of FN at the height of clinical manifestations, when the level of O_2^- is still practically unchanged and the concentration and activity of FN are reliably decreased. The modulating effect of FN becomes clearly apparent by the end of the acute period: the reactivity of neutrophils with respect to FN rises for a low baseline activity, and drops for a high one. The modulating effect of FN is confirmed in an experiment on rats. Stimulation of FN receptors is commonly acknowledged to increase chemotaxic adhesive activity of neutrophils; our findings suggest that FN affects the killer activity of phagocytes by modulating the generation of superoxide anion radical.

Thus, in patients with ATI we observed wave-like changes in the concentration of O_2^- generated by neutrophils into the incubation medium: it rises during the early period of the disease, then drops and bottoms out at day 5, and once again sharply rises during late convalescence. The same dynamics of the O_2^- level in the incubation medium was observed in experimental animals with salmonella endotoxemia. Neutrophil reactivity with respect to various ligands depends on the number of cells and their baseline free radical activity. In the case of a low number of neutrophils in the incubation medium the inductors of O_2^- generation (FN, salmonella endotoxin, and formylpeptide) act as membrane superactivators, i.e., they dramatically increase O_2^- generation.

In ATI of moderate severity and experimental salmonella endotoxemia FN modulated the free radical activity of neutrophils by boosting low baseline O_2^- production and suppressing high production.

REFERENCES

1. N. M. Berezhnaya, *Neutrophils and Immunological Homeostasis* [in Russian], Kiev (1988).
2. I. B. Bogin, N. L. Amosova, and V. B. Poluektova, in: *Pathogenesis, Immunology, Clinical Observations, and Diagnosis of Infectious Diseases* [in Russian], Ed. V. L. Cherkasov, Moscow (1992), pp. 31-34.
3. G. A. Ermolin, I. N. Ovcharuk, A. V. Fedotov, *et al.*, *Vestn. Akad. Med. Nauk SSSR*, No. 2, 55-66 (1991).
4. *Handbook of Clinical and Laboratory Methods* [in Russian]. Ed. E. A. Kost, Moscow (1975), pp. 168-169.
5. P. G. Bradford and P. Rubin, *Mol. Pharmacol.*, **27**, 74-78 (1985).
6. U. Bretz and M. Baggiolini, *J. Cell Biol.*, **63**, 251 (1974).