MICROBIOLOGY AND IMMUNOLOGY

Effect of the Natural Cytokines Complex on the Oxygen-Dependent Function of Phagocytes of the Rat Vagina

K. I. Rubleva, O. A. Mynbaev, and G. T. Sukhikh

UDC 618.15-088.853-02-07-092.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.115, №2, pp.168-169, February, 1993
Original article submitted July 28, 1992

Key Words: natural cytokines complex; chemiluminescence; phagocytosis.

Peptides of the immune system are highly effective regulators of endogenous nature, which orchestrate both the regulatory and effector functions of neutrophils and macrophages and participate in the regulation of phagocytosis well as in the process of the presentation of antigens to lymphocytes [4]. Information is available on the successful use of the complex of cytokines secreted by lymphocytes of healthy donors or cattle in the treatment of experimental wounds. By participating in fibroblast activity regulation, cytokines may prevent scar formation from the coarse-fibered connective tissue [1]. In addition, cytokines have been found to activate macrophage function, in particular, the production of active forms of oxygen [2,3]. However, these processes have still been studied insufficiently: no information is available on the effect of the cytokines complex on reparative processes in mucous membranes.

The purpose of the present work was to study the influence of the cytokines complex on the O_2-dependent function of phagocytes of an experimental wound of the rat vagina.

MATERIALS AND METHODS

Supernatant containing the natural cytokines complex was obtained in a mononuclear cell culture isolated from rat peripheral blood after Boyum [5]. Five $x10^6$ of the mononuclears obtained were stimulated with PHA (Bulgaria) in a concentration of 20 μg/ml for 3 h. The cells were then washed free of mitogen and cultured for 20-24 h in medium 199 containing antibiotics: penicillin (100 IU/ml) and streptomycin (100 μg/ml). Fifteen albino nonpedigree rats (males) weighting 150-200 g were used in the experiments. A standard model of trauma to the vaginal mucosa was used. The mucous wall (3×3 mm) of the upper third of the vagina was dissected with sharp scissors. The treatment consisted in a daily intravaginal injection of 100 μl of the cytokines complex for 10 days after the membrane damage. The animals of the first group were treated with nondiluted cytokines complex; those of the second group were treated with the same complex in a 10-fold dilution with medium 199. The third (control) group received only medium 199. Each test group consisted of 5 animals. Cytological investigations of vaginal smears were carried out. The smears were obtained by the intravaginal introduction of 5 ml of medium 199 before the damage (baseline) and then by daily treatment with medium 199 30 min before the injection of drugs during 10 days and on days 15, 17, 25, and 29. For control of the effectiveness of the cytokines complex, the chemiluminescent (CL) activity of vaginal phagocytes was measured. The smears from each group were combined, centrifuged for 10 min at 200 g, and the supernatant was decanted. The precipitate was resuspended in 1 ml of medium 199, and the

Russian Research Center of Perinatology, Obstetrics, and Gynecology(Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0002-0166$12.50

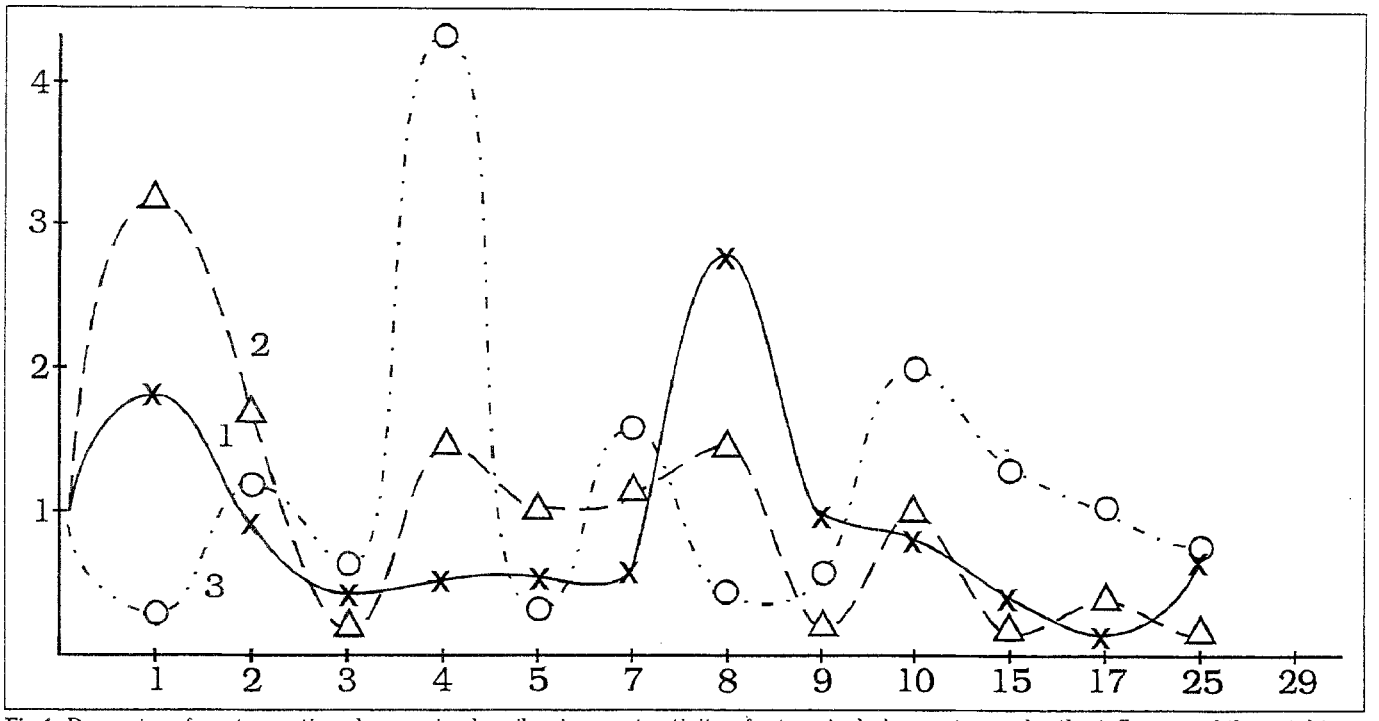

Fig.1. Dynamics of postoperative changes in chemiluminescent activity of rat vaginal phagocytes under the influence of the cytokines complex. 1) control; 2) first group; 3) second group. Abscissa: days; ordinate: mV, $\times 10^3$.

leukocytes count was determined. For a study of the O_2-dependent function of the vaginal phagocytes, their spontaneous as well as zymosan-induces chemiluminescence was measured on an "LKB 1251 Wallak" apparatus (Finland). In measuring cuvettes (two parallels for each group) cell suspension was added in such a way that the final concentration was not greater than 1 mln/ml, and the volume was adjusted to 1 ml by medium 199. The spontaneous luminescence was measured in the presence of luminol (50 µl per sample, final concentration in the sample 10^{-5} M) for 5 min, after which the inductor zymosan (50 µl, concentration 2 mg/ml) was added, and the luminescence was measured prior to peak formation.

RESULTS

The mean value of the baseline induced chemiluminescence (CL) intensity was estimated to be 807±21.5 mV (in the following only data about the induced CL will be reported) .

In the first group on the first day after the operation a 4-fold increase of the CL intensity was observed with respect to the baseline (3237±156 mV). On the 2nd and 4th days it was almost twice as high (1537±117 and 1472±89 mV). On the other days CL was close to the baseline value. No phagocyte activity was detected on the 9th, 15th, and 29th days (see Fig.1.).

In the second group on the first day a marked 4.5-fold drop of CL activity was observed - 162±26 mV; on the 2nd and 3rd days it was found to approach the baseline value, on the 4th day it exceeded the baseline value 5 times (4221±308 mV), and thereafter a CL decrease was observed to value lower than the baseline, on the whole, except for the 7th and 10th days (1445±78 and 1639±95 mV, respectively).

In the control group a 2-fold increase of CL intensity was observed on the first day (1949±202 mV), followed by a gradual decrease to the 7th day (338±54 mV). On the 8th day a new peak was detected (2838±317 mV), after which a new decrease of intensity was noted with a total absence of cell activity, on the 17h and 29th days.

The results of the cytological study of the smears show that the number of neutrophils (NP) increases considerably after the operation: on the 1st-4th days (35-60%) in the first group, and on the 3rd-7th days (27-45%, in the second group with respect to the baseline value (only a few NP in the field of vision) as well as to the control group, in which only on the first day were numerous NP (67%) detected.

In the first group on days 1, 2, 4, and 7 single macrophages (MP) were found in the smears. In the second group on days 4 and 8 from 8 to 20 MP were in the field of vision, and isolated ones were found on days 1, 2, 7, 9, 15, and 25. In the control group MP were observed from the 2nd to the 9th day (from single ones in the field of vision to 29%), as well as on days 17, 25, and 29.

In the smears from the two first groups a significant increase of the number of large epithelial round-

nucleus cells with a large cytoplasm content was detected as compared with the control, in which only isolated epithelial cells were found in the postoperative period. Fibroblasts in smears of the first two groups were found earlier in the postoperative period (1st-10th days) than in the control group (7th-25th days).

It should also be noted that in the baseline as well as control smears on days 2, 4, 10, and 15 a small coccal and bacillus flora was found. No flora was detected in the first two groups during the entire postoperative period.

The results obtained suggest an activating effect of the natural cytokines complex on the O_2-dependent function of vaginal phagocytes. This cell function is attended by phagocytosis and the generation of active forms of oxygen. The absence of flora observed in the experimental groups is assumed to be due to the bactericidal effect and to the activated phagocytosis. The cytokines complex affects the CL activity of cells and also prolongs the period of high intensity.

From the increase of the epithelium content in smears from animals treated with cytokines, it may be assumed that the cytokines complex has an epithelium-forming effect on the vaginal mucosa. The earlier appearance of fibroblasts in smears from the first two groups also confirms the acceleration of the regeneration process in a wound induced by cytokines.

Thus, we established an activating effect of the natural cytokines complex on phagocytes of rat vaginal mucosa during the postoperative period, as well as a bactericidal and epithelium-forming effect.

REFERENCES

1. L. V. Gankovskaya, L. V. Koval'chuk, S. I. Moskvina, *et al.*, *Itogi Nauki i Tekhniki. Immunologiya*, **26**, 165-167 (1988).
2. L. V. Gankovskaya, A. Z. Tskhovrebova, I. A. Gvozdeva, *et al.*, *Byull.Eksp.Biol.*, **102**, № 8, 210-213 (1986).
3. L. V.Koval'chuk, E. V. Karimova, L. V. Gankovskaya, *et al.*, *Dokl.Akad.Nauk SSSR*, **318**, 1503-1506
4. W. E. Paul *et al.*, (Eds), Fundamental Immunology, Raven Press, New York (1984).
5. A. Boyum, *Scand.J.Clin.Lab.Invest.*, **21**, Suppl.7, 77 (1967).

Analysis of Antineuronal Antibodies in Sera of Patients with Amyotrophic Lateral Sclerosis

N. A. Shanina, A. P. Sveikata, I. A. Zavalishin, and V. I. Gel'fand

UDC 616.832−004.2−04:616.153.96−097−078.33

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 2, pp. 169−173, February, 1993
Original article submitted October 8, 1992

Key Words: *amyotrophic lateral sclerosis; brain; cytoskeletal proteins; phosphorylation; immunoblotting*

Amyotrophic lateral sclerosis (ALS) is a chronic progressive brain disease causing motoneuron degeneration, which is manifested clinically by spastic paresis and paralysis, dysphasia and dysphagia, as well as respiratory distress in the final stages of disease. Despite the efforts of many years and numerous studies, the etiology and pathogenesis of ALS remain unclear, and there is no medical treatment for the disease. Genetic factors, the effect of heavy metals, disturbances in parathyroid gland metabolism, hypo-

M. V. Lomonosov State University, Institute of Neurology of the Russian Academy of Medical Sciences, Moscow
(Presented by V. N. Smirnov, Member of the Russian Academy of Medical Sciences)

0007−4888/93/0002−0168$12.50