EFFECT OF RECOMBINANT INTERLEUKIN-2 ON COURSE OF EXPERIMENTAL STAPHYLOCOCCAL PERITONITIS IN MICE

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The widespread use of antibiotics in infectious diseases has led to the development of resistance to many of them, and their prolonged use in clonic infections may also lead to immunodepression. Drug-induced and natural immunodepression are responsible for the ineffectiveness of treatment of chronic infectious diseases. Attempts to use lymphokines, natural regulators of immunity, may therefore be a promising trend.

Interleukin-2 (IL-2), a T-cell growth factor, plays a key role in the regulation of cellular and humoral immunity, and induces proliferation of T-lymphocytes and the lymphokine cascade, followed by activation of macrophages, natural killer cells, and LAK* cells [2, 10].

The aim of the investigation was to compare the effect of IL-2 and antibiotics on the course of experimental peritonitis induced in mice by *Staphylococcus aureus*.

EXPERIMENTAL METHOD

Female BALB/c and C57BL/6 mice weighing 18-20 g and aged 8-12 weeks, obtained from the Stolbovaya laboratory animals nursery in a total number of 550, were used in the experiments.

Chronic intraperitoneal infection or peritonitis [3] was induced by injection of a sublethal dose of *S. aureus*, namely $(1.0\text{-}1.3)\cdot10^9$ bacteria in a 10% solution of polyvinyl alcohol (PVA, from "Fluka"), followed by injection of 2 LD_{100} of the infecting agent on the 2nd, 4th, or 6th day after primary infection.

The lethal dose causing death of 100% of the animals (LD_{100}) was determined by plotting the dose-dependent mortality curve of the mice following intraperitoneal injection of $(0.5\text{-}3)\cdot10^9$ bacteria in a 10% solution of PVA, containing $4\cdot10^9$ bacteria in 1 ml.

Recombinant IL-2 (rIL-2, from "Cetus") in doses of $(5\text{-}10)\cdot10^3$ IU/mouse was used for treatment together with the following antibiotics: penicillin 100 U with streptomycin 0.1 mg/mouse or gentamicin (0.1 mg/mouse).

All the therapeutic substances were diluted in 0.5 ml of isotonic salt solution (ISS) and injected intraperitoneally: a) on day 0 into the group of mice receiving bacteria on days 0 and 2; b) on days 0 and 2 into the group in which bacteria were injected on days 0 and 4, and c) on days 0, 2, and 4 into the group of mice into which bacteria were injected on days 0 and 6. Mice of the control group were given intraperitoneal injections of 0.5 ml of ISS on the same schedule.

*Lymphokine Activated Killer.

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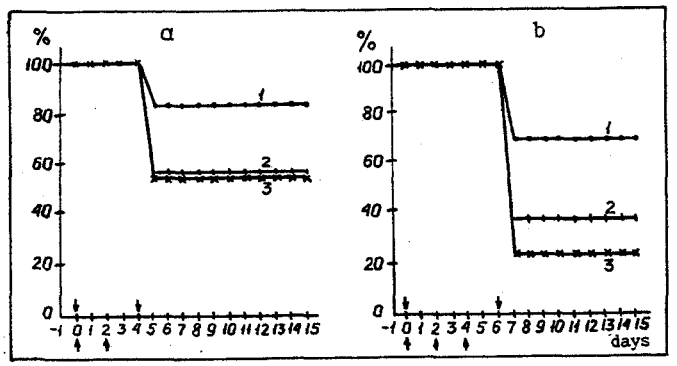

Fig. 1. Treatment of experimental staphylococcal peritonitis with rIL-2 and gentamicin. Sublethal dose of *S. aureus* ($1.3 \cdot 10^9$ bacteria) was injected intraperitoneally into mice on day 0.2 LD_{100} of *S. aureus* was injected intraperitoneally after injection of the cublethal dose of *S. aureus:* a) on day 4; b) on day 6. rIL-2 (7500 U/mouse) (1), gentamicin (0.1 mg/mouse) (3), and ISS (2) were injected intraperitoneally: a) on days 0 and 2; b) on days 0, 2, and 4.

For the hematologic investigation, peripheral blood samples were taken from the caudal vein of the intact, control, and experimental mice 6 days after injection of the sublethal dose of *S. aureus* and 24 h after injection of 2 LD_{100} of *S. aureus,* corresponding to 7 days after primary infection. The total number of leukocytes was counted and the leukocytic formula calculated by traditional methods.

For bacteriologic analysis of the peripheral blood and organs, coarse homogenates of the heart, lung, liver, and spleen of the mice 24 h after injection of a sublethal dose of *S. aureus* were seeded on Abbot nutrient broth, and 48 h later, on "Difco" nutrient agar. Parallel seedings of a bacterial suspension of *S. aureus* 5/2 on "Difco" nutrient agar were carried out to verify the purity of the strain. Gram's stain was used for the cytologic study.

The results were subjected to statistical analysis by Student's test and the chi-square test.

EXPERIMENTAL RESULTS

In this investigation the effect of rIL-2 was studied on the course of experimental staphylococcal peritonitis Averaged results of 16 experiments are given.

The survival rate of mice of the control group, receiving only *S. aureus* 5/2 and ISS varied depending on the dose of bacteria injected and the time of injection after primary infection.

When 2 LD_{100} of the bacterial suspension containing $(4.0\text{-}4.5) \cdot 10^9$ bacteria was injected into the control group 2 days after primary infection, only 18% of the animals survived. On repeated infection with 2 LD_{100}, the mortality among the mice in this group on the 4th day was 44%. After secondary infection with the same doses on the 6th day, 38% of mice survived.

To find the therapeutic dose of rIL-2 and to compare its effect with the action of antibiotics, rIL-2 was injected in different doses on days 0, 2, and 4 after primary infection. In this group of experiments 2 LD_{50} was injected on the 6th day after primary infection.

The results show that the optimal therapeutic dose of rIL-2 is $(5\text{-}7.5) \cdot 10^3$ IU/mouse. Antibiotic treatment of mice receiving 2 LD_{100} of the staphylococcal suspension on the 6th day after primary infection increased the number of animals dying to 75%, whereas injection of rIL-2 increased their survival rate by 42% ($p < 0.05$; Fig. 1b).

When 2 LD_{100} was injected 2 days after primary infection, both rIL-2 and antibiotics were ineffective ($p > 0.05$). When 2 LD_{100} of staphylococcal suspension was injected on the 4th day after primary infection, antibiotics had no therapeutic effect whereas rIL-2 increased the survival rate of the mice by 28% ($p < 0.05$; Fig. 1a).

Investigation of the hematologic parameters in intact mice showed the total leukocyte count to be 12,270 ± 2092 in 1 ml; lymphocytes predominated in the differential count (80.6 ± 9.5%), with neutrophils accounting for 16.0 ± 0.5%, and juvenile neutrophils and stab cells for 1.1 ± 0.12%. Macrophages were not normally found in the peripheral blood, but monocytes accounted for 1.7 ± 0.05% and eosinophils for 2.3 ± 0.6% of the total cell count.

After injection of a sublethal dose of *S. aureus* changes in the peripheral blood were virtually identical in type in all groups and correlated with the high rate of survival in all groups. A small decrease in the number of leukocytes was observed in the mice (greatest in the group receiving gentamicin – 10,160 ± 1902); this was accompanied by relative and absolute lymphocytopenia (maximal in the group receiving gentamicin – 52.6 ± 10.6% or 5600 ± 1133 lymphocytes), and mild neutrophilia. The shift to the left was virtually undetectable, the number of monocytes was increased by 4-6 times compared with normal (the greatest increase in the group receiving gentamicin – 8.8 ± 2.05%), and a few macrophages appeared (up to 5.0 ± 0.89%). The results are evidence of a negligible toxic action of the infecting agent in a sublethal dose.

Changes in the peripheral blood 24 h after injection of 2 LD_{100} of *S. aureus* consisted of marked lymphocytopenia (to 18.2 ± 4.6% – in the group receiving antibiotics), an increase in the number of polymorphonuclear neutrophils (to 43.8 ± 6.8% – in the group receiving rIL-2), a shift to the left (to 9.5 ± 1.5% – in the group receiving antibiotics), eosinopenia, an increase in the number of monocytes (to 5.6 ± 2.06%), and the appearance of macrophages (to 27.7 ± 7.2% in the group receiving antibiotics). The changes were more marked than in mice of the intact group and they indicate a significant toxic action of 2 LD_{100} of *S. aureus* on the peripheral blood parameters. The most marked changes in the peripheral blood were observed in the group receiving antibiotics.

When the results of injection of 2 LD_{100} and of treatment by the various preparations are compared, distinct correlation will be apparent. Minimal toxic changes in the periperal blood and maximal survival of the mice took place in the group of animals receiving rIL-2, whereas the maximal toxic effect on the peripheral blood and the highest mortality among mice took place in the group receiving gentamicin.

The pathological observations revealed diffuse suppurative peritonitis [1]. Bacteriologic analysis of the blood and internal organs of animals receiving a sublethal dose of *S. aureus* revealed the existence of bacteriemia 24 h after infection, confirming the toxic effect of *S. aureus,* revealed by the hematologic tests.

According to our observations and those of other workers, IL-2 injected intravenously or intraperitoneally induces lymphoid proliferation in the lungs, liver, kidneys, and lymph nodes [5, 7]. As a result clones of T and B lymphocytes are generated, and these, in turn, activate other populations of immunocompetent cells, and thus induce the secretion of various lymphokines: interleukin-1 (IL-1), γ-interferon, tumor necrosis factor (TNF), colony-stimulating factors (CSF), and other factors causing powerful stimulation of immunity against infection [2, 10].

Proliferation of B lymphocytes leads to increased production of immunoglobulin. Weyand and co-workers [12, 13] showed that injection of rIL-2 (5000 IU) over a period of 3 days causes the IgM titer to be increased by 3-10 times.

Low doses of rIL-1 reduce mortality among mice with granulocytopenia and infected with *Pseudomonas aeruginosa* [11]. rIL-1-α induces neutrophilia and increases the resistance of mice to intraperitoneal infection with *Klebsiella pneumoniae* and methicillin-resistant *S. aureus* [8]. Murine rIL-1-α has been shown to have a protective effect in mice infected with *Listeria monocytogenes* [3].

IL-2 induces secretion of γ-interferon, which greatly potentiates the antimicrobial effect of macrophages [9]. A therapeutic effect of γ-interferon also has been shown in experiments on mice infected by intraperitoneal injection of *E. coli* and intramuscular injection of *Klebsiella pneumoniae* [6].

γ-Interferon, TNF, macrophages, and CSF or a combination of them potentiate activity of mouse macrophages against protozoal infection caused by *Entamoeba histolytica* [4].

It is thus evident that IL-2 (together with other lymphokines) can be successfully used to treat chronic infectious diseases accompanied by an immunodepressive syndrome.

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