PROPHYLACTIC INTERLEUKIN-2 INCREASES THE SURVIVAL RATE OF MICE WITH ACUTE INTRAPERITONEAL INFECTION WITH *Staphylococcus aureus*

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Stimulation of T lymphocytes by interleukin-2 (IL-2) induces the secretion of several lymphokines and monokines, which are growth and differentiation factors of cells of the blood and immune system [8, 9]. IL-2 also stimulates humoral immunity by activating B lymphocytes, a function which must determine its efficacy in other immunodepressive states also, especially those of an infectious nature. Several recombinant forms of cytokines have now been produced, including IL-2, which has undergone extensive preclinical and clinical trials, chiefly in cancer patients [7]. The aim of this investigation was to study the prophylactic action of recombinant interleukin-2 (rIL-2) in acute intraperitoneal infection caused by the Gram-positive *Staphylococcus aureus* strain 5/2.

EXPERIMENTAL METHOD

Experiments in vivo were carried out with male and female C57BL/6 mice weighing 18-20 g and aged 8-12 weeks, obtained from the "Stolbovaya" Nursery. The total number of animals used was 650.

Acute intraperitoneal infection (AII) was produced in the mice by intraperitoneal injection of a suspension of a 24-h culture of *Staph. aureus* strain 5/2 (obtained from the I. P. Pavlov Research Institute of Experimental Medicine, St. Petersburg) in a 10% solution of polyvinyl alcohol (PVA, from "Fluka") (a modification of the model of experimental pneumonia [2]); 1 ml of suspension contained $4 \cdot 10^9$ bacteria.

To obtain the lethal dose (LD) of *Staph. aureus* the dose-dependent mortality curve was determined by intraperitoneal injection of $(0.5\text{-}3) \cdot 10^9$ bacteria into mice. The animals remained under observation for 30 days after infection.

For treatment we used rIL-2 (Cetus) in doses of $(5\text{-}10) \cdot 10^3$ IU per mouse. The antibiotics used were: penicillin 100 U together with streptomycin 0.1 mg per mouse or gentamicin 0.1 mg per mouse. Mice of the control group received an injection of isotonic salt solution (ISS).

All the therapeutic substances were diluted in 0.5 ml ISS and injected intraperitoneally simultaneously with a bacterial suspension of *Staph. aureus* in 10% solution of PVA, or 3 and 2 days or 1 day before injection of LD_{100} of the bacterial suspension (prophylactic injection). Each group of mice consisted of 10 animals. The total number of experiments was 18.

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

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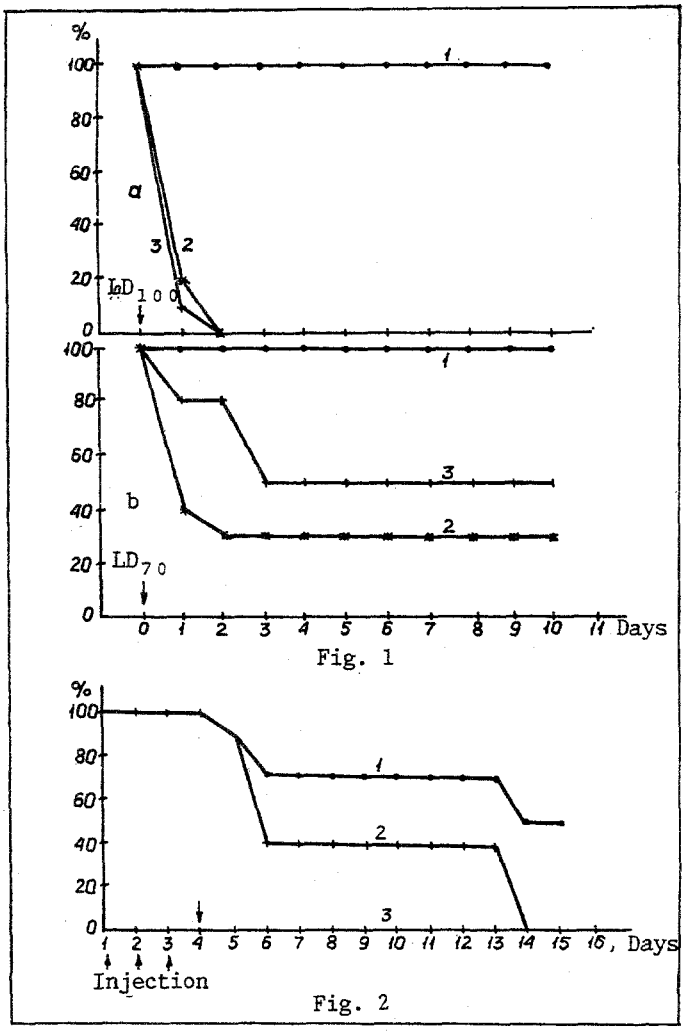

Fig. 1. Treatment of acute intraperitoneal staphylococcal infection by rIL-2 and gentamicin: a) survival rate of mice after injection of LD_{100} of suspension; b) survival rate of mice after injection of LD_{70} of suspension. Survival rate of mice estimated over a period of 30 days. Abscissa, time (days); ordinate, survival rate (per cent): 1) gentamicin, 2) ISS, 3) rIL-2.

Fig. 2. Prophylactic injection of rIL-2 before intraperitoneal injection of LD_{100} of bacterial suspension. rIL-2 and ISS injected intraperitoneally 3 and 2 days and 1 day before injection of LD_{100} of *Staph. aureus* strain 5/2. Survival rate of mice estimated over a period of 30 days: 1) rIL-2 (7500 U); 2) ISS; 3) LD_{100}. Abscissa, time; ordinate, survival rate.

EXPERIMENTAL RESULTS

To determine the dose-dependent mortality curve, mice of the control group were given an injection of *Staph. aureus* in 10% PVA solution simultaneously with ISS. Lethal doses showed a tendency to change, but a lethal outcome occurred in 95% of cases after injection of the bacteria in a dose of $(1.8\text{-}1.9) \cdot 10^9$. In this group about 80% of the animals died in the first 36 h, and a further 10% in the course of 72 h: 5 ± 0.9% of mice survived. No deaths occurred if fewer than $1.3 \cdot 10^9$ bacteria were injected. In this way a dose-dependent mortality curve was obtained and

values of LD_{100} [(1.9-2.0)·10^9 bacterial] and LD_{70} [(1.7-1.8)·10^9 bacteria] of the suspension of *Staph. aureus* were obtained, but at which rIL-2 acted unequally, giving rise to different survival rates of the mice.

As will be clear from Fig. 1a, rIL-2 was ineffective against LD_{100} of the bacterial suspension, whereas antibiotics suppressed the development of the infectious process in 100% of animals ($p < 0.01$). Injection of rIL-2 and a 10% solution of PVA into uninfected mice did not affect their survival rate.

In the group of mice receiving rIL-2 together with LD_{70} of the bacterial suspension, the survival rate was 50%, whereas antibiotics were effective in 100% of cases ($p < 0.01$) (Fig. 1b).

As Fig. 2 shows, preliminary injection of rIL-2 in a dose of 7500 IU/mouse 3, 2, and 1 days before infection of the mice with LD_{100} of *Staph. aureus* led to a survival rate of 50% of the animals, whereas in the control group all the mice died ($p < 0.01$). The longer period of survival of the animals receiving ISS was probably due to irritation of the peritoneum, leading to aseptic inflammation and attraction of immunocytes into the peritoneal cavity.

Antibiotics thus gave rise to a 100% survival rate of the mice after infection with LD_{100} and LD_{70} of the bacterial suspension, whereas rIL-2 was ineffective against LD_{100}, and caused a small but not significant increase in the duration of survival of the animals and an increase of 20% in their survival rate after injection of LD_{70} of the suspension. These results can be explained by the direct bactericidal action of the antibiotics, which is not a property of rIL-2. However, reducing the dose of the bacterial suspension to LD_{70} and, consequently, reducing the toxic action of the staphylococcus, led to an increase in the survival rate of the mice treated with rIL-2. Moreover, prophylactic injection of rIL-2 into the mice 3, 2, and 1 days before infection led to a marked increase in survival rate of the experimental group. This effect can be explained by the ability of IL-2 to cause proliferation of lymphocytes followed by generation of clones of T and B lymphocytes which, in turn, activate other populations of immunocompetent cells, and thereby induce secretion of cytokines: interleukin-1, gamma-interferon, tumor necrosis factor, colony-stimulating factors, and other factors, and cause activation of humoral immunity and of natural killer cells, and to induction of LAK* cells, giving rise to powerful stimulation of immunity [8, 9, 5]. This conclusion is confirmed by results obtained in a pathological investigation of the internal organs of mice receiving injection of rIL-2. According to some investigators [1, 4, 6] and to our own observations, IL-2, injected intraperitoneally or intravenously, induces lymphocyte proliferation in several organs: lungs, liver, spleen, kidneys, and mesenteric lymph nodes.

Reports of the successful use of rIL-2, prophylactic injection of which prevents death of mice with septicemia induced by Gram-negative *E. coli,* have recently been published [3, 10].

Administration of IL-2 and other lymphokines in the treatment and prevention of infectious diseases thus appears a promising development and requires further study.

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*Lymphokine Activated Killer.