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Comparison of the effects of recombinant interleukin 6 and recombinant interleukin 1 on nonspecific resistance to infection

Interleukin 1 (IL 1) is a potent enhancer of nonspecific resistance to infection in mice. Since IL 1 also induces interleukin 6 (IL 6), we tested the hypothesis that IL 6 mediates the effect of IL 1 on nonspecific resistance. In a lethal Pseudomonas aeruginosa infection in granulocytopenic mice, in which 80 ng of recombinant human IL 1α protects against death, IL 6 appeared to be much less effective. Dosages of 8 ng, 80 ng and 320 ng IL 6 did not differ from the control, whereas 800 ng had a marginal protective effect (0.05 < p < 0.1). IL 1 and IL 6 did not potentiate each other in animals treated with suboptimal dosages of both cytokines. Numbers of bacteria cultured from the blood, thigh muscle, liver, spleen, and kidney were similar in animals treated with 800 ng IL 6 and in control animals, arguing against activation of microbicidal mechanisms. The serum concentration profile of IL 6 after an i.p. injection of 80 ng IL 1 was similar to that after 80 ng IL 6 i.p. Only minute amounts of IL 1 were detected in serum after an i.p. injection of IL 6. Taken these data together, it appears that increased resistance to infection induced by IL 1 is not mediated by IL 6.

1 Introduction

Recently, we have reported the beneficial effect of recombinant interleukin 1 (rIL 1) on survival of granulocytopenic mice with a lethal Pseudomonas aeruginosa infection [1]. In this study, we found protection with a single low dosage of IL 1β. Although we could demonstrate that protection was not due to a direct antimicrobial effect of IL 1, to granulocytes or to activation of macrophages, we were unable to elucidate the mechanisms of protection against lethal P. aeruginosa infection. One hypothesis was that treatment with IL 1 protected against the lethal effects of the lipopolysaccharide (LPS) or other toxins of P. aeruginosa. In a subsequent study, in which the effect of IL 1 on a candidal infection in neutropenic mice was investigated, we could demonstrate that the effect of IL 1 on survival is not limited to Gram-negative infection [2].

IL 6 is a 26-kDa cytokine, which is produced by mononuclear phagocytes, fibroblasts and a variety of other types of cells [3–5]. This factor has been described as interferon-β2 [6], hybridoma (plasmocytoma) growth factor [7–9], B cell-stimulating factor 2 (BSF-2) [10] and hepatocyte-stimulating factor [11]. Since IL 1 is a potent inducer of IL 6, the latter could be responsible for the enhanced survival of animals treated with IL 1, e.g., by inducing a hepatic acute-phase protein. Therefore, we have compared the effects of IL 6 and IL 1 on survival of lethally infected mice. In addition, we have investigated the kinetics of injected IL 6 and IL 1, and the mutual induction of these cytokines in vivo.

2 Materials and methods

2.1 Mice

Female, 25 g Swiss Webster mice (Broekman, Someren, The Netherlands), were fed standard laboratory chow and water ad libitum.

2.2 IL

Human recombinant IL 1α (rIL 1α), which was kindly provided by Dr. Peter Lomedico, Hoffmann-La Roche, Nutley, NJ, was used in the majority of the experiments. rIL 1β (kindly provided by Dr. Alan Shaw, Biogen/Glaxo, Geneva, Switzerland) was also used. These IL 1 preparations contained <30 pg lipopolysaccharide (LPS) per mg of protein. Human rIL 6, containing <3 pg LPS/μg of protein was produced as published elsewhere [8].

IL 1 and IL 6 were given as a single i.p. injection in 2% (v/v) normal mouse serum in 0.1 ml pyrogen-free saline. Control mice received heat-inactivated IL 1 (100°C for 20 min).

2.3 Infection model

Mice were rendered granulocytopenic (<0.5 × 10⁸ granulocytes per liter) by means of two i.p. injections of cyclophosphamide (Bristol Myers, Syracuse, NY), 150 and 100 mg/kg of body weight, respectively, 4 days and 1 day before the inoculation of the microorganism. Approximately 2 × 10⁷ Pseudomonas aeruginosa (27853, ATCC, Rockville, MD) were injected into the left thigh muscle. Two doses of gentamycin (Lyomed Inc., Rosemont, IL), 120 mg/kg, were given s.c. 6 and 23 h post infection [1]. The mice in each cage were randomized to receive either IL 1, IL 6 or heat-inactivated IL 1, 24 h before the inoculation of bacteria. Survival was...
over a period of at least 48 h. Survival curves were analyzed using the Kaplan Meier log rank test [12].

### 2.4 Clearance of bacteria

Twenty-four hours after the injection of *P. aeruginosa*, six mice treated with IL6 and six control mice were killed by CO₂ asphyxia. Immediately after death, blood cultures were taken by cardiac puncture, and the muscles of the left thigh (the site of inoculation of the bacteria), the spleen, the kidney and the liver were removed aseptically, weighed and homogenized in sterile saline in a tissue grinder. To bring the counts into the optimal range for reading, samples of thigh muscle were diluted 1:10⁶ and other samples were diluted 1:10 in sterile saline. The suspensions were then plated on sterile DST agar (Oxoid, Ltd., Basingstoke, GB) in tenfold dilutions. After overnight incubation at 37°C the number of colonies was counted.

### 2.5 Pharmacokinetics of rIL6 and rIL1, and induction of IL6 by IL1

At various time points after an i.p. injection of 80 ng of IL6, three mice were killed by CO₂ asphyxia. Immediately after death blood was taken by cardiac puncture. The IL6 concentrations in the sera obtained were measured using the B-9 cell line [5], and IL1 concentrations were measured using D10.G4.1 cells [13], the D10(N4)M subclone; both assays have been described in detail [14]. Similarly, serum concentrations of IL6 and IL1 were measured after an i.p. injection of 80 ng IL1α in mice.

### 3 Results

#### 3.1 Survival of mice

Human rIL1α, given as a single i.p. injection of 80 ng (≈ 3 μg/kg) 24 h before infection, improved the survival of neutropenic mice with a lethal *P. aeruginosa* infection significantly ($\chi^2 = 6.8; p < 0.01$) compared to control mice that received heat-inactivated IL1 (Fig. 1). rIL6 was much less effective than IL1 in these protection experiments (Fig. 1). Even the effect of 800 ng IL6 was not significantly different from the control ($\chi^2 = 3.0; 0.05 < p < 0.1$); dosages of 320 ng, 80 ng and 8 ng did not differ from the control.

To investigate whether IL1 and IL6 would potentiate each other, suboptimal dosages of both cytokines (8 ng and 80 ng, respectively) were injected either alone or in combination (Fig. 2). No potentiation was detected; if anything, there was slight, albeit not significant antagonism between IL1 and IL6.

#### 3.2 Clearance of *P. aeruginosa*

No differences in the numbers of bacteria in blood and tissues were found between mice treated with 800 ng IL6 or control mice 24 h after an i.m. injection of $2 \times 10^7$ *P. aeruginosa* (Fig. 3). When the data were expressed as number of microorganisms per gram of tissue rather than per organ, the data from two groups also did not differ.

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**Figure 1.** The effect of IL1α and of IL6 treatment on the survival of granulocytopenic mice with a *P. aeruginosa* infection. The cytokines were given as single i.p. injections 24 h before infection. Control mice received heat-inactivated IL1 (100°C for 20 min). Only the difference in survival between animals treated with IL1 is significant ($p < 0.01$). Each group consisted of 20 mice.

**Figure 2.** The effect of 8 ng IL1α and 80 ng IL6 injected i.p. 24 h before infection, either alone or in combination, on survival of lethally infected mice. There is no potentiation of IL1 and IL6. Only the difference between survival with 8 ng IL1 and that of the control mice is significant at $p < 0.025$. Each group consisted of 22 mice.
Effects of IL 6 and IL 2 on infection

The next question we addressed was whether IL1 and IL6 of 80 ng IL 1 are quite similar to those after an injection of the inactivated IL 1 (hatched bars) 24 h before inoculation. Each bar represents the mean ± SE of six mice.

Figure 4. Serum concentration profiles of IL 6 (▲) and IL 1 (■) after i.p. injection of 80 ng IL 6 (A) or 80 ng IL 1 (B). Each data point represents mean of serum samples from three mice; bars represent SE.

4 Discussion

In the present study, we have investigated the hypothesis that IL 6 is the central mediator of IL 1-induced protection against lethal bacterial infection in mice. The experiments show that IL 6 is 10–100 times less potent than IL 1 in protecting mice. If IL 1 induced a large amount of IL 6 in vivo, these results still would not rule out an IL 6-mediated pathway. Although we could indeed demonstrate that IL 1 is a potent inducer of IL 6 in mice, the serum concentrations of IL 6 after an i.p. injection of 80 ng IL 1 are quite similar to those after an injection of the (barely protective) 80 ng dose of IL 6 i.p. Taking these data together, the hypothesis mentioned above has to be refuted.

The actual mechanism responsible for increase in survival induced by IL 1, IL 6 and TNF remains unclear. In our previous studies direct antimicrobial effects of IL 1 were ruled out in vitro [1, 2]. Since the mice were profoundly granulocytopenic in those studies, an effect of IL 1 on the neutrophils was considered unlikely. Also, the beneficial effects of IL 1 on hematopoiesis [17] were not demonstrated in our short-term experiments [1, 2]. In the IL 1 studies, no effect of IL 1 on macrophages could be demonstrated [1]. The most convincing argument against an effect on microbial function of macrophages, however, came from the microbial clearance data, which failed to demonstrate a difference between IL 1-treated and control mice [1, 2, 15]. In the present study, we have obtained similar results with IL 6, i.e., the numbers of bacteria in the blood and the various organs were similar in IL 1-treated mice and control mice.

It is assumed that cytokines like TNF and IL 1 contribute to death from infection [18–20]. It could well be that early treatment with IL 1, and to a much lesser extent with IL 6, reduces the lethal effects of these cytokines. This protection could be produced by down-regulation of receptors for these cytokines in the lethal phase. The down-regulation of TNF receptors by IL 1 treatment, which has recently been described to occur in vitro, is in agreement with this concept [21].

In conclusion, whatever the mechanisms of IL 1-induced protection against death due to lethal infection may be, IL 6 does not appear to be a critical intermediate cytokine.

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5 References

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