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## The effects of recombinant interleukin-1 and recombinant tumor necrosis factor on non-specific resistance to infection

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### Abstract

Natural and synthetic immunomodulators that increase non-specific resistance to infection induce the production of interleukin-1 (IL-1) and tumor necrosis factor (TNF). Therefore, we investigated the effect of IL-1 and of TNF on the survival of lethally-infected mice. Mice were injected with  $1 \times 10^6$  *Klebsiella pneumoniae* in the thigh muscle. When recombinant human IL-1 $\beta$  was given as a single i.p. injection 24 h before the infection, survival was increased. Using 80 ng IL-1 $\beta$  per mouse, survival compared to control animals was 80% versus 20% 48 h after the infection ( $p < 0.001$ ). No effect of IL-1 was observed when it was given  $\frac{1}{2}$  h before or 6 h after the infection. IL-1 $\alpha$  proved to be at least as potent as IL-1 $\beta$ .

Numbers of bacteria cultured from the blood, thigh muscle, liver, spleen, and kidney were similar in IL-1-treated and control animals. Protection against death by IL-1 was also investigated in granulocytopenic mice with a *Pseudomonas aeruginosa* infection. Administration of the cyclooxygenase-inhibitor, ibuprofen, did not affect the beneficial effect of IL-1. In this model human recombinant TNF was at least tenfold less active than IL-1 $\beta$ .

Pretreatment with IL-1 also had a significant effect on survival of mice that received a high dose of bacterial lipopolysaccharide.

### Introduction

Several substances have been shown to increase natural resistance mechanisms [1-6]. Most of these substances, such as lipopolysaccharide [1-4], BCG, and muramyl peptides [5, 6] are derived from bacteria. Because of their toxicity, these immunomodulatory substances have not gained acceptance as a therapy in humans.

Substances that increase non-specific resistance appear to be able to stimulate mononuclear phagocytes to synthesize and secrete the

cytokines interleukin-1 (IL-1) and tumor necrosis factor  $\alpha$  (cachectin, TNF) in vitro [7, 8]. IL-1 is the collective name for two 17 kD proteins (IL-1  $\beta$  and  $\alpha$ ) with a number of important biological effects [7, 9]. TNF is also a 17 kD protein, which shares many of its effects with IL-1 [8, 10, 11]. The main difference between the effects of IL-1 and TNF is that TNF does not stimulate T lymphocytes, whereas IL-1 does. Because both IL-1 and TNF are endogenous pyrogens and mediate other acute phase responses [7-10], it is possible that many of the effects

of the immunomodulatory substances mentioned above are mediated by these molecules. Therefore, we investigated the ability of these cytokines to ameliorate the outcome of a lethal infection in mice. In a previous study, we investigated the effect of recombinant interleukin-1 $\beta$  on survival of granulocytopenic mice with a lethal *Pseudomonas aeruginosa* infection [12]. In that study, we found protection with a single low dosage of IL-1 $\beta$ . It appeared that the animals were only protected from death if they were pretreated with IL-1 24 h before infection. Although we could demonstrate that protection was not due to a direct antibacterial effect of IL-1, not due to granulocytes and not to activation of macrophages, we were unable to elucidate the mechanism of protection against lethal *P. aeruginosa* infection. One hypothesis was that pretreatment with IL-1 protected against the lethal effects of the lipopolysaccharide or other toxins of *P. aeruginosa*.

The present paper deals with a number of questions that emerged from these studies: (1) Is IL-1 also able to increase survival in other lethal Gram-negative infections? (2) Does IL-1 protect against death due to bacterial lipopolysaccharide? (3) Is recombinant human TNF also able to increase survival of granulocytopenic mice with a lethal *Pseudomonas aeruginosa* infection?

### Materials and methods

**Mice.** Female, 25 g Swiss Webster mice (Taconic Farms, Germantown NY and Broekman, Someren, the Netherlands), were fed standard lab chow and water ad libitum.

**IL-1 and TNF.** Recombinant human IL-1 $\beta$  (kindly supplied by Cistron Biotechnology, Pine Brook, NJ), which contains less than 30 pg of endotoxin per mg of protein, was used in the majority of the experiments. Recombinant IL-1 $\beta$  from a different source (kindly provided by Dr Alan Shaw, Biogen/

Glaxo, Geneva, Switzerland) and recombinant human IL-1 $\alpha$  (kindly provided by Dr Peter Lomedico, Hoffmann La Roche, Nutley, NY) were also used. Recombinant human TNF $\alpha$  containing less than 30 pg endotoxin per mg of protein was provided by Genetech Inc., South San Francisco.

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

**Infection models.** *Klebsiella pneumoniae* (ATCC 43816), a strain which produces a lethal infection in normal mice [13], was inoculated in the left thigh muscle of non-neutropenic mice as described elsewhere [14]. Unless stated otherwise an inoculum of  $1 \times 10^6$  was used. The mice in each cage were randomized to receive either IL-1 at different time points, or heat-inactivated IL-1. Survival was scored over a period of at least 48 h.

For the *Pseudomonas aeruginosa* infection mice were rendered granulocytopenic ( $<0.5 \times 10^9$  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. Between  $1 \times 10^7$  and  $1 \times 10^8$  *Pseudomonas aeruginosa* (ATCC27853) were injected into the left thigh muscle. Two doses of gentamicin (Lyomed Inc., Rosemont, IL), 120 mg/kg, were given s.c., 6 and 23 h post infection. In one series of experiments, mice were pretreated with a single subcutaneous injection of 10 mg/kg of the cyclooxygenase inhibitor ibuprofen (Upjohn Co, Kalamazoo, MI) 24 h before the infection.

**Clearance of bacteria.** Twenty-four hours after the injection of *Klebsiella pneumoniae*, mice were killed by CO<sub>2</sub> 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 : 10<sup>4</sup> and other samples were diluted 1 : 10 in sterile saline. The suspensions were then plated on sterile Mueller-Hinton agar using the automated spiral plater (Spiral Systems Inc, Cincinnati, OH) as described elsewhere [12]. After overnight incubation at 37°C, the number of cfu was counted.

**Death due to bacterial endotoxin.** A single intraperitoneal injection of 800 µg of *E. coli* endotoxin (lipopolysaccharide, Sigma, St Louis, MI) was given to mice 24 h after the administration of 80 ng IL-1β i.p. or heat inactivated IL-1 i.p. Survival was scored.

**Statistical analysis.** Survival curves were analysed using the Kaplan Meier/log rank test [15].

## Results

### *The effect of IL-1 in Klebsiella pneumoniae infection*

The rate of death of non-neutropenic outbred Swiss mice infected with *K. pneumoniae* in the thigh muscle is greatly dependent on the inoculum. With 1 × 10<sup>6</sup> cfu approximately 80% of the control mice would die within 48 h. Higher inocula resulted in accelerated death.

Recombinant human IL-1β, given as a single i.p. injection of 80 ng (which equals 3 µg/kg) 24 h before infection, improved the survival of these mice significantly compared to mice that received heat-inactivated IL-1β (Fig. 1). When IL-1 was given shortly before injection of *K. pneumoniae*, survival was not significantly better than in the control group (Fig. 1). IL-1 given as late as 6 h after the inoculation of the bacteria also failed to improve survival (data not shown). The results

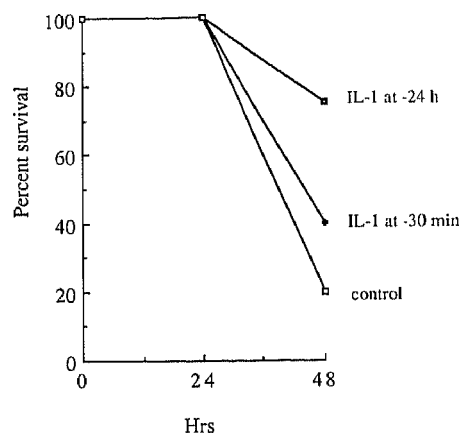


Fig. 1. The effect of IL-1β treatment on the survival of mice with a *Klebsiella pneumoniae* infection. Recombinant human IL-1β was given as a single i.p. injection either 24 h or 30 min before the intramuscular inoculation of 1 × 10<sup>6</sup> bacteria in the thigh muscle. Control mice received heat-inactivated IL-1 (100°C for 20 min). There were at least 30 mice in each group. The difference in survival between the group that received IL-1 24 h before infection and the control group is significant at *p* < 0.01. Survival of the group that received IL-1 30 min before infection does not differ from that of the controls.

obtained with recombinant IL-1β from the two sources were similar. IL-1α proved to be at least as potent as IL-1β (data not shown).

### *Clearance of K. pneumoniae*

No differences in the numbers of bacteria in blood and tissues were found between mice treated with IL-1 or with heat-inactivated IL-1 24 h after an intramuscular injection of 1 × 10<sup>6</sup> *K. pneumoniae* (Fig. 2). When the data were expressed as number of microorganisms per organ rather than per gram of tissue, the data from two groups also did not differ.

### *The effect of ibuprofen on the protection by IL-1*

Pretreatment with a single injection of ibuprofen 30 min before the injection of IL-1 did not significantly influence the survival of either the IL-1 treated mice or the control mice. A representative experiment in granulocytopenic mice inoculated with *P. aeruginosa* is shown in Fig. 3.

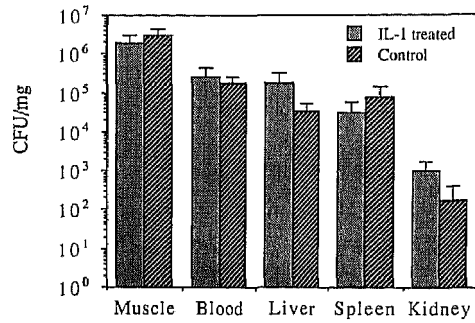


Fig. 2. Counts of colonies of *Klebsiella pneumoniae* in the blood and organs of mice 24 h after an intramuscular injection of  $1 \times 10^6$  cfu of *K. pneumoniae*. Mice received either 80 ng human recombinant IL-1 $\beta$  or heat-inactivated IL-1 $\beta$  24 h before infection. Each bar represents the mean  $\pm$  standard error of log cfu/g tissue in 6 mice.

### The effect of IL-1 on endotoxin-mediated death

Mice pretreated with IL-1 $\beta$ , 24 h before an injection with a LD<sub>70</sub> dose of endotoxin (800  $\mu$ g) showed significantly greater survival than mice pretreated with heat-inactivated IL-1 (Fig. 4).

### Comparison of TNF and IL-1 in *Pseudomonas* infection

Pretreatment with a dose of 80 ng TNF, 24 h before infection did not increase survival of neutropenic mice with a lethal *P. aeruginosa* infection, whereas the same dose of IL-1 $\beta$  did. A representative experiment is shown in Fig. 5. A tenfold higher dose of TNF did increase survival, although not to the same extent as 80 ng IL-1 (Fig. 5). A combination of 8 ng TNF and 8 ng IL-1 $\beta$  did not influence survival (data not shown).

## Discussion

In these studies, we report the efficacy of a low dose of IL-1 $\beta$  and IL-1 $\alpha$  (3.0  $\mu$ g/kg) in prolonging survival in mice with a lethal *Klebsiella pneumoniae* infection. These studies expand on the results we obtained in granulo-

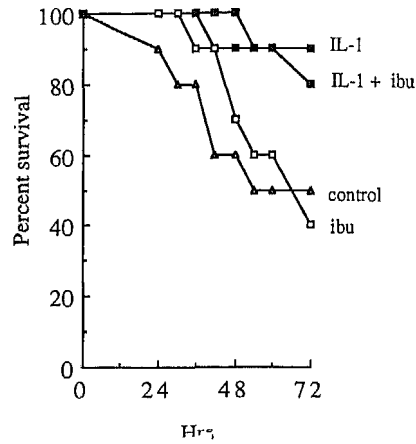


Fig. 3. The effect of pretreatment with a single injection of ibuprofen on the survival of granulocytopenic mice treated with either 80 ng IL-1 $\beta$  or heat inactivated IL-1 24 h before infection with  $1 \times 10^7$  cfu *P. aeruginosa*. The differences between the mice that received active IL-1 and those that did not are significant ( $p < 0.05$ ). Each group consists of 12 mice.

cytopenic mice with a lethal *Pseudomonas aeruginosa* infection [12] and on the results, recently reported by Ozaki *et al.* [16]. These investigators also found increased resistance to infection using human recombinant IL-1 $\alpha$  in non-neutropenic mice that were infected with either *P. aeruginosa* or *Klebsiella pneumoniae*. Increased resistance to infection has

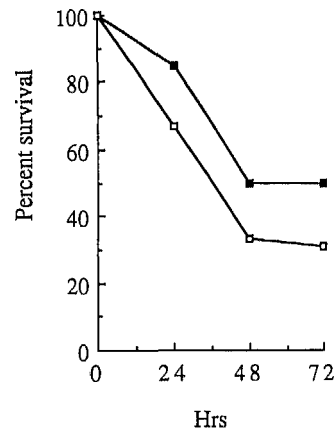


Fig. 4. The effect of treatment with 80 ng IL-1 $\beta$  at -24 h (■) on death induced by intraperitoneal injection of 800  $\mu$ g E. coli lipopolysaccharide in mice. Control mice (□) received heat inactivated IL-1. The differences between the two groups, each of which consisting of 35 mice, are significant ( $p < 0.05$ ).

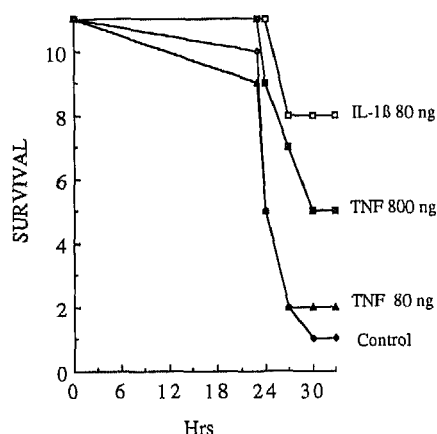


Fig. 5. Comparison of the effect of TNF and IL-1 $\beta$  on survival of granulocytopenic mice infected with  $5 \times 10^7$  *P. aeruginosa*. Each group consists of 11 mice.

been induced with IL-1 in other experimental models with facultative intracellular bacteria. Czuprynski and Brown have protected mice from lethal infection with *Listeria monocytogenes* with recombinant IL-1 [17], and many years ago Pulliam and Kampfschmidt have reported increased survival of rats infected with *Salmonella typhimurium*, using partially purified IL-1 [18].

Like in our previous studies with *P. aeruginosa*, the time of administration of IL-1 in relation to the time of *Klebsiella* infection in the present study was crucial. The lack of a beneficial effect when IL-1 is given shortly before infection might be explained by the rapidity of the spread of these Gram-negative bacteria in mice. Recent experiments with *Candida albicans* in granulocytopenic mice show that IL-1 is effective when given as late as 6 h after infection [19].

Our experiments using TNF show that a low dose of recombinant human TNF does not protect against death from *P. aeruginosa*, whereas a tenfold higher dose (800 ng per mice, which equals 30  $\mu\text{g}/\text{kg}$ ) does to some extent. Similar findings were reported by Parant, who used different infection models [20, 21]. Taverne *et al.* have demonstrated that recombinant mouse TNF at dosages as

high as 5–10  $\mu\text{g}$  per mouse increased survival and reduced parasitemia in experimental malaria [22].

Why TNF was less potent in our studies than IL-1 is presently unclear. One explanation could be that it is due to the suboptimal fit of human TNF in murine TNF receptors [23], a problem which does not seem to exist for IL-1. It is also of interest that we did not find synergism between IL-1 and TNF, since synergistic effects of these cytokines have been described in a variety of conditions *in vitro* and *in vivo* [24–26].

The mechanisms responsible for the cytokine-induced increase in survival remain unclear. In our previous studies a direct antibacterial effect of IL-1 was ruled out *in vitro* [12]. Because 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 [27, 28] were not demonstrated in our short-term experiments [12]; the present experiments in normal mice further argue against such a mechanism as the explanation.

An effect of IL-1 on macrophages could also not be demonstrated [12]. The most convincing argument against an effect on macrophage activation came from the bacterial clearance data, which failed to demonstrate a difference between IL-1-treated and control mice. In the present study, we have obtained similar results with *K. pneumoniae* in non-neutropenic mice, i.e., the numbers of bacteria in the blood and the various organs were similar in IL-1-treated mice and control mice.

We have previously speculated that a humoral factor, for example an acute phase protein, such as an endotoxin-binding protein [29, 30] could be responsible for protection. Although we presently report a certain degree of tolerance against endotoxin, we have obtained other data that refute such a humoral factor as the mechanism of protection. First, we have recently demonstrated that IL-1 gives

considerable protection in lethal infection with *Candida albicans*, a microorganism that does not contain endotoxin [19]. Secondly we were unable to provide protection against *C. albicans* or *P. aeruginosa* by passive transfer of serum.

It is assumed that cytokines like TNF and IL-1 contribute to death from infection [25, 31, 32]. It could well be that early treatment with IL-1 (and TNF) reduces the lethal effects of these cytokines. Since bacterial endotoxin is one of the most potent inducers of these cytokines, the endotoxin tolerance induced IL-1, could fit in such a concept. Interestingly, two other favorable effects of IL-1 treatment in experimental animals, namely protection from lethal radiation [33] and protection against oxygen damage [26], are also presently unexplained, but could depend on similar mechanisms.

In conclusion, our studies and those of others demonstrate that IL-1 $\beta$  and IL-1 $\alpha$  and to a lesser extent TNF are able to increase natural resistance to infection. Although the mechanism of protection is not understood, these results hold promise for the treatment of infections in humans, especially in neutropenic patients. A dose of IL-1 of 3  $\mu\text{g}/\text{kg}$  (which equals 1.2  $\text{mg}/\text{m}^2$ ) is highly protective. Similar dosages of TNF are presently given to humans in phase 1/phase 2 studies. If one assumes that IL-1 is somewhat less toxic than TNF, treatment with the former cytokine should be feasible. Since the protective effect of IL-1 is not blocked by ibuprofen, premedication with a cyclooxygenase inhibitor could probably prevent a large number of side effects.

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