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Effects of Interleukin-8 on Nonspecific Resistance to Infection in Neutropenic and Normal Mice

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The effect of treatment with interleukin-8 (IL-8), a neutrophil-activating cytokine, was investigated in normal and neutropenic mice infected with a lethal dose of Pseudomonas aeruginosa, Klebsiella pneumoniae, or Plasmodium berghei. Intraperitoneal (i.p.) IL-8 treatment was associated with accelerated death when IL-8 was administered shortly before i.p. infection with P. aeruginosa or shortly after i.p. infection with P. aeruginosa and K. pneumoniae. Histopathological analyses demonstrated a tendency to more severe organ lesions in IL-8-treated mice. Only nonneutropenic mice that received IL-8 shortly before the infectious challenge and at the site of infection were protected by IL-8. Whether IL-8 is protective of or detrimental to the survival of infection appeared to depend on the presence of bacteria at the injection site and on the presence of neutropenia. IL-8 may be an important participant in the cascade of interacting cytokines that is induced by the lethal infectious challenge.

Interleukin-8 (IL-8) is an 8-kDa polypeptide that is produced by a wide variety of cell types upon stimulation by cytokines like IL-1 and tumor necrosis factor alpha and by endotoxin and lectins (9, 17, 20; for a review, see reference 21). IL-8 is a chemoattractant for neutrophils, T lymphocytes, and basophils and leads to expression of adhesion molecules, oxidative burst, and degranulation in neutrophils (5, 12, 17, 21).

We have previously shown that treatment of both neutropenic and normal mice with low doses of IL-1 (3 μg/kg of body weight) 24 h before an infection with gram-negative bacteria enhances survival (22, 23). Also, protection against lethal murine malaria has been obtained with low doses of IL-1 (3). The exact mechanism of this protection is still unclear, but since IL-1 induces IL-8 (14, 17, 21), we investigated the effect of IL-8 in lethal bacterial infections in both neutropenic and normal mice and in mice with malaria in order to find out whether IL-8 has a therapeutic potential.

MATERIALS AND METHODS

Cytokines. Recombinant human IL-8 was prepared at Sandoz Research Institute, Vienna, Austria (13). The endotoxin content of this preparation was less than 0.15 ng/mg. Human IL-8 was used because a murine analog (presumably macrophage-inflammatory protein 2 [25]) was not available. Although IL-8 is a human cytokine which has a certain species specificity (19), it has been shown to be effective in mice (7). Recombinant human IL-1β was kindly provided by P. Graber (Glaxo, Geneva, Switzerland). The endotoxin content of this preparation was less than 1.53 ng/mg. The cytokines were stored at −80°C and were diluted immediately before use in pyrogen-free phosphate-buffered saline (PBS) with 2% (vol/vol) mouse serum.

Mice. Female outbred Swiss mice (weight, 20 to 25 g; TNO, Rijswijk, The Netherlands) were used in the bacterial infection studies. Female inbred C57BL/6J mice (weight, 20 to 25 g; from a local colony) were used in the malaria experiments. The mice were allowed to acclimatize for 6 days and were kept under specific-pathogen-free conditions. Standard lab chow (RMH-TM; Hope Farms, Woerden, The Netherlands) and acidified water were available ad libitum. Neutropenic mice received irradiated food.

Infections. For survival experiments, mice were infected with 0.5 × 10^7 to 1 × 10^7 CFU of Pseudomonas aeruginosa ATCC 27853 intramuscularly (i.m.) in the right thigh muscle or intraperitoneally (i.p.) on day 0. Since P. aeruginosa does not kill normal mice, mice were made neutropenic (<0.5 × 10^7 neutrophils per liter) by two subcutaneous injections of cyclophosphamide (150 mg/kg; ASTA Pharma, Frankfurt, Germany) on day −4 and 100 mg/kg on day −1. In a number of experiments, mice infected with 1 × 10^6 to 5 × 10^6 CFU of Klebsiella pneumoniae ATCC 43816 were made neutropenic to a lesser extent (0.8 × 10^7 to 2 × 10^7 neutrophils per liter) by giving them 150 mg of cyclophosphamide per kg on day −1. Nonneutropenic mice were also infected with K. pneumoniae. All bacterial challenges (0.1 ml per mouse) were prepared by appropriate dilution of overnight cultures washed three times in saline. Cytokines were given i.p. at several time points. Six hours after infection, gentamicin (120 mg/kg; Schering, Kenilworth, N.J.) was administered subcutaneously in order to postpone the time of death and thus accentuate the differences between treatment groups. Mortality was recorded for at least 48 h. Since IL-8 is heat stable, control animals received 80 ng of heat-inactivated (90°C for 30 min) IL-1 instead of IL-8. For malaria experiments, 1,000 erythrocytes parasitized with Plasmodium berghei K173 were injected i.p. as described elsewhere (4). Parasitemia was determined in May-Grunwald Giemsa-stained thin blood films made from a drop of tail blood. Mortality was scored twice daily. Mice that succumbed to infection were processed for histopathology.

Differential counts of leukocytes. Thin blood films from tail
blood were stained with May-Grunwald-Giemsa’s solution. Blood was taken from ether-anesthetized mice by orbital or heart puncture and was placed in EDTA. Five milliliters of ice-cold PBS with 0.38% (wt/vol) sodium citrate was injected into the peritoneal cavity of mice that were killed by cervical dislocation. After massage of the abdomen, the peritoneal cells were aspirated and counted by flow cytometry (H-l; Technicon Instruments Corporation, Tarrytown, N.Y.). Cytocentrifuge preparations of the peritoneal cells were stained with May-Grunwald-Giemsa’s solution. Differential counts of blood and peritoneal cells were performed in duplicate on 200 nucleated cells.

**Bacterial counts.** *P. aeruginosa* and *K. pneumoniae* were cultured at 37°C in brain heart infusion broth with various concentrations of IL-8 or vehicle (PBS). At 2, 3, 4, and 5 h after inoculation, 100 μl of 10-fold serial dilutions were plated in duplicate on blood agar to determine bacterial counts.

In the assessment of the effect of IL-8 on bacterial outgrowth in vivo, mice were anesthetized with ether, bled, and killed by cervical dislocation. Organs were removed aseptically, weighed, and homogenized with an Ultraturrax (Janke & Kunkel, Staufen, Germany) in sterile saline; 10 or 20 μl of 10-fold serial dilutions was incubated overnight at 37°C, and the number of CFU was determined.

**Histology.** Observer-masked histopathology was performed on mice sacrificed 30 h after i.p. *P. aeruginosa* challenge and on mice that died after *P. berghei* infection. The frequency of animals with hemorrhages, the frequency of hemorrhages in an organ of a given animal, as well as the size of the hemorrhages were scored in the various experimental groups.

**Statistics.** Survival was analyzed by use of the log rank test (16), bacterial counts were analyzed by using one-way analysis of variance or the Gehan Wilcoxon test (depending on the censoring of data), and differential leukocyte counts were analyzed by the Kruskal Wallis test (11). P values of <0.05 were considered statistically significant.

**RESULTS**

**IL-8 in *P. aeruginosa* infection.** When *P. aeruginosa* was administered i.m. to neutropenic mice, a single dose of 800 ng of IL-8, given i.p. 24 or 0.5 h before or 6 h after infection, did not significantly influence the survival of mice (Fig. 1A). Similar results were found with lower doses. Since no toxicity was observed when 800 ng was given to noninfected normal and neutropenic mice, this dose was used in further experiments. However, as shown in Fig. 1B, i.p. injection of *P. aeruginosa* resulted in a significant enhancement of the death rate if IL-8 was given 0.5 h before or 6 h after infection ($\chi^2 = 5.96$ and $P < 0.05$, and $\chi^2 = 6.69$ and $P < 0.01$, respectively). Administration of IL-8 24 h before infection did not affect survival. IL-1β (800 ng given i.p.) significantly enhanced survival in mice infected i.m. and i.p. (data not shown).

**K. pneumoniae infection.** In normal and neutropenic mice, IL-8 administered i.p. 24 or 0.5 h before or 6 h after i.m. challenge with *K. pneumoniae* had no effect on survival (Fig. 2A and B). In the case of the i.p. route of infection, however, the death rate was enhanced by i.p. IL-8 administration 6 h after the challenge (in normal mice, $\chi^2 = 8.62$ and $P < 0.005$; in neutropenic mice, $\chi^2 = 4.26$ and $P < 0.05$) (Fig. 2C and D). In contrast, IL-8 given to normal mice 0.5 h before the intraperitoneal challenge resulted in a reproducible protective effect in normal mice ($\chi^2 = 12.17$ and $P < 0.001$) but not in neutropenic mice.

Table 1 summarizes the conditions and the outcomes of mouse survival after the bacterial challenges.

**P. berghei infection.** Six daily injections of 800 ng of IL-8 starting on the day of i.p. infection did not alter the degree of parasitemia or the death rate after infection (data not shown).

**Effects of IL-8 on bacterial growth in vivo and in vitro.** To examine whether the effects of IL-8 were due to enhanced bacterial growth, bacterial counts in the blood, livers, spleens, kidneys, and thymus of mice in each treatment group were determined 30 h after the bactericidal infection, a time point at which there was a clear difference in survival between the groups. No significant differences in bacterial counts were observed in any treatment group of mice infected with *P. aeruginosa* i.m. or i.p. and *K. pneumoniae* i.p. (eight mice per group; data not shown). We also examined whether IL-8 enhanced multiplication of *P. aeruginosa* and *K. pneumoniae* in culture, because some pathogenic bacteria use cytokines as growth factors (18). At concentrations from 0.1 to 1,000 ng of IL-8 per ml, no effect of IL-8 on bacterial growth was detected (data not shown).

**Blood and peritoneal leukocytes.** The numbers of peritoneal leukocytes in neutropenic mice were assessed 30 h after i.p. or i.m. *P. aeruginosa* infection, a time point at which a maximal difference in survival between the treatment groups was observed. The peritoneal granulocyte and macrophage numbers did not differ between IL-8-treated and control infected mice; the total leukocyte count and the number of blood granulocytes did not differ either.

In normal mice infected i.p. with *K. pneumoniae*, total and differential leukocyte counts of blood and peritoneal fluid at 30 h after infection did not differ significantly between IL-8-treated and control infected mice (data not shown).

**Histology.** Histologic analyses were performed on the organs of mice killed 30 h after infection with *P. aeruginosa* for the reasons described above. Hemorrhages were found in
The same experiment as that for panel C performed in neutropenic control group consisted of at least 125 mice. Administration of 800 ng of IL-8 i.p. 6 hours after infection also did worse than controls. The same was found for neutropenic mice which received IL-8 0.5 h before infection, but normal mice were protected by this treatment. In mice with P. berghei infections, no effect of IL-8 was found on survival or parasitemia, but histological analyses indicated that IL-8 treatment enhanced the development of cerebral malaria.

The different results obtained between mice with i.m. and i.p. bacterial infections suggest that acceleration of death occurs if both IL-8 and bacteria are present at the same site. Because the acceleration of death occurs only if IL-8 is administered shortly before or after the infectious challenge, the effect of IL-8 seems to be fast and short-lived. It is intriguing that the deleterious effects were most prominent when there was neutropenia.

The deleterious effect of IL-8 could not be explained by an enhanced multiplication of bacteria. Accelerated death might be explained by the chemotactic effect of IL-8 on neutrophils, with the subsequent release of secretory products that cause tissue damage, contributing to death (1). If an IL-8-evoked neutrophil response were the explanation, one would expect to find more obvious effects of IL-8 in non-neutropenic rather than neutropenic mice, but this was not the case. A similar, very low number of neutrophils in the peritoneal cavities of the IL-8-treated group and the control group also argues against such a mechanism.

DISCUSSION

Whereas IL-1 treatment strongly enhances the survival of normal and neutropenic mice that are lethally infected (3, 4, 15, 22–24), IL-8 administered i.p. did not protect mice under most test conditions applied in the present study. IL-8 did not significantly change the survival rate in mice with i.m. infections with P. aeruginosa and K. pneumoniae. Surprisingly, survival and morbidity may even be negatively affected by IL-8 in mice with i.p. infections. After i.p. infection with P. aeruginosa, significantly enhanced mortality was observed in neutropenic mice which received IL-8 shortly before or 6 h after infection. The findings in mice infected with K. pneumoniae i.p. were partially different. Mice treated with IL-8 6 h after infection also did worse than controls. The same was found for neutropenic mice which received IL-8 0.5 h before infection, but normal mice were protected by this treatment. In mice with P. berghei infections, no effect of IL-8 was found on survival or parasitemia, but histological analyses indicated that IL-8 treatment enhanced the development of cerebral malaria.

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FIG. 2. (A) Survival of normal Swiss mice that received 800 ng of IL-8 given i.p. 24 or 0.5 h before or 6 h after infection with 1 \times 10^6 to 5 \times 10^6 CFU of \textit{K. pneumoniae} in the right thigh muscle. Control mice received heat-inactivated (90°C for 30 min) IL-1. Each treatment group consisted of 60 mice. The differences between the groups were not significant. (B) The same experiment as that for panel A performed in neutropenic mice (at least 70 mice per group); differences were not significant. (C) Survival of normal Swiss mice that received 800 ng of IL-8 given i.p. 24 or 0.5 h before or 6 h after i.p. infection with 1 \times 10^6 to 5 \times 10^6 CFU of \textit{K. pneumoniae}. Each treatment group consisted of at least 125 mice. Administration of 800 ng of IL-8 i.p. 6 h after infection resulted in a statistically significant deteriorating effect ($\chi^2 = 8.62$ and $P < 0.005$). Mice that received IL-8 i.p. 0.5 h before the challenge were protected ($\chi^2 = 12.17$ and $P < 0.001$). (D) The same experiment as that for panel C performed in neutropenic mice. The survival of mice (at least 40 mice per group) that received the brains, livers, spleens, kidneys, and lungs of mice infected with \textit{P. aeruginosa}. In most organs, no clear differences between the treatment groups were present. Petechiae were more frequent in the lungs of the control group (seven of eight mice in the control group versus three of eight mice in the IL-8-treated group). Extensive hemorrhages were more frequently observed, however, in the brains of IL-8-treated mice than in those of control mice (four of eight animals versus one of seven animals, respectively) (Fig. 3). In mice with \textit{P. berghei} infections, histopathology confirmed that IL-8 did not prevent the development of cerebral malaria, in contrast to IL-1 treatment (3). All mice that died within 2 weeks after infection exhibited hemorrhages in their brains, and these tended to be more frequent and more severe in IL-8-treated animals. Pathological changes in other organs were similar in both treatment groups.

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Since cyclophosphamide, which was used in our study to induce neutropenia, also strongly affects monocytopoiesis, the effects of IL-8 on monocytes are probably not responsible either. In addition, the numbers of peritoneal macrophages were also virtually identical in IL-8-treated and control mice.

A possible mechanism of the enhancement of death after IL-8 administration is the participation of IL-8 in the lethal cytokinemia, the overshoot of cytokine production which is thought to mediate most symptoms of serious infections and which we think is ameliorated by preexposure to IL-1 (22). In other words, IL-8 administered i.p. could act synergistically with the deleterious effects of other cytokines and mediators like tumor necrosis factor, IL-1, gamma interferon, platelet-activating factor, and leukotriene B4 induced by gram-negative bacteria administered i.p. (6). Since hemorrhages and other pathological changes are similar in mice with cerebral malaria and mice challenged with a lethal dose of bacteria, a common lethal pathway in which these cytokines are involved can be assumed. The observation that IL-8-treated animals had more pronounced brain hemorrhages would be in agreement with the hypothesis presented above and with the findings of Colditz et al. (2) showing plasma leakage after IL-8 administration.

The protective effect of IL-8 administered to nonneutropenic mice shortly before i.p. challenge with *K. pneumoniae* can be reconciled with the latter hypothesis. The administered IL-8 may inhibit local neutrophil adhesion to endothelial cells activated as a consequence of the bacterial challenge, thus limiting inflammatory damage from a neutrophil-endothelial cell interaction (8, 10). This might explain the lack of differences in the leukocyte and bacterial counts that we found, despite the protective effect of IL-8 in this setting. Another possible explanation is, that under the circumstances of the present study, IL-8 initially attracts a sufficient number of neutrophils to the right place and at the right time to be able to phagocytize and destroy the administered microorganisms to a certain extent. This initial event might suppress the initiation of the cascade of lethal cytokinemia. This hypothesis is not necessarily in conflict with the observed similar neutrophil and bacterial counts at later time points. Thus, our results indicate that despite its neutrophil-activating effects reported in the literature, IL-8 is a harmful molecule under a number of experimental conditions. Such

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**TABLE 1. Conditions and survival outcome after bacterial challenges**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Route of bacterial administration</th>
<th>Effect on survival</th>
<th>Polymorphonuclear leukocyte condition</th>
<th>Figure no.</th>
<th>Time (h) IL-8 was given before or after bacterial challenge</th>
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</thead>
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<td><em>P. aeruginosa</em></td>
<td>i.m.</td>
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<td>Neutropenic</td>
<td>1A</td>
<td>-24, -0.5, +6</td>
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<tr>
<td><em>P. aeruginosa</em></td>
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<td>Detrimental</td>
<td>Neutropenic</td>
<td>1B</td>
<td>-0.5, +6</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
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<td>None</td>
<td>Normal</td>
<td>2A</td>
<td>-24, -0.5, +6</td>
</tr>
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<td><em>K. pneumoniae</em></td>
<td>i.m.</td>
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<tr>
<td><em>K. pneumoniae</em></td>
<td>i.p.</td>
<td>Detrimental</td>
<td>Normal</td>
<td>2C</td>
<td>+6</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
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<td>Neutropenic</td>
<td>2D</td>
<td>-0.5, +6</td>
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</tbody>
</table>

FIG. 3. Hemorrhages and ghosts of erythrocytes in the brain of an IL-8-treated mouse 30 h after infection with *P. aeruginosa*. 
deleterious effects are also known for other proinflammatory cytokines. Our results argue against a possible role of IL-8 as a mediator of the protective effect of IL-1 preexposure in mice with infection.

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REFERENCES