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Interleukin-1 (IL-1), two 17-kDa proteins (IL-1α and IL-1β) produced by many different types of cells, possesses a wide spectrum of biological properties (6). In animals, administration of IL-1 has been shown to enhance nonspecific resistance to gram-positive and gram-negative bacteria, fungi, and plasmodia (41). For instance, treatment of both neutropenic and normal mice with a single low dose (3 to 30 μg/kg of body weight) of recombinant human IL-1β (rhIL-1β) 24 h before a lethal infection with gram-negative bacteria enhances survival (36, 37). The exact mechanism of this protective effect is still unclear. A direct antimicrobial effect of IL-1 has been excluded in vitro (37). Whether IL-1 enhances the clearance of microorganisms in vivo is controversial (18, 22, 37); a lack of IL-1 production of eicosanoids like platelet-activating factor (PAF) and arachidonic acid metabolites. We investigated the potential role of these substances in IL-1-induced protection. Low doses of murine TNF-α but not of human TNF-α enhanced survival, suggesting an effect via the type II TNF receptor rather than the type I TNF receptor, which has little species specificity. In line with this TNF-α-induced protection from infection, pretreatment with a low dose of a rat anti-murine TNF-α monoclonal antibody tended to inhibit IL-1-induced protection, suggesting a role of TNF-α as a mediator of IL-1-induced enhanced resistance to infection. Pretreatment with higher doses of anti-TNF-α, however, showed a dose-related protective effect per se, which could be further enhanced by a suboptimal dose of IL-1. A combination of optimal doses of anti-TNF-α and IL-1 produced an increase in survival similar to that produced by separate pretreatments. This lack of further enhancement of survival by combined optimal pretreatments suggests a similar mechanism of protection, most likely attenuation of deleterious effects of overproduced proinflammatory cytokines like TNF-α during lethal infection. Pretreatment with different doses of GM-CSF before a lethal Pseudomonas aeruginosa challenge in neutropenic mice did not enhance survival. Different doses of WEB 2170, a selective PAF receptor antagonist, of MK-886, a selective inhibitor of leukotriene biosynthesis, or of several cyclooxygenase inhibitors did not reduce the protective effect of IL-1 pretreatment. We conclude that IL-1-induced nonspecific resistance is partially mediated by induction of TNF-α and not by GM-CSF, PAF, and arachidonic acid metabolites. The mechanism of action of IL-1 seems to be similar to that of anti-TNF-α.

Roles of Tumor Necrosis Factor Alpha, Granulocyte-Macrophage Colony-Stimulating Factor, Platelet-Activating Factor, and Arachidonic Acid Metabolites in Interleukin-1-Induced Resistance to Infection in Neutropenic Mice

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Treatment with a single low dose (80 to 800 ng) of interleukin-1 (IL-1) 24 h before a lethal bacterial challenge in granulocytopenic and in normal mice enhances nonspecific resistance. The mechanism behind this protection has only partially been elucidated. Since IL-1 induces production of tumor necrosis factor alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet-activating factor (PAF), and arachidonic acid metabolites, we investigated the potential role of these substances in IL-1-induced protection. Low doses of murine TNF-α but not of human TNF-α enhanced survival, suggesting an effect via the type II TNF receptor rather than the type I TNF receptor, which has little species specificity. In line with this TNF-α-induced protection from infection, pretreatment with a low dose of a rat anti-murine TNF-α monoclonal antibody tended to inhibit IL-1-induced protection, suggesting a role of TNF-α as a mediator of IL-1-induced enhanced resistance to infection. Pretreatment with higher doses of anti-TNF-α, however, showed a dose-related protective effect per se, which could be further enhanced by a suboptimal dose of IL-1. A combination of optimal doses of anti-TNF-α and IL-1 produced an increase in survival similar to that produced by separate pretreatments. This lack of further enhancement of survival by combined optimal pretreatments suggests a similar mechanism of protection, most likely attenuation of deleterious effects of overproduced proinflammatory cytokines like TNF-α during lethal infection. Pretreatment with different doses of GM-CSF before a lethal Pseudomonas aeruginosa challenge in neutropenic mice did not enhance survival. Different doses of WEB 2170, a selective PAF receptor antagonist, of MK-886, a selective inhibitor of leukotriene biosynthesis, or of several cyclooxygenase inhibitors did not reduce the protective effect of IL-1 pretreatment. We conclude that IL-1-induced nonspecific resistance is partially mediated by induction of TNF-α and not by GM-CSF, PAF, and arachidonic acid metabolites. The mechanism of action of IL-1 seems to be similar to that of anti-TNF-α.

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IL-1-induced enhanced resistance of neutrophenic mice to lethal gram-negative infection.

(Materials and Methods) Mice. Female outbred Swiss mice (20 to 25 g; TNO, Rijswijk, The Netherlands) were kept under specific-pathogen-free conditions. Standard irradiated lab chow and distilled water were available ad libitum. To render mice neutrophenic (<0.5 × 10^6 granulocytes per liter), two subcutaneous injections of cyclophosphamide were administered (150 and 100 mg/kg) on day -4 and on day -1, respectively, before infectious challenge (39).

Materials. Recombinant human IL-1β (rHIL-1β) was kindly provided by P. Graber (Glaxo, Geneva, Switzerland), and P. Krammer, German Cancer Research Center, provided a neutralizing rat anti-murine TNF-α monoclonal antibody (MAb) V1q (7) was kindly provided by I. Lindley, Sandoz, Vienna, Austria) had a specific activity of 6 × 10^7 U/mg in the murine bone marrow cell proliferation assay, and endotoxin content was <250 pg/ml.

Cytokines were stored undiluted at -70°C and were diluted just before use. A single batch of LPS (serotype O:55:4) was provided by P. Graber (Glaxo, Geneva, Switzerland) and P. Krammer (German Cancer Research Center, Heidelberg, Federal Republic of Germany). The rat MAb IgG2α was purified from ascites by 45% ammonium sulfate precipitation followed by dialysis against pyrogen-free distilled water, protein G chromatography, and another dialysis step. LPS content (Limulus amebocyte lysate assay) was less than 8.2 pg/mg of protein. In the L929 bioassay (1), the rat TNF-α-neutralizing activity was 175 U/mg. Bacteria for infectious challenges (Pseudomonas aeruginosa ATCC 27853) were cultured overnight and washed three times in saline, and appropriate dilutions were prepared.

L929 TNF bioassay. The L929 TNF assay was performed as previously described (1). Briefly, 100,000 L929 cells were added to serial twofold dilutions of rmTNF. The test was performed in the presence of 4 μg of emetine (Sigma GmbH, Munich, Federal Republic of Germany) per ml. After 20 h at 37°C and 5% CO_2 in air at 90% relative humidity, TNF-mediated cytopathic effects on L929 cells were evaluated with crystal violet at 540 nm (Sigma Chemical Co.). The detection limit of the assay was 80 pg/ml.

Experimental protocols. For survival experiments, IL-1, rmGM-CSF, rHIL-1β, rmTNF-α, or control treatment (heat-inactivated IL-1) was administered intraperitoneally (i.p.) (0.1 ml) to neutrophenic mice 24 h before a lethal intramuscular (i.m.) challenge with P. aeruginosa at time zero. To obtain reproducible survival curves, a relatively high bacterial challenge was given. This was combined with gentamicin (120 mg/kg) given subcutaneously 6 h after the bacterial challenge in order to postpone the time of death and thus accentuate the differences between treatment groups. Mortality was recorded for a period of at least 48 h after challenge. For investigations of the respective roles of TNF-α, PAF, and arachidonic acid metabolites in IL-1-induced enhanced survival, V1q, a neutralizing anti-murine TNF-α MAb, was given intravenously (i.v.) 3 h before the i.p. IL-1 injection, and WEB 2170 and MK-886 were administered orally by gavage 1 h before the IL-1 injection. The cyclooxygenase inhibitors ibuprofen, acetylsalicylic acid, and indomethacin were administered orally (gavage) or intraperitoneally 45 min before the IL-1 injection.

In experiments for confirming the in vivo activity of the anti-TNF-α MAb, serum TNF bioactivity at 2 h after i.p. LPS was measured in mice pretreated i.v. with anti-TNF-α or saline 3 h before the LPS challenge. In addition, survival rates were compared in groups of mice pretreated i.v. with anti-TNF-α or the same amount of rat IgG 3 h before a lethal i.p. LPS challenge.

Statistical analysis. Survival data were analyzed with the log rank test (28). P values of less than 0.05 were considered significant.

RESULTS

Effect of TNF-α compared with IL-1-induced protection from infection. We first investigated the role of TNF-α in IL-1-induced protection from infection. Different doses of hTNF-α, mTNF-α, or rHIL-1β were administered i.p. to neutrophenic mice 24 h before a lethal i.m. P. aeruginosa challenge. As shown in Fig. 1A and B, both 80 and 800 ng of rhIL-1β produced significant protection in comparison with results with control mice. Survival rates were not significantly enhanced by 80 or 800 ng of rHIL-1β and 80 ng of rmTNF-α. However, 800 ng of rmTNF-α enhanced survival rates significantly; this protection was not significantly different from protection induced by 80 ng of rhIL-1β (Fig. 1B).

Effects of anti-TNF-α IgG. To investigate the role of TNF-α in IL-1-induced protection from infection further, we assessed the effect of V1q, a neutralizing rat anti-murine TNF-α MAb. In experiments by Echtenacher et al. (7) and in our own experiments, intravenous pretreatment with 5 × 10^4 neutralizing units of anti-TNF-α completely neutralized LPS-induced serum TNF-α bioactivity. In addition, the same dose of anti-TNF-α i.v. gave significant protection against a lethal i.p. LPS challenge (2.5 mg per mouse) administered 3 h later (anti-TNF-α-pretreated mice versus IgG-pretreated mice; P < 0.025; 10 mice per group; data not shown).

Administration of anti-TNF-α (15, 60, or 400 μg per mouse i.v.) 27 h before a lethal P. aeruginosa challenge enhanced...

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survival in a dose-dependent fashion (in comparison with the 
same amount of IgG; not significant, \( P < 0.05 \), and \( P < 0.005 \), 
respectively; at least 11 mice per group; data not shown). 
Survival after administration of 400 \( \mu \)g of anti-TNF-\( \alpha \) at 27 h 
before the infectious challenge did not differ significantly from 
the survival after the same dose of anti-TNF-\( \alpha \) given at \(-3\) h, 
and anti-TNF-\( \alpha \) administered at \(-27\) h gave protection to 
almost the same extent as the same dose administered at \(-3\) h 
(both compared with the same dose of IgG simultaneously: 
\( P = 0.08 \) and \( P < 0.05 \), respectively; 30 or 31 mice per group; 
data not shown). Treatment with the lower doses of anti-
TNF-\( \alpha \) at \(-27\) h combined with a suboptimal dose (400 \( \mu \)g) of 
IL-1 at \(-24\) h enhanced survival rates compared with those 
with anti-TNF-\( \alpha \) or IL-1 separately (Fig. 2A, shown for 15 \( \mu \)g 
per mouse). Anti-TNF-\( \alpha \) (400 \( \mu \)g) administered i.v. at 27 h 
before infection induced protection similar to that induced by 
800 \( \mu \)g of IL-1 administered i.p. at \(-24\) h, and combination of 
the two treatments could not further enhance survival (Fig. 
2B).

Pretreatment at \(-27\) h with a low, nonprotective dose of 
anti-TNF-\( \alpha \) (5 \( \mu \)g i.v.) consistently tended to reduce the 
protective effect induced by 800 \( \mu \)g of IL-1 i.p. at \(-24\) h. 
Although the difference between the effects of IL-1 with 
anti-TNF-\( \alpha \) and with IgG did not reach statistical significance, 
the addition of anti-TNF-\( \alpha \) abolished the significant difference 
of IL-1 treatment from the controls (Fig. 2C).

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**FIG. 1.** (A) Survival of neutropenic mice pretreated i.p. with 
different doses of rhIL-1\( \beta \) or rhTNF-\( \alpha \) 24 h before a lethal i.m. \( P. \) 
aeruginosa challenge. Significantly greater protection in comparison 
with control mice (receiving heat-inactivated IL-1\( \beta \)) occurs in the 
groups pretreated with 80 or 800 \( \mu \)g of IL-1\( \beta \) (\( \chi^2 \) = 7.74 and 10.01 and 
\( P < 0.01 \) and \( P < 0.05 \), respectively; 29 and 31 mice per group). (B) 
Survival of neutropenic mice pretreated i.p. with different doses of 
rhIL-1\( \beta \) or rmTNF-\( \alpha \) 24 h before a lethal i.m. \( P. \) aeruginosa challenge. 
Significantly greater protection in comparison with control mice 
(receiving heat-inactivated IL-1\( \beta \)) occurs in the groups pretreated with 
80 or 800 \( \mu \)g of IL-1\( \beta \) (\( \chi^2 \) as in panel B) and with 800 \( \mu \)g of rmTNF-\( \alpha \) 
(\( \chi^2 = 4.81 \) and \( P < 0.05 \); 30 mice per group). Horizontal axes, time 
(hours) postchallenge.

**FIG. 2.** (A) Survival of neutropenic mice pretreated i.v. with 15 \( \mu \)g 
of the anti-TNF-\( \alpha \) antibody V1q or the same amount of control IgG at 
27 h and/or treated i.p. with 400 \( \mu \)g of rhIL-1\( \beta \) or control substance 
(heat-inactivated IL-1\( \beta \)) at 24 h before a lethal i.m. \( P. \) aeruginosa 
challenge. Significant differences in survival between mice treated 
with IgG plus heat-inactivated IL-1\( \beta \), mice treated with IgG plus IL-1 (\( \chi^2 \) = 
5.68 and \( P < 0.025 \)), and mice treated with anti-TNF-\( \alpha \) plus IL-1 (\( \chi^2 \) = 
8.79 and \( P < 0.005 \); 10 mice per group) are present. (B) Survival of 
neutropenic mice pretreated i.v. with 400 \( \mu \)g of the anti-TNF-\( \alpha \) 
antibody V1q or the same amount of control IgG at 27 h and/or treated 
i.p. with 800 \( \mu \)g of rhIL-1\( \beta \) or control substance (heat-inactivated 
IL-1\( \beta \)) at 24 h before a lethal i.m. \( P. \) aeruginosa infection. Significant 
differences in survival between mice treated with IgG plus heat-
inactivated IL-1\( \beta \), mice treated with IgG plus IL-1 (\( \chi^2 = 12.09 \) and \( P < 0.005 \)), 
and mice treated with anti-TNF-\( \alpha \) plus IL-1 (\( \chi^2 = 8.72 \) and \( P < 0.005 \); 29 
to 31 mice per group) are present. (C) Survival of neutropenic mice 
pretreated i.v. with 5 \( \mu \)g of the anti-TNF-\( \alpha \) antibody V1q or the same 
amount of control IgG at 27 h and/or treated i.p. with 800 \( \mu \)g of 
rhIL-1\( \beta \) or control substance (heat-inactivated IL-1\( \beta \)) at 24 h before a 
lethal i.m. \( P. \) aeruginosa infection. Although survival of mice treated 
with rhIL-1\( \beta \) plus anti-TNF-\( \alpha \) is consistently lower than that of mice 
treated with rhIL-1\( \beta \) plus control IgG, these differences did not reach 
significance. However, no significant difference in survival between 
mice treated with anti-TNF-\( \alpha \) plus heat-inactivated IL-1\( \beta \) and mice 
treated with anti-TNF-\( \alpha \) plus IL-1 is present, whereas the difference in 
survival between mice treated with IgG plus heat-inactivated IL-1\( \beta \) 
and mice treated with IgG plus IL-1 is significant (\( \chi^2 = 12.09 \) and \( P < 
0.001 \); 18 to 20 mice per group).
To assess the role of PAF in IL-1-induced protection, we compared with vehicle treatment (data not shown).

Next, we investigated whether GM-CSF might be a mediator of IL-1-induced protection from infection. A dose range of GM-CSF expected to be physiologically relevant was investigated; GM-CSF was administered i.p. to neutropenic mice 24 h before a lethal i.m. challenge with P. aeruginosa. Survival was not affected by 0.5, 1, 2, 5, or 10 μg of GM-CSF in comparison with survival in control mice (28 mice per group; results not shown).

Effect of pretreatment with inhibitors of PAF, lipooxygenase, and cyclooxygenase on IL-1-induced enhanced survival. To assess the role of PAF in IL-1-induced protection, we administered WEB 2170, a PAF receptor antagonist, in different oral doses (10, 30, and 150 mg/kg of body weight) 1 h before 800 ng of IL-1. Such dosage schedules, reported to be effective in inhibition of PAF effects (14, 15), did not significantly influence IL-1-induced protection in comparison with pretreatment with vehicle (shown for 30 mg/kg in Fig. 3A). The same doses of WEB 2170 per se did not affect survival in comparison with vehicle treatment (data not shown).

Next, we investigated the role of leukotrienes in IL-1-induced protection from infection by administration of MK-886, an inhibitor of lipooxygenase translocation. MK-886 (10, 30, or 150 mg/kg) was administered orally 1 h before i.p. injection of 800 ng of IL-1. Such dosage schedules (reported to be effective in inhibition of leukotriene effects [45]) did not reduce survival in comparison with pretreatment of mice with vehicle. Survival of mice treated with different doses of MK-886 did not differ significantly from that of mice treated with vehicle (shown for 30 mg/kg in Fig. 3B).

Pretreatment with indomethacin, ibuprofen, or acetylsalicylic acid administered orally or intraperitoneally in doses reported to completely inhibit cyclooxygenase effects (5, 45, and 220 mg/kg, respectively) (21) did not affect IL-1-induced enhanced survival. Survival after administration of these cyclooxygenase inhibitors per se did not differ from survival after pretreatment with vehicle (20 mice per group; data not shown).

**DISCUSSION**

In the present study, we investigated the potential role of TNF-α, GM-CSF, platelet-activating factor, and arachidonic acid metabolites in IL-1-induced enhanced resistance to infection. These mediators are induced by IL-1 (2, 3, 8, 26, 29) and potentially increase host resistance to infection. In the experiments presented here, we found that only murine TNF-α gave protection and that administration of GM-CSF, inhibitors of PAF, inhibitors of lipooxygenase, and inhibitors of cyclooxygenase did not influence survival rates.

Although TNF pretreatment has been shown to enhance resistance to bacterial, fungal, viral, and protozoal infections (7, 25, 31), administration of human TNF-α in our infection model in doses expected to be effective in vivo (25) did not mimic the protective effect of IL-1. Since many effects of TNF-α have been shown to be species specific (20), we assessed the effect of murine rTNF-α. Pretreatment with 800 ng of murine TNF-α was found to give protection to about the same degree as 80 ng of rhIL-1. The difference in protection between human and murine TNF-α in mice could indicate that the protective effects are predominantly mediated via the murine type II TNF receptor, since this receptor has a much stronger specificity for murine TNF-α than does the type I TNF receptor (20).

Not only murine TNF-α pretreatment but also pretreatment with a high dose of anti-TNF-α immunoglobulin was shown to induce a dose-related level of protection against a lethal P. aeruginosa challenge. This level of protection could be enhanced by coadministration of a suboptimal dose of IL-1. A higher dose of anti-TNF-α (400 μg) induced a level of protection similar to that induced by 800 ng of IL-1, and combined treatment did not further increase survival rates. This suggests that IL-1 and anti-TNF-α exert their protective effect through a similar pathway and that this pathway is already maximally affected by 400 μg of anti-TNF-α or 800 ng of IL-1. Since the levels of protection induced by this high dose of anti-TNF-α were similar when it was administered at 27 or 3 h before infection, it is likely that the mode of action is interference with deleterious effects of excess TNF-α during the lethal cytokinemic phase of the infection (35, 36). In fact, in neutropenic rats a protective effect of an anti-TNF-α MAb against infection with P. aeruginosa has been shown (27).

The small amount of endotoxin contamination of our anti-TNF-α preparation could interfere with the lethal cytokinemic phase of infection by decreased cytokine production or by downregulation of cyto-
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infection is not mediated by induction of GM-CSF, a role that mouse TNF-α as well as anti-TNF-α given at -24 h gives protection should probably be explained in a similar fashion. TNF pretreatment may enhance host defense mechanisms like phagocyte activation and induction of acute-phase proteins (31, 33, 34). It may also, like anti-TNF-α and IL-1, protect the host by interference with lethal cytokinemia during infection, either by desensitization of target cells to the toxic effects of cytokines, by inducing refractoriness of cytokine-producing cells resulting in reduced production after infection, or by induction of TNF-binding proteins (9, 16, 19, 42). Administration of a low dose of anti-TNF-α, which by itself did not enhance survival, seems to diminish IL-1-induced protection. This result, combined with the observation of a moderate protective effect of pretreatment with rmTNF-α, suggests that endogenously produced TNF-α contributes to the protective effect of IL-1.

GM-CSF would be a good candidate as a mediator of IL-1-induced protection (5). Reports on enhancement of antimicrobial resistance by GM-CSF in vitro are numerous (11). In vivo protective effects of pretreatment with low doses of GM-CSF have been shown in experimental models of bacterial as well as viral infections (10, 17), but this is not a universal finding (24). In addition to stimulating hematopoiesis, GM-CSF is able to activate mature phagocytes and induces several secondary mediators (11, 23, 30, 32). IL-1 might give protective effect, via GM-CSF or directly, by activating macrophages, since 24 h after a single dose of GM-CSF (1 μg) macrophages are still activated (25). Our finding of a lack of protective effect of GM-CSF administration argues against a role of GM-CSF as a mediator of IL-1-induced enhanced resistance, although for conclusive evidence additional studies with inhibitors of endogenous GM-CSF would be necessary.

Studies showing a lack of effect of inhibitors of PAF (14) and leukotrienes (12) administered prior to IL-1 argue against a role of these lipid mediators in IL-1-induced enhanced resistance to infection.

In an extension of previous experiments (36), inhibition of induction of prostaglandins and thromboxanes by IL-1 by pretreatment with several cyclooxygenase inhibitors did not affect IL-1-induced resistance to infection, providing additional evidence against a role of cyclooxygenase products.

We conclude that the protective effect of pretreatment of neutropenic mice with a low dose of IL-1 in a lethal P. aeruginosa infection is not mediated by induction of GM-CSF, PAF, leukotrienes, or cyclooxygenase products, but that TNFα is likely to contribute to IL-1-induced protection.

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REFERENCES

Cytokines in radioprotection. Comparison of the radioprotective effects of IL-1 to IL-2, GM-CSF and IFN-γ. Lymphokine Res. 5:S105-S110.


