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 effect on bacterial counts argues against this mechanism in our infection model (36). The protective effect of IL-1 against infectious challenges in granulocytopenic mice indicates that neutrophils do not play a major role (18, 37, 41). Also, glucocorticosteroids and cytokines like IL-6 and IL-8 do not mediate IL-1-induced enhanced resistance (36-41). Recently, we showed that the protective effect of IL-1 in normal as well as in neutropenic mice is mediated to some extent by hepatic acute-phase proteins (38).

In the present study, the mechanisms of IL-1-induced nonspecific resistance have been further investigated, with special attention to other inflammatory mediators induced by IL-1.

IL-1 generates production of tumor necrosis factor alpha (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in vitro as well as in vivo (26, 29). TNF-α possesses several potentially protective effects on the host resistance to pathogens, many of which are shared with IL-1 (4, 34, 41). GM-CSF has, in addition to its effects on hematopoiesis, potent stimulating effects on macrophage and neutrophil functions, and it induces several inflammatory mediators (43).

In this study, we have investigated the role of TNF-α, GM-CSF, PAF, lipooxygenase, and cyclooxygenase products in
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% CO₂ in air at 90% relative humidity, TNF-mediated cytotoxic 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, rhTNF-α, rmTNF-α, or control treatment (heat-inactivated IL-1) was administered intraperitoneally (i.p.) (0.1 ml) to neutropenic 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 hIL-1β were administered i.p. to neutropenic 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 rhTNF-α 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 × 10⁴ 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...
FIG. 1. (A) Survival of neutropenic mice pretreated i.p. with different doses of rhIL-1β or rhTNF-α 24 h before a lethal i.m. P. aeruginosa challenge. Significantly greater protection in comparison with control mice (receiving heat-inactivated IL-1β) occurs in the groups pretreated with 80 or 800 ng of IL-1β (χ² = 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-1β or rmTNF-α 24 h before a lethal i.m. P. aeruginosa challenge. Significantly greater protection in comparison with control mice (receiving heat-inactivated IL-1β) occurs in the groups pretreated with 80 or 800 ng of IL-1β (χ² as in panel B) and with 800 ng of rmTNF-α (χ² = 4.81 and \( P < 0.05 \); 30 mice per group). Horizontal axes, time (hours) postchallenge.

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 μg of anti-TNF-α at 27 h before the infectious challenge did not differ significantly from the survival after the same dose of anti-TNF-α given at −3 h, and anti-TNF-α 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-α at −27 h combined with a suboptimal dose (400 ng) of IL-1 at −24 h enhanced survival rates compared with those with anti-TNF-α or IL-1 separately (Fig. 2A, shown for 15 μg per mouse). Anti-TNF-α (400 μg) administered i.v. at 27 h before infection induced protection similar to that induced by 800 ng 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-α (5 μg i.v.) consistently tended to reduce the protective effect induced by 800 ng of IL-1 i.p. at −24 h. Although the difference between the effects of IL-1 with anti-TNF-α and with IgG did not reach statistical significance, the addition of anti-TNF-α abolished the significant difference of IL-1 treatment from the controls (Fig. 2C).

FIG. 2. (A) Survival of neutropenic mice pretreated i.v. with 15 μg of the anti-TNF-α antibody V1q or the same amount of control IgG at 27 h and/or treated i.p. with 400 ng of rhIL-1β or control substance (heat-inactivated IL-1β) at 24 h before a lethal i.m. P. aeruginosa challenge. Significant differences in survival between mice treated with IgG plus heat-inactivated IL-1β, mice treated with IgG plus IL-1 (χ² = 5.68 and \( P < 0.025 \)), and mice treated with anti-TNF-α plus IL-1 (χ² = 8.79 and \( P < 0.005 \); 10 mice per group) are present. (B) Survival of neutropenic mice pretreated i.v. with 400 μg of the anti-TNF-α antibody V1q or the same amount of control IgG at 27 h and/or treated i.p. with 800 ng of rhIL-1β or control substance (heat-inactivated IL-1β) at 24 h before a lethal i.m. P. aeruginosa infection. Significant differences in survival between mice treated with IgG plus heat-inactivated IL-1β, mice treated with IgG plus IL-1 (χ² = 12.09 and \( P < 0.005 \); 29 to 31 mice per group) are present. (C) Survival of neutropenic mice pretreated i.v. with 5 μg of the anti-TNF-α antibody V1q or the same amount of control IgG at 27 h and/or treated i.p. with 800 ng of rhIL-1β or control substance (heat-inactivated IL-1β) at 24 h before a lethal i.m. P. aeruginosa infection. Although survival of mice treated with rhIL-1β plus anti-TNF-α is consistently lower than that of mice treated with rhIL-1β plus control IgG, these differences did not reach significance. However, no significant difference in survival between mice treated with anti-TNF-α plus heat-inactivated IL-1β and mice treated with anti-TNF-α plus IL-1 is present, whereas the difference in survival between mice treated with IgG plus heat-inactivated IL-1β and mice treated with IgG plus IL-1 is significant (χ² = 12.09 and \( P < 0.001 \); 18 to 20 mice per group).
Effect of GM-CSF compared with IL-1-induced protection from infection. 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 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 cytokine-medic 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 not explain its protective effect, but the serum half-life of the antibody of about 24 h (7) is sufficient to obtain such an effect in the experimental model used. The way IL-1 treatment could interfere with the lethal cytokine phase of infection is by decreased cytokine production or by downregulation of cyto-
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Aeruginosa would be necessary. We thank G. Poelen and T. van de Ing for their technical assistance. Our finding of a lack of protective effect of secondary mediators (11, 23, 30, 32). IL-1 might give protection. This result, combined with the observation of a moderate protective effect of pretreatment with anti-TNF-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 protection, 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 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 TNF-α is likely to contribute to IL-1-induced protection.

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