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.

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). An in vivo role in antimicrobial host resistance has been shown for both TNF-α and GM-CSF (31, 32, 41).

In addition to these cytokines, IL-1 administration induces production of eicosanoids like platelet-activating factor (PAF) (2) and arachidonic acid (8), the latter resulting in production of a variety of lipoxygenase and cyclooxygenase metabolites. Several effects of these mediators might be beneficial to the host in the enhancement of nonspecific resistance to infection (2, 8, 13, 41). In this study, we have investigated the role of TNF-α, GM-CSF, PAF, lipoxygenase, and cyclooxygenase products in...
IL-1-induced enhanced resistance of neutrophvic mice to lethal gram-negative infection.

(Materials and Methods)

Mice. Female outbred Swiss mice (20 to 25 g; TNO, Rijsjivk, The Netherlands) were kept under specific-pathogen-free conditions. Standard irradiated lab chow and aeridified water were available ad libitum. To render mice neutrophvic (<0.5 × 10⁹ 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. Ghia (Selavo, Siena, Italy). According to the specifications of the suppliers, endotoxin contamination in the Limulus amoebocyte lysate assay was negligible (<1.53 and <1.2 ng/mg, respectively). Human and murine rTNF-α were generously donated by G. Adolf, Ernst-Boehringer Institut, Vienna, Austria. Specific activities were 6 × 10⁷ and 1.2 × 10⁷ U/mg in murine LM cells, and endotoxin contents were <100 and <51 pg/mg, respectively. Murine rGM-CSF (rmGM-CSF; a kind gift of I. Lindley, Sandoz, Vienna, Austria) had a specific activity of 6 × 10⁷ U/mg in the murine bone marrow cell proliferation assay, and endotoxin content was 23 pg/mg. Cytokines were stored undiluted at −70°C and were diluted immediately before use in pyrogen-free isotonic phosphate-buffered saline (pH 7.4). Inactivated IL-1 was prepared by heating IL-1 at 95°C for 30 min. To all these solutions, normal mouse serum was added to an end concentration of 2% (vol/vol). WEB 2170, a specific competitive PAF receptor antagonist (14), was donated by F. Pistel, Boehringer Ingelheim, Ingelheim, Federal Republic of Germany. MK-886, a 5-lipoxygenase translocation inhibitor (12), was provided by J. W. Gillard, Merck Frosst Canada Inc., Quebec, Canada. WEB 2170 and MK-886 were dissolved in 0.5% Tylose solution, freshly prepared by dissolving carboxymethyl cellulose (Tylose; Hoechst, Frankfurt, Federal Republic of Germany) in pyrogen-free distilled water. Indomethacin (Holland Pharmaceuticale Supply, Alphen aan de Rijn, The Netherlands), acetylsalicylic acid (Genpharma, Maarssen, The Netherlands) and ibuprofen (Boots Pharmaceuticals, Nottingham, United Kingdom) were dissolved in pyrogen-free saline. Gentamicin was purchased from Schering, Kenilworth, N.J. Cyclophosphamide (ASTA Pharma, Frankfurt, Federal Republic of Germany) was dissolved in sterile pyrogen-free distilled water. Rat immunoglobulin G (IgG), containing 35 pg of endotoxin per mg, and lipopolysaccharide (LPS, from Escherichia coli serotype O55: B5) were from Sigma Chemical Co., St. Louis, Mo.

The hybridoma producing the neutralizing rat anti-murine TNF-α monoclonal antibody (MAb) V1q (7) was kindly provided by P. Kramer, German Cancer Research Center, Heidelberg, Federal Republic of Germany. The rat MAb IgG2a(x) 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 amoebocyte lysate assay) was less than 8.2 pg/mg of protein. In the L929 bioassay (1), the rmTNF-α neutralizing activity was 175 U/µg. 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% 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 neutrophvic mice 24 h before a lethal intramuscular (i.m.) challenge with P. aeruginosa at zero time. 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 neutrophvic 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 × 10⁸ 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
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. 1.** (A) 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 ($x^2 = 7.74$ 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-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 ($x^2 = 4.81$ 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 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 ($x^2 = 5.68$ and $P < 0.025$), and mice treated with anti-TNF-α plus IL-1 ($x^2 = 8.79$ 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 ($x^2 = 12.09$ 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 ($x^2 = 12.09$ and $P < 0.001$; 18 to 20 mice per group).
Survival was not affected by 0.5, 1, 2, 5, or 10 μg of uosu. The same doses of WEB 2170 per se did not affect survival in comparison with vehicle pretreatment 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 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 cytokinemic phase of infection is by decreased cytokine production or by downregulation of cyto-

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**FIG. 3.** (A) Survival of neutropenic mice treated orally with 30 mg of WEB 2170 per kg or with vehicle (carboxymethyl cellulose [CMC]) at 25 h and injected 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 challenge. Survival of IL-1-treated mice pretreated with WEB 2170 or vehicle is significantly enhanced in comparison with the survival of mice that received vehicle followed by heat-inactivated IL-1β (χ² = 7.43 and 14.88 and P < 0.01 and P < 0.0005, respectively). The survival of IL-1-treated mice after pretreatment with WEB 2170 does not differ significantly from that of vehicle-pretreated mice (χ² = 1.44 and P > 0.2; 29 and 30 mice per group). (B) Survival of neutropenic mice treated orally with 30 mg of MK-886 per kg or with vehicle at 25 h and 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 challenge. Survival of IL-1-treated mice pretreated with MK-886 or vehicle is significantly enhanced in comparison with the survival of mice that received vehicle followed by heat-inactivated IL-1β (χ² = 7.72 and 13.27 and P < 0.01 and P < 0.0005, respectively). The survival of IL-1-treated mice after pretreatment with MK-886 does not differ significantly from that of vehicle-pretreated mice (χ² = 1.44 and P > 0.2; 23 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; data 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).

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 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 cytokinemic phase of infection is by decreased cytokine production or by downregulation of cyto-

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kine receptors resulting in reduced effects of the cytokine overshoot (42, 44). Studies on such IL-1 effects are currently in progress. The paradox 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 (23). 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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