Interleukin-1 (IL-1)-Induced Resistance to Bacterial Infection: Role of the Type I IL-1 Receptor

MARIA T. E. VOGELS,1 WYNAND M. C. ELING,2 ANKE OTTEN,3 AND JOS W. M. VAN DER MEER*1

Department of Medicine,1 Department of Medical Microbiology,2 and Department of Medical Statistics,3 University Hospital, Nijmegen, The Netherlands

Received 19 December 1994/Returned for modification 10 February 1995/Accepted 22 May 1995

Pretreatment with a low dose of recombinant human interleukin-1β (IL-1β) (3 to 30 μg/kg) 24 h before a lethal Pseudomonas aeruginosa infection prolongs survival in neutropenic mice. We investigated the role of the type I IL-1 receptor (IL-1RI) and IL-1RII in this IL-1-induced protection by using a specific IL-1 receptor antagonist (IL-1Ra), which blocks effects mainly via IL-1RI. Pretreatment with IL-1Ra before IL-1 partially blocked the IL-1-induced enhanced survival, whereas pretreatment with a specific neutralizing monoclonal antibody to IL-1RI (35F5) eliminated the IL-1 induced protection. The nonapeptide fragment 163-171 of recombinant human IL-1β, which possesses the immunoadjuvant but not the inflammatory effect of the entire molecule via a non-receptor-mediated signal transduction process, did not reproduce the IL-1-induced protection. IL-1-induced protection was associated with reduced serum aspartate aminotransferase and alanine aminotransferase concentrations in conjunction with ameliorated histopathology of the liver. These findings may be due to reduced cytokine production and cytokine sensitivity of target cells after infection. We conclude that the IL-1-induced nonspecific resistance to infection is mediated by cells bearing IL-1RI and is associated with a reduction of liver damage.

Administration of the proinflammatory cytokine interleukin-1 (IL-1) leads to enhanced nonspecific resistance to gram-positive and gram-negative bacteria, fungi, and plasmodia (27). For example, we have shown that pretreatment with a single low dose (3 to 30 μg/kg) of recombinant human IL-1β (rhIL-1β) 24 h before a lethal gram-negative infectious challenge with Klebsiella pneumoniae or Pseudomonas aeruginosa enhances the survival of normal and neutropenic mice, respectively (19, 20). The mechanism of this protection has been only partially clarified. A direct antimicrobial effect of IL-1 has been excluded in vitro (19), and whether IL-1 induces enhanced clearance of microorganisms in vivo is controversial (27). The protective effect of IL-1 against infectious challenges in granulocytopenic mice indicates that neutrophils do not play a major role (19, 27). Glucocorticosteroids, cicosanoids, or cytokines such as IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor are unlikely to mediate the IL-1-induced resistance to infection (22–26). However, IL-1-induced tumor necrosis factor alpha and IL-1-induced acute-phase proteins play some role as mediators of the IL-1 effect (22, 25).

Two receptors for IL-1, which differ in both size and tissue distribution, have been fully characterized (11, 16). The type I IL-1 receptor (IL-1RI) is an 80-kDa protein present on T lymphocytes, endothelial cells, fibroblasts, and hepatocytes, and IL-1RII is a 68-kDa protein found on B lymphocytes, monocytes, and neutrophils (5). In the present study, we have investigated the roles of IL-1RI and IL-1RII in the IL-1β-induced protection against infection in mice by using neutralizing monoclonal antibodies to murine IL-1RI and IL-1RII, respectively (5, 7). We also used the IL-1 receptor antagonist (IL-1Ra), a naturally occurring 25-kDa glycoprotein belonging to the IL-1 gene family, which blocks IL-1 effects by specifically binding mainly to IL-1RI without agonist activity (1). To further elucidate the role of IL-1 receptors, we investigated whether the immunostimulatory 163-171 nonapeptide fragment of human IL-1β, which acts via a non-receptor-mediated signal transduction pathway (3, 4), exerts protective activity.

Recently, we demonstrated that the IL-1β-induced protection against infection is associated with reduced concentrations of tumor necrosis factor alpha and IL-6 in plasma during infection together with an increased level of mRNA for the anti-inflammatory IL-1α (25). In the present study, we have also investigated whether this IL-1-induced modulation of cytokine responses is reflected in a decrease in liver damage.

MATERIALS AND METHODS

Mice. Female outbred Swiss mice (weight, 20 to 25 g; TNO, Rijswijk, The Netherlands) were kept under specific-pathogen-free conditions. Standard irradiated lab chow (RMH-TM; Hope Farms, Woerden, The Netherlands) and acidified water were available ad libitum.

Materials. rhlL-β was generously provided by P. Graber (Glaxo, Greenen, Switzerland). Human IL-1α (1) was donated by J. Vannice, Synergen, Boulder, Colo. Inactivated rhlL-β and IL-1α (control treatments) were prepared by heating rhlL-β and IL-1α at 95°C for 30 min. For in vivo administration, rhlL-β and IL-1α, and the control proteins were dissolved in pyrogen-free phosphate-buffered saline (pH 7.4) with 2% (vol/vol) mouse serum.

35F5, a rat immunoglobulin G1 (IgG1) anti-mouse IL-1RI monoclonal antibody (5), was a kind gift from R. Chizzonite, Hoffmann-La Roche, Nutley, N.J. As a control antibody, a purified rat IgG1 (I-431) was obtained from Sigma Immunochemicals (St. Louis, Mo.). ALVA-42, a murine IgG1 monoclonal antibody blocking the murine IL-1RI (7), and the IL-1β peptide 163-171 (4) were donated by P. Ghiaia (Sclavo, Siena, Italy). Purified mouse IgG1 (I-5381), used as control antibody for ALVA-42, was obtained from Sigma.

Survival experiments. Since P. aeruginosa (ATCC 27853) does not kill normal mice, mice were rendered neutropenic (<0.5 × 10^9 granulocytes per liter) by two subcutaneous injections of cyclophosphamide (ASTA Pharma, Frankfurt, Germany) on days 4 and 1 before challenge (150 and 100 mg/kg, respectively). On day 1 before challenge, 800 ng of human IL-1β or control treatment was injected intraperitoneally (i.p.) in 0.1 ml; 24 h later (time zero), a lethal challenge with 0.5 × 10^7 to 1 × 10^7 CFU of P. aeruginosa was given intramuscularly in the left thigh muscle. Six hours after the 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.
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Liver enzymes. For assessment of the effect of IL-1 on liver enzymes, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels in non-neutrophilic EDTA-plasma from IL-1-pretreated and control neutrophilic mice were measured 24 h after intramuscular \textit{P. aeruginosa} infection. ASAT and ALAT levels were assessed by routine procedures (BM/Hitachi 747; Boehringer Mannheim, Mannheim, Germany).

Statistical analysis. Survival data were analyzed by use of the log rank test (14). Comparisons of biochemical and histopathologic data between treatment groups were made by the Kruskal-Wallis test. \( P \) values of less than 0.05 were considered significant.

RESULTS

Effect of IL-1Ra and IL-1RI and IL-1RII antibodies on IL-1-induced protection against infection. To investigate the involvement of IL-1RI and IL-1RII in the IL-1-induced enhanced resistance to infection, IL-1Ra, which inhibits IL-1 effects mainly via IL-1RI (10), was administered in a 100 to 1,000-fold molar excess over IL-1. Doses of IL-1Ra varying from 40 to 250 \( \mu \)g administered i.p. 15 min before 800 ng of IL-1 i.p., analogous to the regimen found to be effective by others (10), did not significantly reduce the IL-1-induced protection of neutrophilic mice against a lethal \textit{P. aeruginosa} challenge (not shown). After 800 \( \mu \)g of IL-1Ra, a 1,000-fold molar excess over IL-1, there was a trend to reduction of the IL-1-induced protection, although the difference between the IL-1-treated group given a control pretreatment and that given IL-1Ra pretreatment did not reach significance (Fig. 1A). These results suggest a potential role for IL-1RI in the IL-1-induced protection against infection. IL-1Ra, however, is not completely specific for IL-1RI, since it also binds to the murine IL-1RII, albeit with a much lower affinity (10, 11). Therefore, complete inhibition of the IL-1 effect by using even higher doses of IL-1Ra would not allow one to distinguish between a complete effect via IL-1RI due to more effective blockade and an additional IL-1 effect via IL-1RII.

In an alternative approach to establish the possible role of IL-1RI or IL-1RII, we performed experiments with 35F5, a specific neutralizing rat anti-murine IL-1RI monoclonal antibody (5), and ALVA-42, a monoclonal antibody directed against the murine IL-1RII (2, 7).

Pretreatment with 35F5 (200 \( \mu \)g i.p.), 4 h before IL-1, was almost fully effective, reducing significantly the IL-1-induced enhanced survival (\( \chi^2 = 5.92; P < 0.025 \)) to nonsignificant protection (\( \chi^2 = 2.53; P = 0.12 \)) (12 mice per group) (data not shown). When the same i.p. dose of 35F5 was administered 18 h before IL-1, it abrogated the IL-1-induced protection, corroborating the data obtained with IL-1Ra (Fig. 1B). Survival after pretreatment of control-pretreated infected mice with 200 \( \mu \)g of either rat IgG or 35F5 did not differ significantly from survival after pretreatment with saline, and therefore only results for the saline-pretreated group are presented in Fig. 1B.

When pretreatment with either 35F5 or ALVA-42 was performed within one experiment, the complete inhibition of the IL-1 effect by 200 \( \mu \)g of 35F5 administered 18 h before IL-1
IL-1-induced signal transduction is mediated exclusively via IL-1RI, as reported by Sims et al. (17). The key role of IL-1RI is corroborated by the recent paper of Neta et al. (12) finding that IL-1RI-bearing cells play a role in the IL-1-induced protection against infection. The exact mechanism of the IL-1-induced protection of liver hepatocytes and cells in other organs remains to be elucidated. An excess of cytokines and more distal inflammatory mediators such as leukotrienes, platelet-activating factor, nitric oxide, and superoxide radicals produced during lethal infection may be responsible for many pathologic phenomena. Recently, we were able to show that IL-1 pretreatment reduces cytokine production (25), and a role of this mechanism in the amelioration of liver histopathology (i.e., less glycogen depletion, fatty degeneration, and cell necrosis) (21, 21a). Such protection is reminiscent of the IL-1-induced protection against acetaminophen-induced hepatotoxicity (15).

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In conclusion, the IL-1-induced protection against P. aeruginosa infection in neutropenic mice seems to be mediated via IL-1RI. The nonapeptide fragment of rhIL-1β is not protective. The IL-1-induced protection is associated with a significant reduction in biochemical signs of hepatoocyte damage, possibly reflecting the IL-1-induced reduction of cytokine overshoot during infection.


18. Martel-Pelletier, J., R. McCoilum, and J. P. Pelletier. 1993. The synthesis of IL1 receptor antagonist (IL-1Ra) by synovial fibroblasts is markedly increased by the cytokines TNF and IL-1. Biochim. Biophys. Acta 1175:302-305.


