Interleukin-11 attenuates pulmonary inflammation and vasomotor dysfunction in endotoxin-induced lung injury

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Sheridan, Brett C., Charles A. Dinarello, Daniel R. Meldrum, David A. Fullerton, Craig H. Selzman, and Robert C. McIntyre, J r. Interleukin-11 attenuates pulmonary inflammation and vasomotor dysfunction in endotoxin-induced lung injury. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L861–L867, 1999.—Interleukin (IL)-11, like other members of the gp130 receptor class, possesses anti-inflammatory properties. We hypothesized that IL-11 pretreatment would attenuate endotoxin [lipopolysaccharide (LPS)]-induced lung inflammation and diminish injury to endothelial-dependent and -independent mechanisms of pulmonary vasorelaxation that require cGMP in Sprague-Dawley rats. LPS (20 mg/kg ip) increased lung tumor necrosis factor (TNF-α) compared with the saline control (0.7 ± 0.15 ng/g lung wt for control vs. 3.5 ± 0.09 ng/g lung wt for LPS; P < 0.05). IL-11 (200 mg/kg ip) injected 10 min before LPS administration attenuated the LPS-induced lung TNF-α levels (1.6 ± 0.91 ng/g lung wt; P < 0.05 vs. LPS). IL-11 also diminished LPS-induced lung neutrophil sequestration as assessed by myeloperoxidase units (2.1 ± 0.25 U/g lung wt for saline and 15.6 ± 2.02 U/g lung wt for LPS vs. 7.07 ± 1.65 U/g lung wt for LPS plus IL-11; P < 0.05). Similarly, TNF-α binding protein (175 mg/kg) attenuated LPS-induced myeloperoxidase activity (6.04 ± 0.14 U/g lung wt; P < 0.05). Both IL-11 and TNF-α binding protein similarly attenuated LPS-induced endothelium-dependent vasomotor dysfunction with improved relaxation responses to 10−7 and 10−6 M acetylcholine and A-23187 in phenylephrine-preconstricted isolated pulmonary artery rings (P < 0.05 vs. LPS). Endothelium-independent relaxation responses to sodium nitroprusside were also improved after LPS at 10−6 M (P < 0.05 vs. LPS). Moreover, IL-11 decreased endotoxin-induced mortality in CF1 mice from 90 to 50% (P < 0.05 vs. LPS). Therefore, IL-11 prevents LPS-induced lung TNF-α production, neutrophil sequestration, and pulmonary vasomotor dysfunction. We conclude that IL-11 possesses anti-inflammatory activity that protects against LPS-induced lung injury and lethality.

tumor necrosis factor-α; neutrophil; lung myeloperoxidase; guanosine 3',5'-cyclic monophosphate; gp130

ENDOTOXIN [lipopolysaccharide (LPS)]-induced lung injury is associated with increased production of tumor necrosis factor (TNF)-α (6, 7, 28, 47) and sequestered pulmonary neutrophils (36). This LPS-induced lung inflammation results in pulmonary vascular endothelial and smooth muscle injury (24, 36, 38). Injury of pulmonary vascular endothelium and smooth muscle after LPS impairs endothelium-dependent and -independent mechanisms of vasorelaxation that require the production of cGMP (14). This experimental injury in rats appears to be mediated, in part, by lung sequestration of neutrophils because neutrophil depletion prevents LPS-induced histological injury and vasomotor dysfunction (36). Interleukin (IL)-11, a multifunctional cytokine that stimulates the gp130 transmembrane-receptor subunit (32), is biologically related to IL-6, leukemia inhibitory factor, oncostatin-M, ciliary neurotrophic factor, and cardiopentin-1 (2, 9, 13, 31, 49). IL-11 is currently administered to thrombocytopenic patients as a hematopoietic stimulant (16); however, it also reduces murine circulating TNF-α (42) and alveolar macrophage TNF-α production after LPS treatment (34). Consistent with these anti-inflammatory observations, IL-11 treatment also attenuates swine mortality in staphylococcal enterotoxin-induced toxic shock (1).

We hypothesized that IL-11 would attenuate LPS-induced pulmonary TNF-α production, neutrophil accumulation, and impairment of endothelium-dependent and endothelium-independent cGMP-mediated pulmonary vasorelaxation. To study pulmonary vasomotor function, we examined the effect of IL-11 on the LPS-induced impairment of the following vasorelaxation mechanisms: 1) endothelium-dependent, receptor-dependent cGMP-mediated vasorelaxation [response to acetylcholine (ACh)], 2) endothelium-dependent, receptor-independent cGMP-mediated vasorelaxation (response to A-23187), and 3) endothelium-independent relaxation by direct stimulation of smooth muscle soluble guanylate cyclase with sodium nitroprusside (SNP). To study the role of TNF-α in these LPS-induced parameters of lung injury, we examined the effect of TNF-α binding protein (TNFBP).

MATERIALS AND METHODS

Reagents. Standard reagents with the exception of A-23187 (Calbiochem, La Jolla, CA) were obtained from Sigma (St. Louis, MO). Fresh solutions were prepared daily with either deionized water or normal saline as the diluent.

Animal housing and acclimatization. Animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health [DHEW Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892]. Male Sprague-Dawley rats (Sasco, Omaha, NE) weighing 300–350 g were quarantined in quiet, humidified, light-cycled rooms for 2–3 wk before use. The rats were allowed ad libitum access to food and water throughout

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MPO activity = \( \frac{\Delta A_{460} \times 13.5}{\text{lung weight (g)}} \)

where \( \Delta A_{460} \) is the rate of change in absorbance at 460-nm light between 1 and 3 min after the initiation of the reaction (optical density/min) (15). The coefficient 13.5 was empirically determined such that 1 U of MPO activity is the amount of enzyme that will reduce 1 mmol peroxide/min (14, 36).

RESULTS

Lung TNF-\( \alpha \) levels. Two hours after LPS treatment, lung TNF-\( \alpha \) levels were reduced by 56% with IL-11 pretreatment. After a saline injection into control rats (Fig. 1), 0.67 \( \pm \) 0.15 ng/g lung wet wt of TNF-\( \alpha \) was present; in rats given LPS, the level of TNF-\( \alpha \) increased to 3.5 \( \pm \) 0.09 ng/g lung wet wt at 2 h (P < 0.05 vs. control level). If IL-11 was administered 10 min before LPS, measurable lung TNF-\( \alpha \) levels decreased to 1.6 \( \pm \) 0.91 ng/g lung wet wt (P < 0.05 vs. LPS and control values). IL-11 alone did not change lung TNF-\( \alpha \) levels relative to the control value (0.35 \( \pm \) 0.12 ng/g lung wet wt; P < 0.05 vs. LPS and IL-11 plus LPS values).
Lung neutrophil accumulation (MPO). Endotoxin also increased lung neutrophil accumulation as indirectly assessed with a marker of neutrophil presence, MPO. Similar to the TNF-\(\alpha\) response, IL-11 pretreatment diminished LPS-induced lung neutrophil accumulation (55%) as did TNFBP treatment (61%) (Fig. 2). After saline injection, lung MPO was 2.1 \(\pm\) 0.25 U/g lung wet wt; in rats given LPS, MPO increased to 15.6 \(\pm\) 1.02 U/g lung wet wt after 6 h (P < 0.05 vs. control value). Pretreatment with IL-11 10 min before LPS treatment attenuated lung MPO to 7.07 \(\pm\) 1.65 U/g lung wet wt (P < 0.05 vs. control and LPS values). Similar pretreatment with TNFBP resulted in attenuation of lung MPO, with a measured value of 6.04 \(\pm\) 0.14 U/g lung wet wt (P < 0.05 vs. control and LPS). IL-11 alone did not influence lung neutrophil accumulation (MPO 3.6 \(\pm\) 0.59 U/g lung wet wt).

Influence of IL-11 on cGMP-mediated pulmonary vasorelaxation. We next examined the effect of IL-11 on endothelium-dependent and -independent mechanisms of pulmonary vascular smooth muscle relaxation that require the generation of cGMP. Concentration-response curves to ACh, A-23187, and SNP were generated in pulmonary arteries from rats treated with IL-11 alone.

Administration of IL-11 did not influence cGMP-mediated vasorelaxation (data not shown) as assessed by evaluation of cumulative dose responses for EC\(_{50}\) or by an absolute vasorelaxation response. Neither endothelium-dependent [both receptor-dependent (ACh) and receptor-independent (A-23187)] nor endothelium-independent (SNP) pathways were different from those in control rats.

Effects of IL-11 or TNFBP on endotoxin-induced impairment of cGMP-mediated pulmonary vasorelaxation. Endotoxin administration produced significant impairment of endothelium-dependent, receptor-dependent cGMP-mediated pulmonary vasorelaxation (response to ACh). This impairment was attenuated with IL-11 administration by 52% at \(10^{-6}\) M ACh and by 77% with TNFBP. As shown in Fig. 3, control rings were preconstricted to 277 \(\pm\) 15 mg PE-induced tension and relaxed to 11 \(\pm\) 4 mg tension with \(10^{-6}\) M ACh. In LPS-treated rats preconstricted to 274 \(\pm\) 15 mg tension, 216 \(\pm\) 16 mg PE-induced tension remained in response to \(10^{-6}\) M ACh (P < 0.05 vs. control value). Rings from (IL-11 plus LPS)-treated rats were preconstricted with PE to 298 \(\pm\) 10 mg tension, with 104 \(\pm\) 12 mg tension remaining in response to \(10^{-6}\) M ACh. Rings from (TNFBP plus LPS)-treated rats were preconstricted with PE to 300 \(\pm\) 21 mg tension, with 60 \(\pm\) 17 mg tension remaining in response to \(10^{-6}\) M ACh. Thus (IL-11 plus LPS)- and (TNFBP plus LPS)-treated rats had an impaired response to ACh compared with control rats (P < 0.05). However, pretreatment with IL-11 (or TNFBP) before LPS produced markedly less dysfunction of cGMP-mediated pulmonary vasorelaxation in response to ACh than that in LPS alone-treated rats (P < 0.05).

Endothelium-dependent, receptor-independent cGMP-mediated pulmonary vasorelaxation (response to A-23187) was significantly less impaired in (IL-11 plus LPS)-treated rats compared with LPS alone-treated rats, with a 48% improved relaxation response at \(10^{-6}\) M A-23187 (Fig. 4). This same response was paralleled with TNFBP treatment before LPS, with a 72% improvement in the relaxation response at \(10^{-6}\) M A-23187. Control rats were preconstricted to 303 \(\pm\) 23 mg PE-induced tension and relaxed to 11 \(\pm\) 6 mg tension with \(10^{-6}\) M A-23187. In LPS-treated rats preconstricted to 281 \(\pm\) 15 mg tension, 185 \(\pm\) 26 mg PE-induced tension remained in response to \(10^{-6}\) M A-23187 (P < 0.05 vs. control value). Rings from (IL-11 plus LPS)-treated rats were preconstricted with PE to 304 \(\pm\) 18 mg tension with 96 \(\pm\) 9 mg tension remaining in response to \(10^{-6}\) M A-23187. Rings from (TNFBP plus LPS)-treated rats were preconstricted to 311 \(\pm\) 17 mg, with 51 \(\pm\) 14 mg tension remaining in response to
10−6 M A-23187. Thus (IL-11 plus endotoxin)- or (TNFB-P plus LPS)-treated rats had an impaired response to A-23187 compared with control rats ($P < 0.05$). However, [IL-11 (or TNFB-P) plus LPS]-treated rats had significantly less dysfunction of cGMP-mediated pulmonary vasorelaxation in response to A-23187 than LPS alone-treated rats ($P < 0.05$).

Endothelium-independent cGMP-mediated vasorelaxation by direct stimulation of soluble guanylate cyclase (response to SNP) was less impaired after IL-11 in LPS-treated rats (74%) compared with that in LPS alone-treated rats only at the single concentration of 10−6 M. Again, this response was paralleled with TNFB-P treatment before LPS, with a similar 74% improvement in the relaxation response at the same, single dose of SNP. As shown in Fig. 5, control rings were preconstricted to 298 ± 14 mg PE-induced tension and relaxed to 0 mg tension with 10−6 M SNP. In LPS-treated rats preconstricted to 299 ± 24 mg tension, 53 ± 10 mg PE-induced tension remained in response to 10−6 M SNP ($P < 0.05$ vs. control value). Rings from (IL-11 plus LPS)-treated rats were preconstricted with PE to 306 ± 15 mg tension, with 14 ± 4 mg tension remaining in response to 10−6 M SNP. Rings from (TNFB-P plus endotoxin)-treated rats were preconstricted with PE to 327 ± 22 mg tension, with 14 ± 7 mg tension remaining in response to 10−6 M SNP. Thus [IL-11 (or TNFB-P) plus LPS]-treated rats had an improved relaxation response at the level of the smooth muscle after LPS treatment only at the single concentration of 10−5 M SNP ($P < 0.05$).

Survival study. As shown in Fig. 6, IL-11 attenuated LPS-induced mortality in CF1 mice. The LPS-treated mice had a 90% mortality by 60 h. Endotoxin-injected mice pretreated with IL-11 experienced an overall mortality of 50% at 72 h, which did not change at 7 days. Survival differences were stable after 60 h. Thus overall LPS lethality was diminished by IL-11 treatment at 60 h ($P = 0.02$) and was sustained through 7 days ($P = 0.05$).

DISCUSSION

IL-11 is a member of the gp130 receptor subunit cytokine family; others include IL-6, leukemic inhibitory factor, oncostatin-M, cardiotrophin-1, and ciliary neurotrophic factor (33). First discovered in 1990, IL-11 has a variety of in vitro biological activities within the hematopoietic, lymphopoietic, hepatic, adipose, bone, gastrointestinal, and nervous systems (13, 48). In the present study, pretreatment with IL-11 attenuated LPS-induced lung inflammation as demonstrated by...
IL-11 attenuates LPS-induced acute lung injury

Fig. 6. Kaplan-Mier plot of survival in CF1 mice (10/group) after intraperitoneal LPS. ○, Mice pretreated with IL-11; ■, mice pretreated with saline before LPS. 7d, 7 Days. *χ² P values ≤ 0.05.

diminished pulmonary tissue TNF-α levels and neutrophil accumulation. This anti-inflammatory influence of IL-11 also diminished pulmonary vasomotor dysfunction. Specifically, IL-11 attenuated LPS-induced dysfunction of endothelial-dependent and -independent mechanisms of pulmonary vasorelaxation that require the generation of cGMP. These findings suggest that IL-11 inhibits the inflammatory cascade initiated by systemic LPS in the lung, with a concomitant decrease in pulmonary vascular endothelial and smooth muscle impairment. The effects of IL-11 also included reduced LPS-induced mortality in mice.

Several investigators have demonstrated beneficial effects of IL-11 in experimental sepsis. Pretreatment with IL-11 significantly reduced mortality in a murine model of toxic shock syndrome (1) and in experimental group B streptococcal sepsis in neonatal rats (5). In a rabbit model of endotoxemia, IL-11 pretreatment prevented hypotension and decreased gastrointestinal mucosal damage induced by LPS (30). The anti-inflammatory effects of IL-11 on both murine and rabbit models of endotoxemia appear to be due to inhibition of the production of proinflammatory mediators. Trepicchio et al. (43) demonstrated that IL-11 reduced circulating levels of proinflammatory cytokines such as TNF-α, IL-1β, and interferon-γ after endotoxin administration in a dose-dependent fashion in mice. Furthermore, these investigators found that in vitro treatment of peritoneal macrophages with IL-11 before an endotoxin challenge decreased TNF-α production by 60%.

The results of the present study demonstrate that LPS increases lung TNF-α levels and lung neutrophil accumulation in rats. These findings are associated with impairment of pulmonary vasorelaxation mechanisms and histological endothelial injury (14, 36). After LPS treatment, investigators (12, 17, 40) have observed increased steady-state levels of TNF-α mRNA in the murine macrophage. TNF-α levels rise with endotoxemia, with subsequent lung edema and neutrophil sequestration, increased protein extravasation, and histological evidence of alveolar damage in bovine and guinea pig models (19, 39). Other investigators (27) observed that administration of TNF-α alone to guinea pigs results in lung injury as determined by increased pulmonary arterial pressures, lung edema, and lung protein permeability. Others (21, 27, 46) have observed in sheep, guinea pigs, and rodents that TNF-α impairs endothelium-dependent mechanisms of vasorelaxation in the aorta and pulmonary artery. In the present study, pretreatment with TNFBP attenuated LPS-induced acute lung injury as measured by lung neutrophil accumulation and impairment of the mechanisms of cGMP-mediated pulmonary vasorelaxation. The present results support the role of TNF-α as a mediator of LPS-induced lung injury. The novel finding of this study is the observation that IL-11 appears to attenuate LPS-induced lung injury comparable to that of TNFBP. IL-11 substantially decreases lung TNF-α levels 2 h after LPS treatment. TNFBP and IL-11 similarly attenuate lung neutrophil accumulation and impairment of cGMP-mediated mechanisms of pulmonary vasorelaxation 6 h after LPS treatment. The data of the present study suggest that IL-11 has anti-inflammatory effects in a rat model of LPS-induced lung injury that may function through the attenuation or downregulation of TNF-α production.

IL-11 is characterized as a member of the IL-6 superfamily of proteins that share the gp130 transmembrane-receptor subunit. This superfamily of proteins includes leukemic inhibitory factor, oncostatin-M, cardiotrophin-1, and ciliary neurotrophic factor. The IL-6-receptor complex is composed of two distinct transmembrane molecules: 1) a ligand-binding subunit (IL-6R) and 2) a signal-transducing subunit (gp130) (22). Some members of the IL-6 family (IL-6 and IL-11) induce homodimerization of gp130 (20, 31), whereas others (leukemic inhibitory factor, oncostatin-M, and ciliary neurotrophic factor) induce heterodimerization, with the 190-kDa leukemic inhibitory factor receptor (9). After dimerization, these receptors activate the transcription factor nuclear factor-IL-6 via the Ras-mitogen-activated protein kinase cascade and activate the Janus kinase-signal transducer and activator of transcription signaling pathway (8, 23). Anti-inflammatory effects of gp130 signaling extends to other cytokines including IL-6. Leukemic inhibitory factor, cardiotrophin-1, and ciliary neurotrophic factor appear to blunt the inflammatory response after a stimulus. Specifically, pretreatment with these cytokines is associated with attenuation of LPS-induced TNF-α production (3, 4, 11, 35, 44). Whether IL-6 affords the same protection as IL-11 in acute lung injury remains unclear. However, IL-6 inhibits TNF-α and IL-1 production by mononuclear cells in vitro and reduces TNF-α release in endotoxemic mice in vivo (41, 45). The role of IL-6 in the pathogenesis of endotoxin- or TNF-α-induced inflammation appears to be limited (25, 26). Libert and colleagues (25, 26) found that IL-6 antibody and anti-IL-6-receptor antibody conferred protection to lethal doses of TNF-α and LPS. However, both antibodies failed to protect against higher doses of TNF-α and LPS. The anti-IL-6 antibody was unable to protect against TNF-α in mice sensitized by galactosamine, corticoid-receptor antagonist RU-
38486, or human IL-1β. Furthermore, protection did not correlate with serum concentrations of IL-6.

Alternative explanations for the LPS-attenuating effects of IL-11 include influences of IL-11 on circulating neutrophil counts, increased LPS clearance, and/or immunomodulation secondary to the species differences between the recombinant human IL-11 protein and the rodent and murine models. IL-11 has well-described effects on bone marrow. It appears that IL-11 acts synergistically with other early- and late-acting growth factors to stimulate various stages and lineages of hematopoiesis. IL-11 has been associated with megakaryocytopenia, thrombocytopenia, and erythropoiesis (13). Although in vivo IL-11 increases the cycling rates and absolute myeloid progenitors in both the bone marrow and spleen of normal mice (18), it has no effects on peripheral leukocyte counts when administered to normal rodents (50) and nonhuman primates (29). Increased LPS clearance could produce results similar to those reported. It is currently unknown whether IL-11 alters LPS clearance (i.e., upregulation of LPS binding protein, enhanced metabolism and/or excretion, and/or increased sequestration by neutrophils and/or macrophages). Because IL-11 is a recombinant human protein, it is theoretically possible that this cross-species exposure induces an immunomodulatory effect observed as attenuated lung TNF-α and neutrophil sequestration and diminished pulmonary vascular endothelial and smooth muscle injury.

The results of the present examination of LPS-induced lung injury contribute to the growing body of evidence that IL-11 is associated with attenuation of the inflammatory response. By blunting the proinflammatory response, IL-11 diminishes pulmonary vasomotor dysfunction in rats and ultimately improves survival in mice to a lethal LPS challenge.

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