Interleukin-18 Impairs the Pulmonary Host Response to Pseudomonas aeruginosa

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Interleukin-18 (IL-18) is a potent cytokine with many different proinflammatory activities. To study the role of IL-18 in the pathogenesis of Pseudomonas pneumonia, IL-18-deficient (IL-18−/−) and wild-type mice were intranasally inoculated with Pseudomonas aeruginosa. IL-18 deficiency was associated with reduced outgrowth of Pseudomonas in the lungs and diminished dissemination of the infection. In addition, pulmonary inflammation (histopathology) and levels of tumor necrosis factor alpha, IL-6, and macrophage inflammatory protein-2 in lungs and plasma were lower in IL-18−/− mice. Consistent with results obtained for IL-18−/− mice, treatment of wild-type mice with a neutralizing IL-18 binding protein-immunoglobulin G Fc fusion construct also attenuated outgrowth of Pseudomonas compared with that for mice treated with a control protein. These results demonstrate that the presence of endogenous IL-18 activity facilitates inflammatory responses in the lung during Pseudomonas pneumonia, concurrently impairing bacterial clearance.

Interleukin 18 (IL-18) was originally identified as a gamma interferon (IFN-γ)-inducing factor (IGIF) (25). IL-18 is mainly produced by activated macrophages and is first synthesized as a precursor protein (pro-IL-18), which requires splicing by IL-1β-converting enzyme (ICE, or caspase 1) to liberate the mature active protein (5, 8). IL-18 synergistically enhances IL-12-stimulated IFN-γ production (20) and promotes cell-mediated immunity (12, 20, 26, 35). Direct proinflammatory effects of IL-18 include activation of nuclear factor κB (NF-κB) (18), induction of cytokines such as tumor necrosis factor alpha (TNF-α), IL-1β, IL-6, and IL-8 (23, 26), and activation of neutrophils (16). Hence, IL-18 can be considered a pluripotent mediator with strong proinflammatory properties. Endogenous IL-18 activity is negatively regulated by IL-18 binding protein (IL-18BP). For human IL-18BP, four isoforms resulting from mRNA splicing have been described; of these, the a and b isoforms neutralize IL-18 with high affinity (11).

Several studies have implicated IL-18 as an important mediator in the innate immune response to bacterial infection. Plasma IL-18 levels are elevated in patients with severe sepsis (7, 15, 24), and such elevated circulating concentrations contribute to the development of a lethal systemic inflammatory response syndrome during endotoxic shock in mice (9, 22). In contrast to its apparent detrimental role during fulminant shock, IL-18 likely is required for an adequate antibacterial host defense, as indicated by reduced resistance of IL-18-deficient or -depleted mice to infections by Salmonella enterica serovar Typhimurium (17), Shigella flexneri (28), and Listeria monocytogenes (21). Recently, our laboratory studied the role of IL-18 in the pathogenesis of pneumonia caused by Streptococcus pneumoniae (14). Although survival of IL-18 gene-deficient (IL-18−/−) mice was not different from that of wild-type mice, the absence of IL-18 caused the host defense to deteriorate, as reflected by enhanced outgrowth of bacteria in the lungs of IL-18−/− mice relative to that in wild-type mice. Moreover, IL-18−/− mice were more susceptible for progression to systemic infection. While S. pneumoniae is the most common causative microorganism in community-acquired pneumonia (2), the pathogen most frequently involved in nosocomial pneumonia is the gram-negative bacterium Pseudomonas aeruginosa (3). The role of IL-18 in the pathogenesis of gram-negative bacterial pneumonia is unknown. Therefore, we compared host defense in IL-18−/− and wild-type mice during respiratory tract infection with P. aeruginosa.

MATERIALS AND METHODS

Animals. Female IL-18−/− mice (C57BL/6 background) (35) and normal C57BL/6 wild-type mice (Harlan, Horst, The Netherlands), 8 to 10 weeks old, were used in all experiments. The Institutional Animal Care and Use Committee of the Academic Medical Center approved all experiments.

IL-18BP-Fc construct. Recombinant human IL-18BP isoform a (kindly provided by Giorgio Sendi, Amgen Inc.) was produced as a fusion construct with human immunoglobulin G1 (IgG1) Fc as described previously (4). This construct, designated IL-18BP-Fc, binds and neutralizes human, mouse, and rat IL-18. At the dose given in the present study (5 mg/kg of body weight), IL-18BP-Fc prevented lipopolysaccharide (LPS)-induced IFN-γ release and lethality in mice; the inhibitory effect of IL-18BP-Fc on LPS-induced IFN-γ production was long-lasting, with >90% inhibition when IL-18BP-Fc was injected up to 6 days before LPS challenge (4). In the present investigation IL-18BP-Fc was given as a single intraperitoneal injection 2 h before induction of pneumonia at a dose of 5 mg/kg (100 μg). Purified human IgG1 (Nordic Immunology, Tilburg, The Netherlands) was used as a control.

Induction of pneumonia. Pneumonia was induced as described previously (31–33). P. aeruginosa (strain PA103 or strain PA01), grown to mid-logarithmic

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RESULTS

Induction of pneumonia and IL-18. Control mice had high levels of IL-18 in their lungs (Fig. 1). Although infection with P. aeruginosa slightly increased IL-18 concentrations in lung homogenates, the difference was not significant.

Inoculation with P. aeruginosa induced signs of pneumonia in all mice. Twenty-four hours after inoculation with P. aeruginosa, lungs appeared swollen and reddish, with multiple hemorrhages on the surface. Wet weights of lungs from wild-type mice inoculated with P. aeruginosa increased by more than 150% relative to weights of lungs from control mice inoculated with sterile saline (Fig. 2). IL-18−/− mice also demonstrated increases in wet lung weights after induction of Pseudomonas pneumonia, although the increases were smaller than those in wild-type mice (P < 0.05).

Inoculation with P. aeruginosa induced a diffuse pneumonia in all mice. At 24 h after inoculation with P. aeruginosa, lungs of wild-type mice displayed pneumonia characterized by a diffuse and heavy inflammatory infiltrate mostly composed of neutrophils. Endothelialitis was a prominent feature (Fig. 3A). In contrast, the inflammation in IL-18−/− mice was less severe and was limited to perivascular and interstitial inflammatory infiltrates (Fig. 3B).

Bacterial clearance. Next, we determined the role of endogenous IL-18 in the clearance of Pseudomonas from the pulmonary compartment. For this purpose, wild-type and IL-18−/− mice were inoculated with P. aeruginosa, and CFU were counted in lungs harvested after 24 h (Fig. 4). IL-18−/− mice had significantly fewer CFU in their lungs at 24 h after induction of pneumonia than wild-type mice (P < 0.05). In addition, the number of IL-18−/− mice that developed bacteremia was markedly lower than that of wild-type mice. At 24 h after infection, 30% of the IL-18−/− mice had blood cultures positive for P. aeruginosa, while 66.7% of the wild-type mice had bacteria in their blood.

Bacterial clearance in mice treated with IL-18BP–Fc. Compensatory immune mechanisms may develop in mice that genetically lack the IL-18 signaling pathway. To determine whether the differences between IL-18−/− and wild-type mice were caused solely by the absence of IL-18, we inoculated wild-type mice with P. aeruginosa 2 h after intraperitoneal injection of IL-18BP–Fc. The results of the experiments with IL-18−/− mice could be replicated in this experiment, i.e., IL-18BP–Fc treatment reduced the number of CFU recovered from lungs at 24 h postinfection relative to that observed after treatment with control IgG1 (Fig. 5).

Clearance of PA01 in IL-18−/− and wild-type mice. To determine whether the differences between IL-18−/− and wild-type mice were related to the Pseudomonas strain used in these experiments, we inoculated IL-18−/− and wild-type mice with P. aeruginosa strain PA01. The results of the experiments de-
scribed above could be replicated in this experiment, i.e., IL-18−/− mice had significantly fewer P. aeruginosa strain PA01 CFU in their lungs at 24 h after induction of pneumonia than wild-type mice (Fig. 6).

**Cytokine and chemokine levels.** Local production of cytokines and chemokines within the pulmonary compartment can influence antibacterial host defense mechanisms during pneumonia (19, 30). Therefore, we measured the concentrations of TNF, IL-6, and MIP-2 in lung homogenates after inoculation with P. aeruginosa (Fig. 7). TNF, IL-6, and MIP-2 levels were all significantly lower in lung homogenates from IL-18−/− mice than in those from wild-type mice (P < 0.05). High concentrations of TNF, IL-6, and MIP-2 in plasma were found for both IL-18−/− and wild-type mice, whether bacteremic or not. At 24 h after induction of pneumonia, higher concentrations of TNF and MIP-2 in plasma were found for wild-type mice than for IL-18−/− mice (P < 0.05) (Fig. 7). Local concentrations of IFN-γ, IL-12p40, and IL-12p70 in IL-18−/− mice were not statistically different from those measured in wild-type mice (data not shown).

**DISCUSSION**

In pneumonia, the initiation, maintenance, and resolution of inflammation involve expression of the complex network of proinflammatory and anti-inflammatory cytokines (19, 30).
Here we describe a series of experiments in which we evaluated the role of IL-18 in the innate immune response in the pulmonary compartment during pneumonia induced by *P. aeruginosa*. IL-18−/− mice were found to have increased resistance to *Pseudomonas* pneumonia, as reflected by fewer bacteria in lungs and reduced dissemination of infection, which was associated with a diminished inflammatory response upon histopathologic examination and suppressed local and systemic cytokine and chemokine concentrations. The enhanced antibacterial defense could be reproduced in normal wild-type mice treated with IL-18BP–Fc, which potently neutralizes IL-18 (4), indicating that compensatory immune mechanisms that could have developed in mice that genetically lack IL-18 are unlikely to be responsible for the present findings.

Notably, IL-18 was expressed constitutively in lungs of normal mice, confirming earlier reports (1, 14, 36), and IL-18 concentrations increased only marginally during pneumonia with *P. aeruginosa*. Similarly, a modest, nonsignificant rise in pulmonary IL-18 levels during pneumococcal pneumonia was recently reported (14). Nonetheless, in both the present and the previous investigation, IL-18 deficiency had a large impact on antibacterial defense in the pulmonary compartment. These findings suggest either that constitutively expressed IL-18 influences the innate immune response during respiratory tract infection or that the modest rise in IL-18 levels is biologically significant in the context of murine pneumonia.

We used strain PA103 because we were experienced in using this *Pseudomonas* strain in this acute pneumonia model. This strain is not a clinical isolate but a laboratory strain that produces large amounts of *Pseudomonas* exotoxin A and reduced amounts of proteins. Although we did not consider it possible that the differences found in the first series of experiments were caused by the characteristics of the bacterium (since both *IL-18−/−* and wild-type mice were infected with this strain), we determined clearance of *P. aeruginosa* strain PA01 (a clinical isolate). These additional experiments showed similar results, i.e., clearance of *P. aeruginosa* PA01 is hampered by IL-18.

The results of this study are in line with other reports demonstrating a detrimental role for proinflammatory cytokines in host defense during *Pseudomonas* pneumonia. Indeed, it was recently reported that mice deficient in either the type I TNF receptor or the IFN-γ receptor display enhanced bacterial clearance of *P. aeruginosa* (31, 33). Similarly, mice deficient in the type I TNF receptor demonstrated accelerated early clearance of *P. aeruginosa* from the lungs (34), whereas elimination of the anti-inflammatory cytokine IL-10 resulted in diminished bacterial outgrowth (29).

While proinflammatory cytokines seem to impair host defense against *P. aeruginosa*, they are important for host defense in murine pneumonia models with other pathogens. In experimental pneumonia with the gram-negative bacterium *Klebsiella pneumoniae* or the gram-positive bacterium *S. pneumoniae*, proinflammatory cytokines such as TNF and IL-1 (13, 27, 37) are important for the clearance of bacteria from the lungs, whereas the anti-inflammatory cytokine IL-10 impairs host defense in these models (6, 38). Importantly, it was recently demonstrated that IL-18 contributes to pulmonary host defense against *S. pneumoniae* pneumonia (14). A possible explanation for the differences between the pneumonia models with different pathogens includes differences in the extent and rapidity with which these strains induce inflammation in the lung.

Absence of endogenous IL-18 activity was associated with reduced levels of TNF, IL-6, and MIP-2 at 24 h postinoculation. The lower bacterial load in lungs of *IL-18−/−* mice (providing lower levels of proinflammatory stimuli) could have been responsible for this finding. However, IL-18 may also be involved in cytokine and chemokine production during pneumonia in a more direct way, considering that IL-18 is capable of stimulating the secretion of these mediators by different cells in vitro (23, 26). Further support for the latter possibility comes from the recent observation that neutralization of endogenous IL-18 reduced vascular leakage and production of TNF in the lung during immune complex alveolitis in rats (10). Importantly, IFN and IL-12 levels in wild-type mice and *IL-18−/−* mice were not different, indicating that the differences found in clearance of *P. aeruginosa* are not caused by a compensatory change in the production of IFN or IL-12.

In conclusion, we found increased bacterial clearance in *IL-18−/−* mice during pneumonia caused by *P. aeruginosa*. The difference from clearance in wild-type mice was associated with an attenuated inflammatory response. Together with earlier findings of diminished clearance of *S. pneumoniae* from the lungs of *IL-18−/−* mice (14), these data exemplify the complex role of IL-18 in innate immunity during pulmonary infection and may have important implications for the development and use of cytokine/anticytokine therapies in the future.

FIG. 7. Reduced local and systemic levels of TNF, IL-6, and MIP-2 at 24 h after inoculation in *IL-18−/−* mice. Both *IL-18−/−* (solid bars) and wild-type mice (open bars) were inoculated with 10^7 CFU of *P. aeruginosa*. Data are means ± SE; n = 9 to 10 mice per group. P < 0.05 for mice inoculated with bacteria versus mice inoculated with sterile saline for all three inflammation mediators (data not shown).
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