Role of interleukin-1 in the pulmonary immune response during Pseudomonas aeruginosa pneumonia

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Departments of 1Experimental Internal Medicine, 2Intensive Care Medicine, 3Pathology, and 6Infectious Diseases, Tropical Medicine, and AIDS, Academic Medical Center of Amsterdam, 1105 AZ Amsterdam, The Netherlands; 4Amgen, Incorporated, Thousand Oaks, California 91320; and 5University of Colorado Health Sciences Center, Denver, Colorado 80262

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Schultz, Marc J., Anita W. Rijneveld, Sandrine Florquin, Carl K. Edwards, Charles A. Dinarello, and Tom van der Poll. Role of interleukin-1 in the pulmonary immune response during Pseudomonas aeruginosa pneumonia. Am J Physiol Lung Cell Mol Physiol 282: L285–L290, 2002.—Pneumonia is associated with IL-1 in the pulmonary compartment. To study the role of IL-1 in the pathogenesis of Pseudomonas pneumonia, IL-1 receptor type 1 gene-deficient (IL-1R −/−) mice and wild-type mice were intranasally inoculated with Pseudomonas aeruginosa. The absence of the IL-1 signal attenuated the outgrowth of Pseudomonas in lungs, as reflected by an increasing number of colony-forming units (cfu) during pneumonia in wild-type mice and a concurrently decreasing number of cfu during pulmonary infection in IL-1R −/− mice (P < 0.05, IL-1R −/− mice vs. wild-type mice). Influx of neutrophils was decreased in bronchoalveolar lavage fluids in IL-1R −/− mice compared with wild-type mice. Similarly, lung levels of cytokines (tumor necrosis factor-α, IL-6) and chemokines (macrophage inflammatory protein-2 and KC) were lower in IL-1R −/− mice 24 h post-inoculation. Consistent with results obtained in IL-1R −/− mice, treatment of wild-type mice with IL-1R antagonist also diminished outgrowth of Pseudomonas when compared with wild-type mice treated with vehicle (P < 0.05). These results demonstrate that an absence or reduction in endogenous IL-1 activity improves host defense against Pseudomonas pneumonia while suppressing the inflammatory response.

INTERLEUKIN (IL)-1 is a potent proinflammatory cytokine that has been implicated in numerous physiological processes as well as inflammatory diseases (5). Evidence exists that IL-1 is an important mediator of pulmonary inflammation induced by bacteria and bacterial products. IL-1 is produced in lungs after intratracheal administration of lipopolysaccharide (LPS), and inhibition of IL-1 activity attenuates lung inflammation caused by LPS (28, 30). In addition, recombinant IL-1 causes neutrophilic infiltration in the lung comparable to LPS (29, 30). Elevated IL-1 levels have been found in pleural fluids of patients with empyema (25), and in patients with unilateral community-acquired pneumonia, significantly higher IL-1 concentrations have been measured in bronchoalveolar lavage fluids (BALF) from infected lungs, compared with BALF from noninvolved lungs or serum (4). Moreover, alveolar macrophages recovered from infected lungs spontaneously released more IL-1 into cell culture supernatants than macrophages evacuated from the non-involved lung (4).

IL-1 signaling is required for the containment of infections with intracellular microorganisms such as Listeria monocytogenes and Leishmania major and infections with the respiratory pathogen Mycobacterium tuberculosis (9, 12, 20). However, little is known about the role of IL-1 in host defense against respiratory bacterial pathogens. IL-1 can bind two receptors. Whereas the IL-1R type 2 is a “decoy” receptor (5), binding of IL-1 to IL-1R type 1 results in signal transduction. As a consequence, IL-1R type 1 gene-deficient (IL-1R −/−) mice do not respond to IL-1 (15). In the present study, we determined the role of endogenous IL-1 in lung inflammation during pneumonia caused by Pseudomonas aeruginosa, the most frequent gram-negative pathogen involved in nosocomial pneumonia (10, 11). Therefore, we compared bacterial outgrowth, local production of cytokines and chemokines, and neutrophil influx in IL-1R −/− and wild-type mice during respiratory tract infection with P. aeruginosa.

METHODS

Animals. Female IL-1R −/− mice backcrossed six times to a C57Bl/6 background (kindly provided by Immunex, Seattle, WA) and normal C57Bl/6 wild-type mice (Harlan, Horst, The Netherlands), 8–10 wk old, were used in all experiments. IL-1R −/− mice were normal in size and weight and displayed no abnormalities in leukocyte subsets (6). The protocol was approved by the Institutional Animal Care and Use Committee of the Academic Medical Center.

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IL-1R antagonist. Recombinant human IL-1R antagonist (IL-1Ra) in hyaluronic acid vehicle was kindly provided by Amgen (Thousand Oaks, CA) and was given as a single intraperitoneal injection 12 h before induction of pneumonia at a dose of 100 mg/kg of body wt (1, 2). Control mice received the hyaluronic acid vehicle only.

Induction of pneumonia. P. aeruginosa (strain PA103) pneumonia was induced as described previously (22, 23). Briefly, bacteria were grown to midlogarithmic phase in Luria broth for 6 h at 37°C, harvested by centrifugation at 1,500 g for 15 min, washed twice in pyrogen-free 0.9% NaCl, and resuspended in 10 ml of 0.9% NaCl. The number of bacteria was determined by serial dilution in sterile isotonic saline and culture on blood agar plates for 16 h. Before we administered an inoculum of 50 μl of the bacterial solution intranasally, mice were lightly anaesthetized with inhaled isoflurane (Forene; Abbott, Queensborough, Kent, UK). Control mice were inoculated with 50 μl of the bacterial solution in PBS. The number of colony-forming units (cfu) does not cause mortality, survival decreases to 60% and 20% after administration of 10⁵ and 10⁶, respectively.

Preparation of blood samples and lung homogenates. At 6 and 24 h after inoculation, mice were anesthetized with Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) and midazolam (Roche, Mijdrecht, The Netherlands), and blood was collected from the vena cava inferior in heparin-containing vacutainer tubes. Whole lungs were harvested and homogenized at 4°C in 5 volumes of sterile 0.9% NaCl in a tissue homogenizer that was carefully cleaned and disinfected with 70% alcohol after each homogenization. Serial 10-fold dilutions in sterile isotonic saline were made of these homogenates (and blood), and 50-μl volumes were plated onto sheep blood agar plates and incubated at 37°C and 5% CO₂. Colony-forming units were counted after 24 h. For cytokine measurements, lung homogenates were spun at 1,500 g for 15 min at 4°C, and supernatants were filtered through a 35-μm filter (Becton Dickinson, Lincoln Park, NJ) and frozen at −20°C until cytokine measurement.

Bronchoalveolar lavage. At 24 h after inoculation, the trachea was exposed through a midline incision and cannulated with a sterile 22-gauge Abbocath-T catheter (Abbott, Sligo, Ireland). Bronchoalveolar lavage (BAL) was performed by instilling two 0.5-ml aliquots of 0.9% NaCl. BAL fluid (BALF; 0.9–1 ml/mouse) was retrieved, and total cell numbers and differential cell counts were determined from cytospins on each sample. BALF differential cell counts were done on cytospin preparations stained with modified Giemsa stain (Diff-Quick; Baxter, McGraw Park, IL).

Histological examination. For histopathological examination, lungs were fixed in 10% buffered Formalin, embedded in paraffin, and 4-μm sections were stained with hematoxylin and eosin.

Assays. Cytokine and chemokine levels were measured by ELISA according to the manufacturers’ recommendations: IL-1α (R&D, Minneapolis, MN), IL-1β (R&D), tumor necrosis factor (TNF)-α (Genzyme, Cambridge, MA), IL-6 (Pharmin- gen, San Diego, CA), macrophage inflammatory protein (MIP)-2 (R&D), and KC (R&D).

Statistical analysis. All data are expressed as means ± SE. Comparisons between means were conducted using Wilcoxon’s test. Significance was set at P < 0.05.

RESULTS

Induction of pneumonia and IL-1α and IL-1β production. Inoculation with P. aeruginosa induced signs of pneumonia in all mice. Six and 24 h after inoculation with 10⁵ cfu P. aeruginosa, lungs appeared swollen and reddish with multiple hemorrhages on the surface. Wet lung weights from wild-type mice inoculated with P. aeruginosa increased by >61%, compared with lungs from control mice inoculated with sterile saline (P < 0.05; Fig. 1). IL-1R −/− mice demonstrated a similar increase in wet lung weights after induction of Pseudomonas pneumonia (nonsignificant vs. wild-type mice). Inoculation with P. aeruginosa induced a diffuse pneumonia in all mice. At 24 h after inoculation, lungs of mice displayed peribronchial and perivascular inflammatory infiltrates with endothelialitis. A heavy interstitial infiltrate of neutrophils was observed together with influx of neutrophils in the alveoli (Fig. 2). At this time point, the intensity and composition of the inflammatory infiltrate were comparable in wild-type mice and IL-1R −/− mice inoculated with P. aeruginosa.

Mice inoculated with isotonic saline had low levels of IL-1α and IL-1β in lung homogenates after intranasal inoculation. Induction of pneumonia was associated with elevated IL-1α and IL-1β levels in lung homogenates (Fig. 3). IL-1β levels were >10-fold higher than IL-1α concentrations in both strains. While in IL-1R −/− mice IL-1α and IL-1β concentrations decreased in lung homogenates from 6 to 24 h postinoculation, IL-1R levels rose in wild-type mice during this time period. As a consequence, wild-type mice displayed higher IL-1β concentrations in lungs than did IL-1R −/− mice at 24 h after induction of pneumonia (P < 0.05).

Bacterial clearance. Having established that IL-1α and IL-1β concentrations are elevated in lungs of mice suffering from Pseudomonas pneumonia, we determined the role of endogenous IL-1 in the clearance of Pseudomonas from the pulmonary compartment. For this purpose, wild-type and IL-1R −/− mice were inoculated with 10⁵ cfu P. aeruginosa, and cfu were counted in lungs harvested after 6 and 24 h (Fig. 4).

![Fig. 1. Wet lung weights from wild-type mice (open bars) and interleukin-1 receptor type 1 gene-deficient (IL-1R −/−) mice (filled bars) inoculated with Pseudomonas aeruginosa and from mice inoculated with sterile saline (hatched bar). Data are means ± SE; n = 6–8 mice per group at each time point. P < 0.05 for mice inoculated with bacteria vs. mice inoculated with sterile saline at all time points.](http://ajplung.physiology.org/)

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After 6 h, the number of cfu recovered from lungs was similar in IL-1R/H11002/H11002 mice and wild-type mice. In wild-type mice, the number of cfu in lungs slightly increased between 6 and 24 h postinoculation. By contrast, IL-1R/H11002/H11002 mice demonstrated a 2 log decrease in P. aeruginosa cfu during this time period. Consequently, IL-1R/H11002/H11002 mice had significantly fewer cfu in their lungs at 24 h after the induction of pneumonia than did wild-type mice (P < 0.05).

To obtain further evidence that endogenous IL-1 impairs bacterial clearance in this model, additional experiments were performed in which IL-1R/H11002/H11002 and wild-type mice were inoculated with a lower (2 × 10^4 cfu) and a higher (2 × 10^6 cfu) Pseudomonas dose and were killed after 24 h. The number of Pseudomonas cfu was lower in IL-1R/H11002/H11002 mice after administration of either dose (P < 0.05 and P = 0.09, respectively). None of the mice became bacteremic after inoculation with 2 × 10^4 or 2 × 10^5 cfu. After inoculation with 2 × 10^6 cfu, both wild-type and IL-1R/H11002/H11002 mice were bacteremic after 24 h (5/8 wild-type mice and 5/8 IL-1R/H11002/H11002 mice). However, the blood of wild-type mice contained more bacteria than blood of IL-1R/H11002/H11002 mice [2.2 ± 0.8 × 10^3 cfu/ml vs. 7.5 ± 3.4 × 10^1 cfu/ml (P < 0.05)].

Bacterial clearance in mice treated with IL-1Ra. Compensatory immune mechanisms may develop in mice that genetically lack the IL-1 signaling pathway. To determine whether the differences between IL-1R/H11002/H11002 and wild-type mice were caused solely by the absence of the IL-1 receptor, we inoculated wild-type...
mice with $2 \times 10^5$ cfu of *P. aeruginosa* 12 h after intraperitoneal injection of IL-1Ra or vehicle. The results from the first series of experiments could be recapitulated in this experiment, i.e., IL-1Ra treatment reduced the number of cfu recovered from lungs at 24 h postinfection compared with treatment with vehicle ($P < 0.05$; Fig. 5).

**Fig. 5.** Clearance of bacteria is enhanced in mice pretreated with IL-1Ra. Means ± SE of *P. aeruginosa* cfu in lungs 24 h after intranasal inoculation with $2 \times 10^5$ cfu in mice receiving IL-1Ra intraperitoneally before inoculation (filled bars) and mice receiving the vehicle without IL-1Ra intraperitoneally before inoculation (open bars); $n = 8$ mice per group at each time point.

Cytokine and chemokine levels in lung homogenates. Local production of cytokines and chemokines within the pulmonary compartment can influence antibacterial host defense mechanisms during pneumonia (18, 24). Therefore, we measured the concentrations of TNF, IL-6, MIP-2, and KC in lung homogenates after inoculation with *P. aeruginosa* (Fig. 6). At 6 h postinoculation, the lung levels of these mediators were similar in wild-type mice and *IL-1R*−/− mice. At 24 h, TNF, IL-6, KC, and MIP-2 levels were all significantly lower in lung homogenates from *IL-1R*−/− mice compared with wild-type mice ($P < 0.05$).

**Fig. 6.** Levels of tumor necrosis factor (TNF; A), IL-6 (B), macrophage inflammatory protein (MIP)-2 (C), and KC (D) were higher in wild-type mice (open bars) compared with *IL-1R*−/− mice inoculated with *P. aeruginosa* (filled bars) at 24 h after inoculation. Data are means ± SE of 6–8 mice per group at each time point. Control mice were inoculated with sterile saline (hatched bars). $P < 0.05$ for mice inoculated with bacteria vs. mice inoculated with sterile saline at all time points except for TNF at 24 h *IL-1R*−/− mice.

**DISCUSSION**

In local infection, like pneumonia, the initiation, maintenance, and resolution of inflammation are considered to be dependent upon the expression of the complex network of proinflammatory and anti-inflammatory cytokines (18, 24). In the present study, we evaluated the role of IL-1 in the innate immune response in the pulmonary compartment during pneumonia induced by *P. aeruginosa*. *IL-1R*−/− mice were found to have an increased resistance against *Pseudo-
Pseudomonas pneumonia, as reflected by an enhanced clearance of bacteria from the lungs, which was associated with reduced local cytokine and chemokine concentrations and a diminished neutrophil recruitment. These results could be recapitulated in normal wild-type mice treated with IL-1Ra, indicating that compensatory immune mechanisms that could have developed in mice that genetically lack the type 1 IL-1R are unlikely to be responsible for the present findings.

The role of cytokines in host defense against Pseudomonas pneumonia has been investigated in several previous studies. The overall conclusion that can be drawn from these investigations is that proinflammatory cytokines induced by P. aeruginosa in models of subacute pneumonia likely impair bacterial clearance from the pulmonary compartment. Indeed, treatment of mice with the anti-inflammatory cytokine IL-10 resulted in a diminished bacterial outgrowth in a model of subacute Pseudomonas pneumonia highly similar to our model (21). Furthermore, mice deficient for the type 1 TNF receptor demonstrated an enhanced early clearance of P. aeruginosa from the lungs during subacute pneumonia (26). Similarly, we recently found that interferon-γ receptor-deficient mice had an accelerated clearance of Pseudomonas from their lungs when compared with normal wild-type mice (23). Our present results are in line with these published reports, i.e., the absence of the proinflammatory cytokine IL-1 signal was associated with an improved clearance of Pseudomonas from the lung compartment. The difference between IL-1R −/− and wild-type mice became apparent after 24 h, which differs somewhat from earlier data in TNF receptor-deficient mice that demonstrated lower Pseudomonas cfu already at 4 h postinoculation (26). It should be noted in this context that the role of TNF in Pseudomonas pneumonia is not undebated, considering that other authors have reported no effect (19) or an adverse effect (14) of inhibition of endogenously produced TNF on the clearance of Pseudomonas during subacute pneumonia.

Leukocyte counts and differentials in BALF at 24 h after induction of pneumonia.

<table>
<thead>
<tr>
<th>BALF Control Mice, ×10^6 cells/ml</th>
<th>BALF, ×10^6 cells/ml</th>
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<tbody>
<tr>
<td>Wild type IL-1R (−/−)</td>
<td>Wild type IL-1R (−/−)</td>
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<tr>
<td>Leukocyte count</td>
<td></td>
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<tr>
<td>Polymorphonuclear cells</td>
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<tr>
<td>D. W. 9.1 ± 0.7*</td>
<td>9.7 ± 0.7*</td>
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<tr>
<td>6.6 ± 0.9*</td>
<td>6.0 ± 0.7*</td>
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<tr>
<td>Lymphocytes</td>
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<tr>
<td>D. W. 0.4 ± 0.1*</td>
<td>0.4 ± 0.1*</td>
</tr>
<tr>
<td>Macrophages</td>
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<tr>
<td>D. W. 0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
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| Data represent means ± SE of 5–6 mice per group at each time point.

For subacute pneumonia, the absence of endogenous IL-1 activity was associated with reduced neutrophil numbers in BALF at 24 h postinoculation after induction of pneumonia. The role of IL-1 in neutrophil recruitment during lung inflammation has been documented previously by observations that IL-1α and IL-1β can induce neutrophil influx in lungs after intratracheal administration to rodents (13, 17, 30) and that inhibition of endogenous IL-1 activity attenuated neutrophil influx in BALF induced by LPS (17). The lower lung concentrations of the potent chemoattractants MIP-2 and KC may also have contributed to the diminished neutrophil recruitment in IL-1R −/− mice, considering that they have been found to play an important role in this inflammation response (8, 27). Alternatively, the lower bacterial load in lungs of IL-1R −/− mice (providing less proinflammatory stimuli) could have been responsible for the attenuated neutrophil recruitment at later time points. Similarly, the higher concentrations of TNF, IL-6, KC, and MIP-2 in lungs of wild-type mice compared with IL-1R −/− mice at 24 h after inoculation with P. aeruginosa could be directly proportional to the number of bacteria present at that moment.

Interestingly, IL-1R −/− mice were recently reported to have a reduced susceptibility to persistent Staphylococcus epidermidis infection in a model of biomaterial-associated infection (3). Hence, these data taken together with the present results suggest that endogenous IL-1 may hamper antimicrobial defense in at least some infections.

In conclusion we found an increased bacterial clearance in mice with a disrupted IL-1R gene during pneumonia caused by P. aeruginosa. These data exemplify the complex role of IL-1 in innate immunity during pulmonary infection and may have important implications for the development and use of cytokine/anticytokine therapies in the future.

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