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; 10.1152/ajplung.00461.2000.—Pneumonia is associated with evidence that interleukin-1 is an important mediator of respiratory 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.
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 pyrogenic-free isotonic saline alone. We previously demonstrated that in this model of subacute Pseudomonas pneumonia, mortality is dependent on the number of bacteria administered intranasally (22, 23). While inoculation with 10^4 colony-forming units (cfu) does not cause mortality, survival decreases to 60% and 20% after administration of 10^5 and 10^6, 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 Abbooth-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 cytopsins on each sample. BALF differential cell counts were done on cytopsin 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 (Pharminogen, 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 10^5 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-1 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 10^5 cfu P. aeruginosa, and cfu were counted in lungs harvested after 6 and 24 h (Fig. 4).
After 6 h, the number of cfu recovered from lungs was similar in IL-1 R−/− 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−/− mice demonstrated a >2 log decrease in P. aeruginosa cfu during this time period. Consequently, IL-1R−/− 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−/− and wild-type mice were inoculated with a lower (2 × 10⁴ cfu) and a higher (2 × 10⁶ cfu) Pseudomonas dose and were killed after 24 h. The number of Pseudomonas cfu was lower in IL-1R−/− mice after administration of either dose (P < 0.05 and P = 0.09, respectively). None of the mice became bacteremic after inoculation with 2 × 10⁴ or 2 × 10⁵ cfu. After inoculation with 2 × 10⁶ cfu, both wild-type and IL-1R−/− mice were bacteremic after 24 h (5/8 wild-type mice and 5/8 IL-1R−/− mice). However, the blood of wild-type mice contained more bacteria than blood of IL-1R−/− mice [2.2 ± 0.8 × 10³ cfu/ml vs. 7.5 ± 3.4 × 10¹ 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−/− 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 *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).

**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 ($P < 0.05$).

**Cell influx.** *Pseudomonas* pneumonia was associated with a profound influx of neutrophils into the BALF (Table 1). At 24 h postinoculation, *IL-1R*−/− mice had less neutrophils in their BALF than did wild-type mice ($P < 0.05$).

**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-
Table 1. Leukocyte counts and differentials in BALF at 24 h after induction of pneumonia

<table>
<thead>
<tr>
<th>BALF, (\times 10^6) cells/ml</th>
<th>BALF Control Mice, (\times 10^6) cells/ml</th>
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<tbody>
<tr>
<td><strong>IL-1R (−/−)</strong></td>
<td><strong>Wild type</strong></td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>9.1 ± 0.7*</td>
</tr>
<tr>
<td>Polymorphonuclear cells</td>
<td>8.7 ± 0.7*</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.4 ± 0.1*</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.0 ± 0.0*</td>
</tr>
</tbody>
</table>

Data represent means ± SE of 5–6 mice per group at each time point. Mice were intranasally inoculated with *Pseudomonas aeruginosa* (10³ colony-forming units) at \(T = 0\) h. Control mice received sterile saline intranasally and were killed after 24 h. †\(P < 0.05\) vs. wild-type mice; *\(P < 0.05\) compared with control mice. BALF, bronchoalveolar lavage fluids; **IL-1R** (−/−), interleukin-1 receptor type 1 gene-deficient mice.

*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.

It is important to emphasize that the role of cytokines in the innate immune response to respiratory tract infections differs in models in which different pathogens are used. Indeed, in experimental pneumonia caused by the gram-negative bacterium *Klebsiella pneumoniae*, proinflammatory cytokines like TNF and IL-1 (16, 31) are important for the clearance of bacteria from the lungs, whereas the anti-inflammatory cytokine IL-10 impairs host defense in this model (7). At present the cause of these overt differences with the role of cytokines in *Pseudomonas* pneumonia is unclear. A possible explanation includes differences in the extent and rapidity by which these strains induce inflammation in the lung. One could speculate that during *Pseudomonas* pneumonia, inflammation is excessive and thereby harms the host, whereas inflammation is more limited in *Klebsiella* and pneumococcal pneumonia, thereby facilitating antimicrobial effector mechanisms.

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 recruitments 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.

We thank Dr. B. Iglewski for providing *P. aeruginosa* strain 103. T. van der Poll is a fellow of the Royal Netherlands Academy of Arts and Sciences.
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REFERENCES