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

MARC J. SCHULTZ,1,2 ANITA W. RIJNEVELD,1 SANDRINE FLORQUIN,3 CARL K. EDWARDS,4 CHARLES A. DINARELLO,5 AND TOM VAN DER POLL1,6

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

Received 20 December 2000; accepted in final form 17 October 2001

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 IL-1 activity caused by LPS (28, 30). In addition, recombinant 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 Pseudomonas 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 postinoculation. 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.

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.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajplung.org 1040-0605/02 $5.00 Copyright © 2002 the American Physiological Society

Address for reprint requests and other correspondence: M. J. Schultz, Academic Medical Center, Univ. of Amsterdam, Dept. of Intensive Care Medicine, C3–326, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands (E-mail: m.j.schultz@amc.uva.nl).
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 anesthetized 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 10^6 and 10^8, 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 Dickenson, 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 (Pharmingen, 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 10^8 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 10^8 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/doi/10.1152/ajplung.00506.2001)
After 6 h, the number of cfu recovered from lungs was similar in IL-1 R/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% wild-type mice and 5% 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 IL-1Ra.

After 6 h, the number of cfu recovered from lungs was similar in IL-1 R/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.

Fig. 4. Clearance of bacteria is enhanced in mice lacking the IL-1 signal. Means ± SE P. aeruginosa colony-forming units (cfu) in lungs 6 and 24 h after intranasal inoculation with 2 × 10^4, 2 × 10^5, and 2 × 10^6 cfu in wild-type (open bars) and IL-1R/H11002/H11002 mice (filled bars); n = 6–8 mice per group at each time point.
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).

**Fig. 5.** Clearance of bacteria is enhanced in mice pretreated with IL-1Ra. Means ± SE *P. aeruginosa* cfu in lungs 24 h after intranasal inoculation with $2 \times 10^6$ 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.

**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.

**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 *Pseudomonas* pneumonia.
The role of cytokines in host defense against *Pseudomonas* pneumonia has been investigated in several previous studies. 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. Igleswski for providing *P. aeruginosa* strain 103. T. van der Poll is a fellow of the Royal Netherlands Academy of Arts and Sciences.

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

<table>
<thead>
<tr>
<th>BALF, ×10⁶ cells/ml</th>
<th>BALF Control Mice, ×10⁶ cells/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td><em>IL-1R−/−</em></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* (10⁵ 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 vs. control mice. BALF, bronchoalveolar lavage fluids; *IL-1R−/−*, interleukin-1 receptor type 1 gene-deficient mice.
REFERENCES


