Pneumomia: an investigation of host defence mechanisms
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Tumor necrosis factor-α compensates for the impaired host defense of interleukin-1 receptor type I deficient mice during pneumococcal pneumonia
TUMOR NECROSIS FACTOR-α

To determine the role of interleukin 1 (IL-1) in the host defense against pneumonia, IL-1 Receptor type I deficient (IL-1R−/−) and wild type (Wt) mice were intranasally inoculated with Streptococcus (S.) pneumoniae. Pneumonia resulted in elevated IL-1α and IL-1β mRNA and protein levels in the lungs. Survival rates did not differ between IL-1R−/− and Wt mice after inoculation with 5 x 10⁴ or 2 x 10⁵ CFU. At early time points (24 and 48h), IL-1R−/− mice had two-log more S. pneumoniae CFU in lungs than Wt mice; at 72h, bacterial outgrowth in lungs was similar in both groups. Upon histopathological examination, IL-1R−/− mice displayed a reduced capacity to form inflammatory infiltrates at 24h after the induction of pneumonia. IL-1R−/− mice also had significantly less granulocyte influx in bronchoalveolar lavage fluid at 24h after inoculation. Since tumor necrosis factor-α (TNF) is known to enhance host defense during pneumonia, we determined the role of endogenous TNF in the early impairment and subsequent recovery of defense mechanisms in IL-1R−/− mice. All IL-1R−/− mice treated with anti-TNF rapidly died (0/14 survivors after 4 days), while 10-day survival in IL-1R−/− mice (control Ab), Wt mice (anti-TNF) and Wt mice (control Ab) was 7/13, 3/14 and 12/13 respectively. These data suggest that TNF is more important for host defense against pneumococcal pneumonia than IL-1, and that the impaired early host defense in IL-1R−/− mice is compensated for by TNF at a later phase.

Introduction

Community acquired pneumonia caused by S. pneumoniae remains a major cause of morbidity and mortality especially in the elderly.¹² Emergence and spread of penicillin-resistant S. pneumoniae have become a worldwide problem.³⁻⁵ Therefore, in order to develop novel therapeutic strategies, it is crucial to study the host response during pneumonia caused by S. pneumoniae.

Activation of the cytokine network plays an important role in the early response to severe infection.⁶ In models of systemic infection, TNF is the first cytokine that becomes detectable in the circulation, followed shortly thereafter by IL-1β.⁷⁻⁹ TNF and IL-1β have highly overlapping biological activities, and synergize in inducing systemic toxicity in animals in vivo.¹⁰,¹¹ Elimination of either TNF or IL-1 activity during severe bacteremia in baboons largely prevents lethality, suggesting that excessive systemic production of these cytokines is of pivotal importance for the development of organ injury during sepsis syndrome.¹²,¹³ However, evidence indicates that the local production of proinflammatory cytokines is crucial for the clearance of bacterial infections from the lung. Indeed, passive immunization against TNF impairs host defense during pneumococcal, Legionella and Klebsiella pneumonia in mice.¹⁴⁻¹⁶ The role of IL-1 during bacterial pneumonia is less well defined.

IL-1 is a pleiotropic pro-inflammatory cytokine, mainly produced by mononuclear phagocytes, which affects nearly all cell types. The IL-1 family consists of three members, namely IL-1α, IL-1β and IL-1 receptor antagonist (IL-1ra).¹⁷,¹⁸ IL-1 can bind to two
receptors, IL-1 receptor (IL-1R) type I and type II. Type I receptors are found on most cell types, whereas expression of type II receptors is limited to blood neutrophils, monocytes, bone marrow progenitor cells and B-lymphocytes. IL-1R type II is not able to transduce a signal and is therefore generally referred to as a decoy receptor.\textsuperscript{19,20} The type I IL-1R has equal affinities for IL-1\(\alpha\), IL-1\(\beta\) and IL-1ra. After binding of IL-1 to IL-1 R type I, IL-1 - IL-1R type I forms a complex with the IL-1R accessory protein, which results in signal transduction and biological effects, including induction of an acute phase response to sterile inflammation, fever and synthesis of other proinflammatory cytokines and chemokines such as IL-6, TNF and IL-8.\textsuperscript{17,21}

To determine the role of IL-1 in the pathogenesis of pneumococcal pneumonia, IL-1R type I gene deficient (IL-1R\(^{-}\)) mice were compared to wild type (Wt) mice after induction of pneumonia with \textit{S. pneumoniae}.\textsuperscript{22} In addition, the possible interaction between endogenous IL-1 and TNF during pneumonia was evaluated by treatment of IL-1R\(^{-}\) and Wt mice with a neutralizing anti-TNF antibody.

**Materials and Methods**

**Animals.** All experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center. IL-1R\(^{-}\) mice back-crossed 6 times to a C57Bl/6 background (kindly provided by Immunex Corporation, Seattle, WA) and normal C57Bl/6 Wt mice (Harlan Sprague Dawley Inc., Horst, the Netherlands) were used. Male (10-12 weeks old) mice were used in all experiments. IL-1R\(^{-}\) mice are normal in size, weight and fertility, and display no abnormalities in leukocyte subsets.\textsuperscript{22}

**Induction of pneumonia.** Pneumonia was induced as described earlier.\textsuperscript{16,23} Briefly, \textit{S. pneumoniae} serotype 3 was obtained from American Type Culture Collection (ATCC 6303; Rockville, MD). Pneumococci were grown in Todd-Hewitt broth (Difco, Detroit, MI) for 6 hours to midlogarithmic phase at 37°C in 5% CO\(_2\), harvested by centrifugation at 1500 x g for 15 minutes, and washed twice in sterile isotonic saline. Bacteria were then resuspended in sterile isotonic saline at different concentrations (2 x 10\(^5\) - 4 x 10\(^6\) colony forming units (CFU)/ml), as determined by plating serial 10-fold dilutions onto sheep-blood agar plates. Mice were lightly anesthesized by inhalation of isoflurane (Abott, Queensborough, Kent, UK), and 50 \(\mu\)l of bacterial suspension or an equal volume of sterile isotonic saline as control was inoculated intranasally.

**Antibodies.** Rat anti-mouse TNF mAb, was kindly provided by D. Shealy (Centocor, Malvern, PA). Rat IgG2a (clone R7D4) was used as control antibody. Antibodies were given intraperitoneally in two doses of 0.5 mg, 2h before and 24h after induction of pneumonia.

**Reverse transcriptase-PCR (RT-PCR).** Mouse lungs were harvested and snap-frozen in liquid nitrogen 24 and 48h after inoculation with \textit{S. pneumoniae}, and 48h after saline inoculation. Total RNA was isolated from mouse lungs using Trizol reagents (Gibco BRL, Life Technologies, Berlin, Germany). Briefly, cells were lysed in Trizol reagents and the RNA
TUMOR NECROSIS FACTOR-α

was isolated following chloroform extraction and isopropanol precipitation. Reverse transcription was performed using 2 μg of total cellular RNA and 0.5 μg Oligo (dT) (Gibco BRL), and incubating the solution (12 μl) for 10 minutes at 72 °C. The final 20 μl reaction mixture contained the following components at the final concentrations indicated: 1x first strand-buffer (Gibco BRL), 10 mM DTT, 1.25 mM each of dNTPs, and 100 U Superscript RNase H Reverse transcriptase (Gibco BRL). The reaction was incubated for 60 minutes at 42°C followed by 72°C for 10 minutes. Finally, 180 μl H₂O was added to the reaction mixture and samples were stored at -20 °C. For PCR, cDNA from 3 mice was pooled and 5 μl of RT product was used in a total volume of 25 μl of a solution containing 0.5 U AmpliTaq polymerase (Perkin Elmer Corp., Norwalk, CT), 1.25 mM dNTPs, 2.5 μl 10x Pol buffer (0.67 M Tris-HCl pH 8.8, 67 mM MgCl₂, 0.1 M BME, 67 μM EDTA, 0.166 M (NH₄)₂SO₄), 1% DMSO, 0.5 mg/ml BSA and 200 ng of each primer. The following sequence was performed on a thermocycler (Perkin-Elmer Corp.) for each PCR reaction: 94°C for 5 min (1 cycle), followed immediately by 94°C for 1 min, 55°C for 1 min and 72°C for 1 min (with variable numbers of cycles) and a final extension phase of 72°C for 10 min. A variable number of cycles was used to ensure that amplification occurred in the linear phase and that differences between control and experimental conditions were maintained by adopting a limited number of cycles. To ensure that differences between samples were not a result of unequal concentrations of cDNA, a PCR using β-actin as internal standard was performed on each sample. β-Actin was shown to be linear at 27 amplification cycles, IL-1α and IL-1β at 30 amplification cycles. The primer sequences are as follows: β-actin (F): 5’GTCAGAAGGACTTAT GTG3’; β-actin (r): 3’GCTCGTGGCCAATAGTGATG5’; IL-1α (F): 5’CTCTAGAGCACCATGCTACA GAC3’; IL-1α (r): 3’TGGAAATCCAGGGGAAA CACTG5’; IL-1β (F): 5’TCATGGGATGTGTA TAACCTGCT3’; IL-1β (r): 3’CCCATACT TTAGGAA GACACGGAT5’; The PCR products were separated on a 1.5% agarose gel and visualized by UV illumination.

Histological examination. After 24 hours fixation of lungs in 4% paraformaldehyde in PBS and embedding in paraffin, 4 μm thick sections were stained with haematoxylin and eosin. All slides were coded and semi-quantitatively scored by a pathologist without knowledge of the type of mice and treatment.

Preparation of lung homogenates. Mice were anesthetized with Hypnorm® (Janssen Pharmaceutica, Beere, Belgium) and midazolam (Roche, Meidrecht, the Netherlands), and blood was collected from the inferior vena cava. Whole lungs were harvested and homogenized at 4°C in 5 volumes of sterile isotonic saline with a tissue homogenizer (Biospect Products, Bartlesville, OK) which was carefully cleaned and desinfected with 70% alcohol after each homogenization. Serial 10-fold dilutions in sterile isotonic saline were made from these homogenates (and blood), and 50 μl volumes were plated onto sheep-blood agar plates and incubated at 37°C and 5% CO₂. CFU were counted after 16 hours. For cytokine measurements lung homogenates were lysed in lysisbuffer (300 mM NaCl, 15 mM
Tris, 2 mM MgCl₂, 2 mM Triton(X-100), Pepstatin A, Leupeptin, Aprotinin (20 ng/ml), pH 7.4) and spun at 1500 x g at 4°C for 15 minutes; the supernatant was frozen at -20°C until cytokine measurement.

**Bronchoalveolar lavage.** The trachea was exposed through a midline incision and cannulated with a sterile 22-gaugeAbbocath-T catheter (Abbott, Sligo, Ireland). Bronchoalveolar lavage (BAL) was performed by instilling two 0.5 ml aliquots of sterile isotonic saline. 0.9-1 ml of lavage fluid was retrieved per mouse, and total cell numbers were counted from each sample in a hemocytometer. BAL fluid (BALF) differential cell counts were carried out on cytospin preparations stained with modified Giemsa stain (Diff-Quick; Baxter, McGraw Park, Ill).

**Pulmonary cell influx.** Single cell suspensions were obtained by crushing lungs through a 40-μm cell strainer (Becton Dickinson). Erythrocytes were lysed with ice-cold isotonic NH₄Cl solution (155 mM NH₄Cl, 10 mM KHCO₃, 100 mM EDTA, pH 7.4), and the remaining cells were washed. Total leukocyte count was determined using a hemacytometer. The number of polymorphonuclear cells (PMNs) was calculated from these totals, using cytospin preparations stained with modified Giemsa stain (Diff-Quick).

**Cytokine and chemokine determination.** Cytokine and chemokine levels were measured by using commercially available ELISA’s, in accordance with the manufacturers recommendations: IL-1α, IL-1β, IL-1ra, TNF, Interferon gamma (IFN-γ), macrophage inflammatory protein (MIP-2), and KC (all R&D systems, Minneapolis, MN).

**Statistical analysis.** Data were analysed using the SPSS statistical package. Data are expressed as means ± SEM, unless indicated otherwise. Comparisons between groups were conducted using the Mann Whitney U test. For survival studies the logrank test was used. P-value <0.05 was considered to represent a statistically significant difference.

**Results**

**Induction of IL-1α, IL-1β and TNF in lungs** (Figure 1). Administration of *S. pneumoniae* induced an increased production of IL-1α and IL-1β in lungs at both mRNA and protein level. Control mice inoculated with saline showed only vague IL-1α and IL-1β mRNA bands, whereas pneumonia was associated with clear bands at both 24 and 48h postinoculation. High IL-1α and IL-1β protein levels were detected in lung homogenates of mice with pneumonia at these same time points (peak levels: IL-1α 4.1 ± 0.3 and IL-1β 5.0 ± 0.3 ng/g lung at 48h; both P<0.05 vs. control). IL-1ra did not increase in lungs of mice with pneumonia (data not shown). In line with previous findings in this model of pneumococcal pneumonia, induction of pneumonia in Wt mice resulted in a sustained increase in TNF concentrations in lung homogenates reaching a plateau between 12 and 72h (233 ± 27 ng/g at 72h, P<0.05 vs. control).
Figure 1. **IL-1α and IL-1β mRNA and protein expression in lungs.** A, B: IL-1α (A) and IL-1β (B) mRNA expression in lung homogenates of Wt mice 24h and 48h after intranasal inoculation with *S. pneumoniae*. Control mice were inoculated with sterile saline and sacrificed after 48h. Bands represent PCR products raised from pooled lungs of three mice. β-actin mRNA expression was similar in all samples (data not shown) C, D: IL-1α (C) and IL-1β (D) protein concentrations in lung homogenates measured by ELISA. Data are mean ± SEM; N=8 for each time point. *P<0.05, vs. control

**Survival does not differ between IL-1R−/− and Wt mice** (Figure 2). Once we had established that IL-1α and IL-1β are produced in lungs during pneumococcal pneumonia, we wanted to evaluate the contribution of these cytokines to survival after inoculation with *S. pneumoniae*. Survival did not significantly differ between IL-1R−/− and Wt mice up to 10 days

Figure 2. **Survival study.** Survival after intranasal inoculation with 5 x 10^4 (A) and 2 x 10^5 (B) CFU *S. pneumoniae* in Wt (closed circles) and IL-1R−/− mice (open squares). Mortality was assessed twice daily for 10 days. N = 12 (A) and 15 (B) per group for each bacterial dose.
after inoculation with $5 \times 10^4$ CFU (7-day survival 64% and 73% resp.) or $2 \times 10^5$ CFU (0% and 15% respectively). Mice surviving for 10 days postinoculation appeared to be permanent survivors. Further experiments were performed with $10^5$ CFU *S. pneumoniae*.

**IL-1 is important for the early host defense.** To determine the role of IL-1 in the early host defense against pneumonia, we assessed the outgrowth of pneumococci in the lungs of IL-1R$^{-/-}$ and Wt mice 24, 48 and 72 hours after intranasal inoculation with $10^5$ CFU *S. pneumoniae*. At early time points (24 and 48h), IL-1R$^{-/-}$ mice had more *S. pneumoniae* CFU in lungs than Wt mice (P<0.05); but at 72h, the number of CFU recovered from lungs was similar in both groups. *S. pneumoniae* could not be cultured from the blood of any of the Wt mice (Figure 3). On the other hand, 25% and 50% of the blood cultures obtained from the IL-1R$^{-/-}$ mice at 24 and 48h respectively were positive for *S. pneumoniae*. These results are in accordance with the survival study which did not show a difference in the eventual survival and thus suggest that while endogenous IL-1 activity is important for the early antibacterial host defense, a defect in IL-1 signalling does not influence the survival in this model.

![Figure 3. Bacterial outgrowth in lungs.](image)

*Figure 3. Bacterial outgrowth in lungs.* CFU *S. pneumoniae* in lungs of Wt and IL-1R$^{-/-}$ mice 24 (A), 48 (B) and 72 (C) hours after inoculation with $10^5$ CFU *S. pneumoniae*. Horizontal lines represent the medians within the group. N = 8 per group per time point.

**Histopathology.** Twenty-four hours after inoculation with *S. pneumoniae*, Wt mice displayed more inflammatory infiltrates than IL-1R$^{-/-}$ mice. Wt mice suffered from a bronchopneumonia involving 5-20% of the lung parenchyma. As illustrated in Figure 4a, the inflammation was characterized by extensive vasculitis and diapedesis of inflammatory cells through small and medium sized vessels. At this stage, neutrophils were dominant and filled bronchi, bronchioles and adjacent alveolar spaces. Necrosis was locally present, leading to the formation of small abscesses. On the other hand, IL-1R$^{-/-}$ mice displayed slight inflammatory infiltrates at 24h post-inoculation, predominantly composed of lymphocytes concentrated around bronchioles and small vessels but without signs of bronchopneumonia (Figure 4b). After 48h, all Wt mice presented interstitial inflammatory infiltrates composed of lymphocytes, monocytes and a few granulocytes, compatible with clearance of the inflammation (Figure 4c). At this time point, 80% of IL-1R$^{-/-}$ mice showed accumulation of foamy cells in alveolar spaces (alveolar macrophages) together with interstitial inflammatory
infiltrates as depicted in Figure 4d.

![Histopathology of lungs](image)

**Figure 4. Histopathology of lungs.** Histological sections of lungs of Wt (A + C) and IL-1R\(^{+/−}\) (B + D) 24h (A + B) and 48h (C + D) respectively after inoculation with \(10^5\) CFU *S. pneumoniae*. Haematoxylin and eosin staining, original magnification x 50. Representative slides are shown.

**Granulocyte recruitment** (Figure 5). Granulocytes play an essential role in antibacterial host defense during pneumonia. In a first attempt to obtain insight into the mechanism by which IL-1 exerts a protective effect in the early phase of pneumococcal pneumonia, we compared cell influx in BALF in IL-1R\(^{−/−}\) and Wt mice. Wt mice had fewer granulocytes in their BALF at 24h (P<0.05) when compared to IL-1R\(^{+/−}\) mice. On the other hand, at 48h post-inoculation, IL-1R\(^{−/−}\) mice demonstrated a 3.5-fold higher influx of granulocytes in BALF than Wt mice (P<0.05). Hence, these data suggest that the recruitment of granulocytes to the inflammatory site is delayed in IL-1R\(^{−/−}\) mice.

**Cytokine and chemokine concentrations.** Local production of specific cytokines and chemokines plays an important role in the pathogenesis of pneumonia. Mediators that have been found to improve host defense include the cytokines TNF and IFN-\(γ\) and the chemokines KC and MIP-2.\(^{15,16,24,26}\) To determine whether alterations in the local expression of these mediators could contribute to the relatively impaired antibacterial defense in IL-1R\(^{−/−}\) mice, we
measured their concentrations in lung homogenates of IL-1R<sup>−/−</sup> and Wt mice. We found that the lung concentrations of all these “protective” cytokines and chemokines were similar or higher in IL-1R<sup>−/−</sup> mice than in Wt mice (data not shown). Thus, these data suggest that IL-1 does not enhance host defense by inducing protective cytokines or chemokines during pneumococcal pneumonia.

![Figure 5. Granulocytic influx in BALF.](image)

**Figure 5. Granulocytic influx in BALF.** Mean ± SEM granulocyte influx in BALF 24h (A) and 48h (B) after intranasal inoculation of 10<sup>5</sup> CFU S. pneumoniae in Wt (open bars) and IL-1R<sup>−/−</sup> mice (closed bars). N = 8 per group

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<th>Table 1. Effect of anti-TNF on bacterial outgrowth and immune responses in IL-1R&lt;sup&gt;−/−&lt;/sup&gt; mice.</th>
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<td>CFU (Log 10 /ml lung)</td>
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Data are mean ± SEM of 5 mice per group, 48h after inoculation with S. pneumoniae CFU. *P<0.05 vs. IL-1R<sup>−/−</sup> mice.

Both IL-1 and TNF are necessary for effective host defense during pneumococcal pneumonia. Since TNF is known to enhance host defense during pneumonia, and IL-1 and TNF can exert synergistic proinflammatory effects in vivo, we next determined the role of endogenous TNF in the early impairment and subsequent recovery of host defense in IL-1R<sup>−/−</sup> mice. All IL-1R<sup>−/−</sup> mice treated with anti-TNF rapidly died after inoculation with 10<sup>5</sup> CFU S. pneumoniae (0% survivors after 4 days), while 10-day survival in IL-1R<sup>−/−</sup> mice (control Ab), Wt mice (anti-TNF) and Wt mice (control Ab) were 62%, 29% and 92% survivors respectively (Figure 6). Mice surviving for 10 days appeared to be permanent survivors. To obtain further insight in the concerted action of IL-1 and TNF in the protective immune response to pneumococcal pneumonia, we compared bacterial outgrowth in IL-1R<sup>−/−</sup> mice treated with anti-TNF or control Ab at 48h post-infection. Treatment with anti-TNF was associated with more S. pneumoniae CFUs in lung homogenates (P<0.05 vs. control Ab;
Figure 6. Role of IL-1 and TNF in lethality induced by pneumococcal pneumonia. (Upper panel) 10-day survival after intranasal inoculation with $10^5$ CFU *S. pneumoniae* in mice pre-treated with a neutralizing anti-mouse TNF mAb (open circles Wt and open squares IL-1R$^{-/-}$ mice) or an equivalent amount of an isotype-matched control mAb (closed circles Wt, closed squares IL-1R$^{-/-}$ mice). N=12-15 per group. *P <0.05 vs. Wt, †P<0.05 vs. IL-1R$^{-/-}$, #P<0.05 vs. Wt + anti-TNF. (Lower panel) Representative haematoxylin and eosin staining of the lung of an anti-TNF treated IL-1R$^{-/-}$ mouse 48h after inoculation showing a severe pneumonia with destruction of the lung parenchyma and edema (magnification x 50).

Table 1), and an enhanced dissemination of the infection as reflected by the fact that all anti-TNF treated IL-1R$^{-/-}$ mice had positive blood cultures vs. 40% of IL-1R$^{-/-}$ mice treated with control Ab. Anti-TNF tended to increase the influx of neutrophils into lungs (Table 1), whereas IL-6 and KC concentrations were lower and MIP-2 concentrations were higher in anti-TNF treated IL-1R$^{-/-}$ mice (P<0.05 vs. IL-1R$^{-/-}$ mice with control Ab for KC and MIP-2). The histopathology showed that all IL-1R$^{-/-}$ mice treated with anti-TNF suffered from a severe pneumonia 48h after inoculation. The lungs showed a dense and diffuse infiltration of granulocytes, destruction of lung parenchyma and a pronounced edema around the vessels (Figure 6).

**Discussion**

TNF and IL-1 are proinflammatory cytokines which function proximally in the cytokine cascade after the initiation of an inflammatory response, and their combined action results in additive or even synergistic biological effects. In an earlier study it was found that TNF is produced in the lung during pneumococcal pneumonia, where it plays a major role in antibacterial host defense. In the present study, using the same model, we show that IL-1α and IL-1β are both produced in the lung and that deficiency of the functional receptor for
these cytokines impairs the early pulmonary defense. Pre-treatment of IL-1R−/− mice with a neutralizing anti-TNF antibody resulted in a strongly diminished survival, suggesting that the combined action of endogenous IL-1 and TNF is required for an effective pulmonary defense against *S. pneumoniae*.

IL-1β has been shown to be locally produced during pneumonia in humans. In patients with unilateral community acquired pneumonia, the inflammatory reaction within the lung was limited to the site of infection, as reflected by higher IL-1β concentrations in BALF from the involved lung than is BALF from the noninvolved lung or in serum. Furthermore, alveolar macrophages recovered from the involved lung spontaneously released more IL-1β than alveolar macrophages from the noninvolved lung. Patients with pleural empyema showed a significant elevation of IL-1β in the pleural fluid when compared to patients with pleural fluid due to other etiologies. Children with a bacterial pulmonary infection had significantly higher levels of IL-1β and IL-1 activity in BALF than children without such an infection. However, knowledge of the role of IL-1 in host defense against pneumonia is limited. An earlier study suggested a protective role for IL-1 during *P. carinii* pneumonia. Reconstitution of SCID mice with immunocompetent spleen cells resulted in clearance of the naturally acquired pulmonary infection with *P. carinii*. Treatment of these mice with anti-IL-1R type I antibodies at 2 days post-reconstitution, inhibited this clearance. In addition, IL-1β deficient mice were more sensitive to pneumonia caused by influenza virus. Together with our present results, these data suggest that locally produced IL-1 contributes to defense mechanisms during bacterial, protozoal and viral lung infections.

The results of the present study suggest that endogenous IL-1 is mainly required in the early stage of the inflammatory response. At early time points (24h and 48h) IL-1R−/− mice showed enhanced bacterial outgrowth in the lungs, while at 72h post-inoculation the number of pneumococci in the lungs was similar in both groups. This is in accordance with the fact that survival curves during 10 days post-inoculation did not reveal marked differences between IL-1R−/− and Wt mice. It should be noted that the number of *S. pneumoniae* CFUs measured at 72 h was considerably lower than the number of CFUs found at 48h. The 24 and 48h data were obtained in one experiment using the same inoculum. The 72h data were generated in a subsequent experiment, in which in retrospect the bacterial inoculum was slightly lower (i.e. 8 x 10⁴ CFU vs. 1 x 10⁵ CFU in the earlier experiment). This, together with the fact that some biological variation between mouse experiments separated in time exists, may have caused the difference between the 48 and 72h time point. The impaired antibacterial defense in IL-1R−/− mice can at least in part be explained by their apparently reduced capacity to mount an inflammatory response in the pulmonary compartment, as reflected by histopathology and an attenuated recruitment of granulocytes, shortly after infection. The local production of protective cytokines was not reduced in IL-1R−/− mice. The finding that the influx of granulocytes in BALF was delayed in IL-1R−/− mice, is in keeping with previous observations that IL-1α and IL-1β can induce granulocyte recruitment to lungs after...
intratracheal administration to rodents,\textsuperscript{32-34} and that inhibition of IL-1 activity reduces endotoxin-induced neutrophil influx in BALF.\textsuperscript{34}

It should be noted that in the final survival studies (Figure 6), IL-1R\textsuperscript{-/-} mice had a slightly reduced survival when compared to normal Wt mice, while in the first two survival experiments IL-1R\textsuperscript{+/-} mice only tended to have an increased mortality (Figure 2). These findings suggest that the absence of an intact IL-1 signal results in a diminished early antibacterial defense that at most influences survival in a modest way. Nonetheless, it is clear that anti-TNF has a more profound detrimental effect in this model (this study and reference 16), indicating that endogenous TNF is more important for host defense against pneumococcal pneumonia than IL-1. Moreover, our data show that TNF and IL-1 act synergistically to combat pneumococci in the lung. Indeed, neutralization of endogenous TNF rendered IL-1R\textsuperscript{-/-} mice highly susceptible to pneumococcal pneumonia. In this respect the acutely fatal outcome of IL-1R\textsuperscript{-/-} mice treated with anti-TNF relative to Wt mice treated with anti-TNF was striking, suggesting that during the early phase of murine pneumococcal pneumonia endogenous IL-1 can compensate in part for the absence of TNF. Considering the different survival curves of IL-1R\textsuperscript{-/-} mice treated with either anti-TNF or control Ab, at later stages of the infection endogenous TNF can compensate for the absence of an intact IL-1 signal.

IL-1 and TNF are potent proinflammatory cytokines that play a pivotal role in the occurrence of organ failure and death in animal models of severe sepsis induced by intravenous administration of high doses of bacteria.\textsuperscript{12,13} However, the clinical relevance of such models is doubtful in light of the acute and fulminant course, and the lack of a local infectious source. In addition, clinical trials in patients with sepsis failed to show any beneficial effect of IL-1 or TNF neutralizing strategies.\textsuperscript{35} Evidence is accumulating that the local activity of proinflammatory cytokines is required for an adequate antibacterial response at the site of an infection.\textsuperscript{6,23,25} Our present data suggest that TNF is more important for the protective host immune response during pneumococcal pneumonia than IL-1, and that these two proinflammatory cytokines contribute to the local defense against pneumococci in the lung by a concerted action. These findings may not only add to our understanding of the role of IL-1 and TNF in pneumococcal pneumonia, but also warrant caution for combined anti-IL-1 and anti-TNF treatments for inflammatory conditions like rheumatoid arthritis.

References


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