Pneumonia: an investigation of host defence mechanisms
Rijneveld, A.W.

Citation for published version (APA):
Rijneveld, A. W. (2003). Pneumonia: an investigation of host defence mechanisms

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Interleukin-1 receptor antagonist transiently impairs antibacterial defense but not survival in murine pneumococcal pneumonia
Recombinant human interleukin-1 (IL-1) receptor antagonist (IL-1ra) has been investigated in several controlled clinical trials to inhibit the biological activity of IL-1, and encouraging results have been reported in particular in patients with rheumatoid arthritis. In the present study we investigated the influence of treatment of wild type mice with IL-1ra, resulting in an incomplete and transient inhibition of IL-1 activity. Treatment with recombinant human IL-1ra resulted in an enhanced bacterial outgrowth in lungs of BALB/c and C57BL/6 mice early after induction of pneumococcal pneumonia, without influencing survival or the pulmonary inflammatory response. The effect of IL-1ra on the host response to Streptococcus (S.) pneumoniae pneumonia is modest and transient.

Introduction
IL-1 and tumor necrosis factor-α (TNF) are potent proinflammatory cytokines that are targets of therapeutic intervention in a variety of inflammatory and autoimmune conditions. Recombinant human IL-1 receptor antagonist (IL-1ra) has been evaluated in several controlled clinical trials to inhibit the biological activity of IL-1, and promising results have in particular been reported in patients with rheumatoid arthritis (RA),2,4 this resulted in approval of IL-1ra as a treatment of RA in the US and several European countries. Approaches to block endogenous TNF activity, such as with monoclonal antibodies or soluble TNF receptor constructs, have yielded beneficial effects in inflammatory disorders like RA, Crohn’s disease, psoriasis and ankylosing spondylitis.5-9 However, concern has been raised about the possibility of enhanced susceptibility for infections in patients treated with anti-cytokine strategies.

This concern originated from experimental data showing that blocking either TNF or IL-1 in animals reduced host defense against live bacterial, protozoal, mycobacterial and some viral infections.10-16 Indeed, since the approval of antibodies against TNF, a higher incidence of disseminated and extrapulmonary tuberculosis in patients treated with an anti-TNF antibody has been reported.17 An increased incidence of opportunistic infections has been observed in patients receiving soluble TNF receptor p75.5,7,18,19 Although the number of patients treated with anti-TNF antibody or soluble TNF receptors is large compared to those receiving IL-1ra, in controlled clinical trials, thus far no mycobacterial or opportunistic infections have been registered in patients treated with recombinant IL-1ra.20 In addition, the number of bacterial pneumonias in IL-1ra treated RA patients is in the range of that expected for RA patients receiving conventional therapy. However, experimental evidence exists that such treatment may hamper antibacterial defense mechanisms. Indeed, we recently found that IL-1 receptor type I gene deficient (IL-1R-) mice, which completely lack the ability to transfer IL-1 signals into the cell, have a reduced ability to mount a pulmonary inflammatory response during pneumonia caused by S. pneumoniae, the most frequently isolated pathogen in community-acquired pneumonia, which was associated with an increased outgrowth of pneumococci in lungs during the first two days after the infection.10 The present study was
initiated to investigate the influence of treatment of wild type mice with IL-1ra, resulting in an incomplete and transient inhibition of IL-1 activity and therefore more appropriately mimicking the clinical situation of patients with a pharmacologically reduced bioavailability of IL-1, on host defense and mortality during pneumococcal pneumonia.

**Materials and methods**

Pneumococcal pneumonia was induced by intranasal inoculation of $10^5$ CFU *S. pneumoniae* serotype 3 (ATCC 6303; Rockville, MD), exactly as described previously. All experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, Amsterdam, the Netherlands. Since the effect of IL-1ra may differ in mice with different genetic backgrounds, the influence of recombinant IL-1ra on the course of pneumonia was evaluated in BALB/c and C57BL/6 mice (female BALB/c and C57BL/6 mice, 10 weeks of age; Harlan Sprague Dawley Inc., Horst, the Netherlands). Recombinant human IL-1ra in hyaluronic acid vehicle, serving as a sustained delivery system, was provided by Amgen (Thousand Oaks, CA) and was given intraperitoneally at 0, 24 and 48h after induction of pneumonia at a dose of 100 mg/kg of body weight. Control mice received hyaluronic acid vehicle only. Lung and blood samples were processed as described earlier. TNF (R&D systems, Abingdon, United Kingdom), IL-6 (Pharmingen, San Diego, CA) and KC (R&D systems) were measured by ELISA. Lowest detection limits were 150 pg/ml for TNF, 37 pg/ml for IL-6 and 55 pg/ml for KC. Myeloperoxidase activity was measured as described. All values are expressed as mean ± SEM. Comparisons were done with Mann-Whitney U test. Survival curves were compared by log-rank test. P-value <0.05 was considered to represent a statistically significant difference.

**Results**

IL-1ra did not influence mortality in either BALB/c or C57BL/6 mice (Figure 1). To evaluate whether the early phase of antibacterial defense was influenced by IL-1ra, we determined the number of pneumococci in the lungs 24 and 48h after inoculation (Figure 2). BALB/c mice treated with IL-1ra displayed an increased outgrowth of *S. pneumoniae* in lungs compared to vehicle treated mice at both timepoints (P<0.005). After 24h 50% of vehicle treated and 75% of IL-1ra treated BALB/c mice were bacteremic, whereas 100% and 88% resp. were bacteremic after 48h. C57BL/6 mice injected with IL-1ra had more bacteria in their lungs after 24h, but not after 48h. After 24h 75% of vehicle treated C57BL/6 mice had pneumococci in their blood, and 88% of IL-1ra treated mice; after 48h, 88% of both groups had positive blood cultures. To determine whether IL-1ra treatment affected the pulmonary inflammatory response to pneumococcal infection, we measured MPO activity and the concentrations of TNF, IL-6 and KC in lung homogenates. None of these parameters were altered by IL-1ra treatment in either BALB/c or C57BL/6 mice (data not shown). In addition, we compared the
histopathology of the lungs of each group at 24 and 48h after after inoculation with *S. pneumoniae*. IL-1ra treated mice displayed inflammatory infiltrates to the same extent as vehicle treated mice (Figure 3).

![Figure 1](image1)

**Figure 1. Recombinant human IL-1ra does not influence survival.** Survival after intranasal inoculation with *S. pneumoniae* of mice treated with recombinant human IL-1ra (100 mg/kg at 0, 24 and 48h; open squares) or vehicle (closed circles) in BALB/c (A) and C57BL/6 (B) mice. Mortality was assessed twice daily for 10 days. N=20 per group.

![Figure 2](image2)

**Figure 2. Recombinant human IL-1ra enhances bacterial outgrowth in lungs.** CFU *S. pneumoniae* in lungs of BALB/c (A) and C57BL/6 (B) mice treated intraperitoneally with vehicle (open bars) or recombinant human IL-1ra (100 mg/kg at 0 and 24h; closed bars) 24 and 48h after intranasal inoculation with *S. pneumoniae*. Data are mean ± SEM. N=8 per group per time point. *P<0.05 vs. vehicle treated mice.

**Discussion**

We here report that although IL-1ra treatment impaired the early host defense against pneumococcal pneumonia, it did not influence survival. These data are in line with our recent data obtained in the same model, revealing an increased bacterial outgrowth in lungs of IL-1R−/− mice relative to lungs of wild type mice during the first two days of the infection, without a difference in mortality. Importantly, whereas the complete absence of an IL-1 signal (as in IL-1R−/− mice) resulted in a profound reduction in the pulmonary inflammatory
response to *S. pneumoniae* infection, partial inhibition of IL-1 activity by IL-1ra did not result in a clearly altered inflammatory reaction. This latter finding should be viewed upon in the context of an earlier study in which this dose of IL-1ra in a slow-release hyaluronic acid vehicle strongly reduced joint inflammation in a rat model of rheumatoid arthritis. The difference between our study with IL-1R-/− mice and the present study could also in part be related to the fact that knockout mice may not only differ from wild type mice with respect to the product of the deleted gene, and that hereditary deficiency of IL-1R may result in compensatory changes that are not directly related to the absence of an IL-1 signal in adult life. The effect of IL-1ra did not markedly differ in BALB/c and C57BL/6 mice, although only the former strain had higher bacterial numbers in their lungs at 48h post infection.

Anti-cytokine therapy offers new expectations for the management of inflammatory diseases. We previously demonstrated that TNF is of critical importance in host defense against pneumococcal pneumonia. The present data, together with our findings in IL-1R-/− mice, suggest that IL-1 occupies a role in the pulmonary immune response to *S. pneumoniae* that by far is less prominent than that of TNF. There are two possible explanations why blocking IL-1 is not as harmful as blocking TNF. Firstly, TNF induces IFN-γ which plays a protective role against mycobacterial and fungal infections, while IL-1 does not. Secondly, anti-TNF antibodies and soluble receptors have a more prolonged TNF inhibitory effect relative to the IL-1 antagonizing effect of IL-1ra. Thus, treatment with IL-1ra results in a partial and transient blockage of IL-1 function and this might result in a return of host defense.

Figure 3. Recombinant IL-1ra does not affect inflammatory infiltrates in lungs. Representative histopathology of lungs of BALB/c mice treated with IL-1ra (A) and vehicle (B) intraperitoneally, 24h after intranasal inoculation with *S. pneumoniae* showing comparable inflammatory infiltrates. HE staining, magnification x 33.
in between the administration of IL-1ra. Further studies are warranted to establish the influence of IL-1ra therapy on host defense against other pathogens.

References