Interleukin-10 Impairs Host Defense in Murine Pneumococcal Pneumonia

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The effects of recombinant interleukin (IL)-10 and the role of endogenous IL-10 were determined in C57Bl/6 mice with pneumonia induced by intranasal inoculation with 10^6 cfu of Streptococcus pneumoniae. Pneumonia induced sustained expression of IL-10 mRNA and protein in lungs, but IL-10 remained undetectable in plasma. Intranasal inoculation of S. pneumoniae in combination with IL-10 (1500 U/mouse) resulted in decreased lung concentrations of tumor necrosis factor-α (TNF) and interferon (IFN)-γ, increased bacterial counts in lungs and blood, and early lethality. Conversely, pretreatment (−2 h) of mice with an anti–IL-10 monoclonal antibody (2 mg/mouse) was associated with increased lung levels of TNF and IFN-γ, reduced bacterial counts in lungs and plasma 40 h after the inoculation, and prolonged survival. These results indicate that during pneumococcal pneumonia, IL-10 attenuates the proinflammatory cytokine response within the lungs, hampers effective clearance of the infection, and shortens survival.

Streptococcus pneumoniae is the most common pathogen causing community-acquired pneumonia [1–3]. The estimated annual incidence of pneumococcal pneumonia is 10–250 cases/100,000 population, with a total case fatality rate of 5% and a case fatality rate of up to 60% in elderly persons with bacteremia [3]. Knowledge of the pathogenesis of pneumonia caused by S. pneumoniae is not only important because of its relatively high incidence but also because of the increase in pneumococcus resistant to penicillin and other antimicrobial agents [4, 5].

Interleukin (IL)-10 is an 18-kDa cytokine produced under different conditions of immune activation by a variety of cell types, including T cells, B cells, and monocytes/macrophages [6]. IL-10 can inhibit the stimulated production of a number of proinflammatory cytokines [7–9]. Both exogenously administered and endogenously produced IL-10 exert protective effects in various models of inflammation, including endotoxemia [10–12], staphylococcal enterotoxin B–induced shock [13, 14], peritonitis [15, 16], shock induced by administration of anti–CD3 monoclonal antibody (MAb) [17], and pancreatitis [18]. Of importance, IL-10 also abrogates local inflammatory reactions within the lung in response to intratracheal administration of lipopolysaccharide (LPS) [19], immune complex injury [20, 21], and allergic airway inflammation [22]. In the present study, we sought to determine the effects of both exogenous and endogenous IL-10 on inflammatory responses to pneumococcal pneumonia.

Methods

**Animals and reagents.** Female C57Bl/6 mice (16–20 g) were used in all experiments. Murine recombinant IL-10 was obtained as culture supernatant from CHO-K1 cells stably transfected with the corresponding CDNA, as described previously [10]. Supernatant collected from mock-transfected cells was used as control [10]. JESS-2A5 is a neutralizing rat anti-mouse IL-10 IgG1 MAb [23]. LO-DNP (gift from Hervé Bazin, University of Leuven, Leuven, Belgium), a rat IgG1 MAb, was used as isotype-matched control antibody [12, 14, 16].

**Experimental protocol.** S. pneumoniae serotype 3 (ATCC 6303) was obtained from American Type Culture Collection (Rockville, MD). Pneumococci were grown at 37°C in 5% CO₂ to midlogarithmic phase in Todd-Hewitt broth (Difco, Detroit) supplemented with 0.5% yeast extract. After incubation, aliquots were stored at −70°C until use. For each experiment, 10 mL of fresh Todd-Hewitt broth supplemented with 0.5% yeast extract was seeded with 100 μL of thawed suspension and incubated in 5% CO₂ for 6 h at 37°C. Bacteria were pelleted by centrifugation at 3000 g for 15 min, washed twice in sterile isotonic saline, and resuspended in sterile isotonic saline, and the bacterial concentration was determined by plating serial 10-fold dilutions onto sheep-blood agar plates. Mice were lightly anesthetized by inhalation of methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL), and 60 μL of the bacterial suspension was inoculated intranasally. If not stated otherwise, 10^6 cfu of S. pneumoniae was administered to each mouse. To assess whether this procedure per se induced an inflammatory response in the lungs, some mice were inoculated intranasally with 60 μL of isotonic saline only (i.e., without bacteria).
In experiments in which the effects of recombinant IL-10 were studied, washed pneumococci were resuspended in supernatant containing IL-10 or in control supernatant, after which bacteria and IL-10 were intranasally administered simultaneously (total inoculum, 60 μL). IL-10 was administered at 1500 U/mouse, an amount 1.5 times the dose previously shown to modulate cytokine release in different models of inflammation and to prevent LPS-induced lethality [10, 12, 17, 18]. We chose to administer IL-10 intranasally because other investigators had shown that systemically injected IL-10 does not exert significant antiinflammatory effects within the lung [22]. In experiments in which the effects of anti–IL-10 were studied, JESS-2A5 or LO-DNP (2 mg/mouse) was given intraperitoneally 2 h before inoculation with S. pneumoniae. This dose of anti–IL-10 enhances proinflammatory cytokine release and mortality induced by peritonitis [16] or by administration of LPS [12] or staphylococcal enterotoxin B [14]. In each case, IL-10–treated mice and their controls and anti–IL-10–treated mice and their controls were compared in one experiment, and cytokine and bacteriology data in these treatment/control groups were always determined in one run.

**Histologic examination.** Lungs for histologic examination were excised en bloc and inflated with 1 mL of 4% paraformaldehyde in PBS to improve resolution of anatomic relationships. Multiple 5.0-μm sections were obtained and stained with hematoxylin-eosin.

**Cytokine measurements.** To assess the production of IL-10 during pneumonia, blood and lungs were collected before and at 12, 24, 48, and 72 h after the administration of 10^6 cfu of S. pneumoniae (6 mice/time point). It should be noted that C57Bl/6 mice started dying from pneumonia from 48 h after the administration of 10^6 cfu of S. pneumoniae. Therefore, data obtained 72 h after inoculation represent animals that were still alive. Blood and lungs were also collected at 24 and 48 h after intranasal inoculation with isosonic saline only (5 mice/time point). At the designated time points, mice were anesthetized with inhaled methoxyflurane, blood was collected by cardiac puncture, and whole lungs were harvested for measurement of IL-10. Further, to assess the effects of recombinant and endogenous IL-10 on the production of other cytokines, blood and lungs were obtained from IL-10–treated mice at 12, 24, and 40 h after the administration of bacteria and from anti–IL-10–treated mice at 24 and 40 h (5–7 mice/group/time point). The 40-h time point (rather than 48 h) was chosen because preliminary experiments had established that IL-10–treated mice died relatively early after induction of pneumonia.

Lungs were processed exactly as described previously [16]. In brief, lungs were wrapped in aluminum foil, quick-frozen by immersion in liquid nitrogen, and stored at −70°C until homogenized. They were homogenized in 5 vol of sterile isotonic saline at 4°C with a Polytron tissue homogenizer (Brinkman Instruments, Cincinnati). Homogenates were centrifuged at 1600 g for 30 min at 4°C to remove cell debris. The cell-free supernatants were then centrifuged at 5000 g for 30 min at 4°C, after which the supernatants were filter sterilized and stored at −70°C. IL-6, IL-10, and IFN-γ were measured by ELISA (Pharmingen, San Diego). Tumor necrosis factor-α (TNF) activity was measured by use of the WEHI 164 clone 13 fibroblast cytotoxicity assay [24]. IL-1β was measured by ELISA (Genzyme, Cambridge, MA). Cytokine levels are expressed as nanograms per milliliter in plasma and as nanograms per gram of tissue in lung homogenates.

**IL-10 mRNA detection by reverse transcription–polymerase chain reaction (RT-PCR).** RT-PCR for lung IL-10 mRNA was done as described previously [16]. Lungs for RNA isolation were harvested before and at 12, 24, 48, and 72 h after intranasal inoculation with S. pneumoniae and 24 and 48 h after intranasal inoculation with isosonic saline. Lungs from 3 mice per time point were pooled. Total cellular RNA was extracted from snap-frozen lungs by use of a commercially prepared solution containing 5 M guanidine isothiocyanate, acid phenol, and 2-mercaptoethanol (RNA-zol-B; Biotec, Friendswood, TX). One microgram of total cellular RNA was reverse transcribed using 2.5 U of murine leukemia virus reverse transcriptase and 0.05 nmol of oligo(dT) (Perkin-Elmer Cetus, Norwalk, CT). cDNA was amplified (PTC-100 thermocycler; MJ Research, Watertown, MA) with 2.5 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus) and oligonucleotide primers specific for β-actin [16] or IL-10 [25]. Each of 35 amplification cycles included denaturation at 95°C, reannealing of primer and fragment at 55°C, and primer extension at 72°C.

The primers used for IL-10 were 5′-TTCCCTAATGCAGGACCTTAAGGTACTTGC-3′ (sense) and 5′-GACACCTTGCTGTTAGGACCTATTTAAAATC-3′ (antisense) [25]. Target sequences for the primers used are in different exons of the IL-10 gene [25]. The upstream primer was chosen such that it overlapped a splice junction, thereby eliminating the possibility of inadvertent genomic DNA amplification. Fifteen microliters of 100-μL reaction mixture was fractionated on a 1% agarose gel. Samples were electrophoresed at 100 V for 1.5 h and stained with ethidium bromide (1 μg/mL).

**Determination of bacterial counts.** Forty hours after intranasal inoculation with S. pneumoniae, blood was obtained aseptically from mice by retroorbital puncture. The mice (6/group) then were sacrificed by cervical dislocation, and lungs were removed aseptically and placed in 10 vol of sterile isotonic saline. Lungs were homogenized in the Polytron tissue homogenizer that was carefully cleaned and disinfected with 70% alcohol after each homogenization. Serial 10-fold dilutions in sterile isotonic saline were plated onto sheep-blood agar plates and incubated for 18 h at 37°C in 5% CO₂, after which colonies were counted.

**Statistical analysis.** All values are expressed as mean ± SE. Two-sample comparisons were made by unpaired t test. Survival curves were compared with the log-rank test. P < .05 was considered to represent a significant difference.

**Results**

**Pathology.** From 16–24 h after inoculation with S. pneumoniae, mice developed signs of systemic toxicity, including lethargy and piloerection. From 36–48 h, progressive respiratory distress occurred. Administration of 10^6 cfu of S. pneumoniae resulted in 100% lethality by 5 days after inoculation.

Gross pathologic examination revealed slightly congested but otherwise unremarkable lungs 24 h after inoculation. At 48 h after inoculation, lungs were red, wet, and firmer than normal. Histologic examination of the lungs 24 h after inoculation revealed areas of acute inflammation associated with the terminal airways. The inflammatory cell infiltrates consisted mainly of neutrophils. Pulmonary vessels were congested and contained large numbers of...
Induction of IL-10. Neither normal mice nor mice 24 or 48 h after intranasal inoculation with sterile saline had detectable IL-10 (>0.20 ng/mL) in their circulation. Similar amounts of IL-10 were detectable in lung homogenates from normal mice (1.23 ± 0.24 ng/g) and from mice after inoculation with saline (24 h: 1.35 ± 0.28 ng/g; 48 h: 1.12 ± 0.16 ng/g; nonsignificant difference). Administration of S. pneumoniae was associated with a marked increase in IL-10 concentrations in lungs (figure 1). The highest lung IL-10 levels were measured after 72 h, at the end of the observation period (25.79 ± 3.02 ng/g). IL-10 was scarcely detectable in plasma during pneumonia (data not shown).

Lung samples obtained from normal mice and from mice inoculated with saline revealed faint bands, indicating that some IL-10 mRNA is expressed constitutively in mouse lungs (figure 2). Intranasal administration of S. pneumoniae resulted in the induction of IL-10 mRNA in the lung within 12 h, as indicated by equal intensity of β-actin bands and significant differences in band intensity between control and pneumonia samples for IL-10 RT-PCR products (figure 2). The expression of IL-10 mRNA was sustained for up to 72 h after inoculation.

IL-10 levels in mice treated with recombinant IL-10. Intranasal administration of recombinant IL-10 together with S. pneumoniae resulted in lung IL-10 levels that, at 12 h after inoculation, were 1 log higher than in lungs from control mice (56.92 ± 4.71 ng/g vs. 5.49 ± 0.29, respectively, P < .001; figure 3); however, thereafter, lung IL-10 levels decreased rapidly in IL-10–treated mice. At 40 h after inoculation, lung IL-10 concentrations were similar in both groups (figure 3). None of the IL-10–treated mice had detectable IL-10 in plasma.

Effect of IL-10 and anti–IL-10 on induction of other cytokines. Treatment with recombinant IL-10 was associated with a decrease in lung TNF levels. The inhibition of TNF production was especially evident at 12 h (by 90%; P < .001), and remarkably, at 40 h (by 70%; P < .01) after the induction of pneumonia (figure 4). IL-10 treatment did not significantly influence lung IL-1β and IL-6 levels, although mean IL-6 levels were considerably lower in mice inoculated with IL-10. Further, IFN-γ concentrations in lung homogenates were lower in IL-10–treated mice at 40 h (P < .05; figure 4).

Treatment with anti–IL-10 was associated with a 3.5-fold increase in lung TNF levels 40 h after inoculation (P < .01; figure 5). Lung IFN-γ levels were also higher in anti–IL-10–treated animals at 40 h (P < .05), while lung IL-1β and IL-6 concentrations were not influenced (figure 5).

TNF (>0.02 ng/mL) and IL-1β (>0.10 ng/mL) were scarcely detectable in plasma (data not shown). IL-6 was detectable (>0.10 ng/mL) in the plasma of most mice, except for 24 h after inoculation in IL-10–treated mice, which at that time point, had significantly lower plasma IL-6 levels than control mice (<0.10 ng/mL vs. 1.80 ± 0.74 ng/mL, respectively). Interferon-γ was detectable marginating neutrophils. At 48 h after inoculation, lung lesions were more severe, with alveolar cell necrosis, alveolar hemorrhage, fibrinous exudate, and perivascular edema.

Figure 1. Mean (±SE) lung concentrations of IL-10 at various times (hours) before (0) and after (1–3) intranasal inoculation of mice with 10⁶ cfu of S. pneumoniae. At each time point, 6 mice were sacrificed, and their were lungs harvested for measurement of IL-10.

Figure 2. IL-10 mRNA and β-actin mRNA expression in lungs of mice, as determined by reverse transcription–polymerase chain reaction at indicated times (hours) before (0) and after (12–48) intranasal inoculation with 10⁶ cfu of S. pneumoniae. Saline = 24 or 48 h after inoculation with sterile isotonic saline only (i.e., without bacteria). Lungs from 3 mice were pooled at each time point.

Figure 3. Mean (±SE) lung concentrations of IL-10 at 12, 24, and 40 h after intranasal inoculation with 10⁶ cfu of S. pneumoniae in combination with recombinant murine IL-10 (1500 U/mouse) or control supernatant. n = 5–7 mice/group/time point. * P < .001 vs. control.
Figure 4. Mean (±SE) lung concentrations of tumor necrosis factor-α (TNF), IL-1β, IL-6, and interferon-γ at 12, 24, and 40 h after intranasal inoculation with 10^6 cfu of *S. pneumoniae* in combination with recombinant murine IL-10 (1500 U/mouse) or control supernatant. n = 5–7 mice/group/time point. *P < .05 or **P < .01 vs. control.

Figure 5. Mean (±SE) lung concentrations of tumor necrosis factor-α (TNF), IL-1β, IL-6, and interferon-γ at 24 and 40 h after intranasal inoculation with 10^6 cfu of *S. pneumoniae*, in mice pretreated (−2 h) with either anti-IL-10 monoclonal antibody (MAb) (2 mg/mouse) or control MAb (2 mg/mouse). n = 6–7 mice/group/time point. *P < .05 or **P < .01 vs. control.
Table 1. Effect of IL-10 and anti–IL-10 on growth of *Streptococcus pneumoniae* in lungs and blood of study mice.

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<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
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<tbody>
<tr>
<td></td>
<td>Lung (×10^7/g)</td>
<td>Blood (×10^4/mL)</td>
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<tr>
<td>IL-10</td>
<td>167 ± 118*</td>
<td>17 ± 7†</td>
</tr>
<tr>
<td>Control</td>
<td>40 ± 17</td>
<td>4 ± 2</td>
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<tr>
<td>Anti–IL-10</td>
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<td>Control MAb</td>
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NOTE. Results are from 6 mice per treatment group 40 h after intranasal inoculation with 10^6 cfu of *S. pneumoniae* in combination with either recombinant murine IL-10 (1500 U/mouse) or control supernatant (experiment 1) or in combination with pretreatment (−2 h) with either anti–IL-10 monoclonal antibody (MAb; 2 mg/mouse) or control MAb (2 mg/mouse) (experiment 2).

although 8% of anti–IL-10–treated mice were permanent survivors, compared with no control mice (figure 7). Considering that a 10^6-cfu challenge could have been too severe for a potential protective effect of anti–IL-10 to become evident, we next inoculated mice with 3 × 10^3 cfu of *S. pneumoniae*. In this experiment, anti–IL-10 afforded significant protection (*P* < .001); 64% of anti–IL-10–treated mice were permanent survivors, compared with 22% of control mice (figure 7).

Discussion

The production of IL-10 is enhanced during clinical infections [26, 27] and in various animal models of infection [12, 14, 16, 28]. The present study showed the sustained production of IL-10 within the lung during pneumococcal pneumonia.

Inhibition of IL-10 led to enhanced pulmonary production of proinflammatory cytokines, reduced bacterial growth, and an improved outcome, while intrapulmonary administration of recombinant IL-10 resulted in opposite responses. These results suggest that IL-10 impairs host defense during pneumococcal pneumonia.

Intranasal inoculation of *S. pneumoniae* with recombinant IL-10 caused a transient 10-fold elevation in lung IL-10 levels during the early phase of infection; however, 24 h after inoculation, the difference between lung IL-10 concentrations in IL-10–treated and control mice was no longer significant. Nonetheless, the effects of initially elevated IL-10 levels persisted throughout the sampling period (i.e., TNF concentrations were still markedly reduced 40 h after inoculation, and IFN-γ levels were reduced only at that time point). Thus, brief exposure to IL-10 may result in prolonged antiinflammatory effects within the lung.

While our studies were in progress, Greenberger et al. [29] reported on the effect of polyclonal anti–IL-10 antiserum in

![Figure 6](https://academic.oup.com/jid/article-abstract/174/5/994/806086)

**Figure 6.** Effect of recombinant IL-10 on survival of mice treated with recombinant murine IL-10 (1500 U/mouse) or control supernatant after intranasal inoculation with 10^6 cfu of *S. pneumoniae*. *n* = 18 mice/group. *P* indicates difference between groups by log-rank test.

![Figure 7](https://academic.oup.com/jid/article-abstract/174/5/994/806086)

**Figure 7.** Effect of anti–IL-10 on survival of mice pretreated (−2 h) with 2 mg of anti–IL-10 monoclonal antibody (MAb) or control MAb after intranasal inoculation with 10^6 or 3 × 10^3 cfu of *S. pneumoniae*. *n* = 24–27 mice/group/experiment. Anti–IL-10 did not influence survival after inoculation with 10^6 cfu of *S. pneumoniae* but improved survival after inoculation with 3 × 10^3 cfu of *S. pneumoniae* (*P* < .001, log-rank test). NS = nonsignificant.
a murine model of *Klebsiella pneumoniae* pneumonia. Their findings were similar to those in our gram-positive model: Clinical and pathologic changes developed gradually, and 100% lethality was noted 5 days after the induction of gram-negative pneumonia. However, IL-10 mRNA and protein was detected 48 h after inoculation, substantially later than in the present study (from 12 h). Administration of anti-IL-10 antiserum was associated with reduced growth of *K. pneumoniae* in lungs and plasma and prolonged survival [29]. Hence, pulmonary IL-10 may serve a similar detrimental role in the pathogenesis of pneumonia caused by *S. pneumoniae* or *K. pneumoniae*.

During overwhelming immune activation, especially in the absence of a localized infectious source, such as after administration of LPS or staphylococcal enterotoxin B, the anti-inflammatory effects of IL-10 can be beneficial [10-14]. In such models, high levels of proinflammatory cytokines appear in the circulation, and inhibition of their systemic effects confers protection against tissue injury and lethality [10-14]. However, these acute models do not provide insight into the potential beneficial effects of proinflammatory cytokines at the site of an infection and should therefore be interpreted with caution. Moreover, neutralization of IL-10 augments mouse resistance to systemic infection with *Mycobacterium avium* [30, 31]. Therefore, it seems likely that IL-10 produced during at least some active infections hampers an adequate proinflammatory response crucial for effective clearance of an infectious agent. The antiinflammatory properties of IL-10 may also explain why containment of pneumonia was compromised further by administration of recombinant IL-10. Indeed, the reduced TNF levels in mice treated with IL-10 may have contributed to reduced bacterial killing within the lung, since TNF can enhance leukocyte bactericidal activity [32]. Further, IL-10-induced inhibition of IFN-γ production may have resulted in an impaired antibacterial defense [33, 34].

It should be noted that IL-10 does not impair host defense during bacterial peritonitis induced by cecal ligation and puncture [15, 16]. Cecal ligation and puncture result in spillage of bacteria from the gut into the peritoneal cavity, leading to polymicrobial peritonitis and systemic infection [35]. Inhibition of IL-10 in this model is associated with enhanced lethality [16], while administration of recombinant IL-10 improves survival [15]. Thus, the effects of IL-10 during bacterial infections seem complex and are likely determined by the source of the infection and the functional balance between proinflammatory and antiinflammatory forces within the cytokine network.

Despite the development of potent antibiotics, pneumonia remains the sixth leading cause of death and the most frequent cause of death from infectious diseases [2]. *S. pneumoniae* is the most frequently isolated bacterium in patients with community-acquired pneumonia [1-3]. Knowledge of the role of cytokines in the pathogenesis of pneumococcal pneumonia is important, especially in an era in which patients with bacterial infections are being treated with immunomodulatory drugs meant to inhibit the activity of proinflammatory cytokines. Here we show that pneumococcal pneumonia results in sustained and compartmentalized production of IL-10 within the lung. The antiinflammatory effects of this endogenous IL-10 were associated with reduced bacterial clearance and enhanced lethality. Elevating IL-10 by intrapulmonary administration of recombinant IL-10 impaired host defense further. Although IL-10 may be useful to attenuate lung injury induced by sterile inflammatory reactions [19-22], our results together with those of Greenberger et al. [29] indicate that IL-10 is detrimental in the setting of bacterial pneumonia. These findings have particular relevance for the application of IL-10 as a treatment for patients with bacterial sepsis.

References


