Activation of neutrophils and inhibition of the proinflammatory cytokine response by endogenous granulocyte colony-stimulating factor in murine pneumococcal pneumonia


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Activation of Neutrophils and Inhibition of the Proinflammatory Cytokine Response by Endogenous Granulocyte Colony-Stimulating Factor in Murine Pneumococcal Pneumonia

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Granulocyte colony-stimulating factor (G-CSF) is considered to improve host defense during infection, via increased recruitment of and enhanced performance of neutrophils and subsequent inhibition of potentially harmful proinflammatory mediators. The present study sought to determine the role of endogenous G-CSF in host defense against pneumococcal pneumonia. Patients with unilateral community-acquired pneumonia demonstrated elevated concentrations of G-CSF in bronchoalveolar lavage fluid obtained from the infected, but not from the contralateral, site. Treatment of mice with pneumococcal pneumonia with an anti–G-CSF antibody reduced neutrophil counts in lung tissue and diminished CD11b expression on pulmonary neutrophils but increased the lung concentrations of tumor necrosis factor–α, interleukin-1β, and cytokine-induced neutrophil chemoattractant. Treatment with anti–G-CSF did not influence the outgrowth of pneumococci in lungs, the dissemination of the infection, or survival in murine pneumonia. During pneumococcal pneumonia, G-CSF is produced locally at the site of the infection, where it exerts both pro- and anti-inflammatory effects.

Pneumonia is a leading cause of morbidity and mortality and is the most frequent source of infection in severe sepsis [1, 2]. The gram-positive bacterium Streptococcus pneumoniae is responsible for >50% of cases of community-acquired pneumonia (CAP) [3–5]. Despite adequate antimicrobial therapy, mortality rates of up to 25% have been reported for hospitalized patients with pneumococcal pneumonia [5, 6]. In addition, the growing resistance of S. pneumoniae to antibiotics is an issue of worldwide concern [4, 7] that warrants expansion of our knowledge of the pathogenesis and host defense mechanisms, to identify adjunctive treatment options.

The recruitment of polymorphonuclear cells (PMNs) to the lungs is a crucial component of the host defense against pneumonia. Because of their potent phagocytic properties, PMNs are critical for the elimination of bacteria in the lower respiratory tract [8]. Among host mediators involved in the function and production of PMNs, granulocyte colony-stimulating factor (G-CSF) features prominently. G-CSF is generally known as a hematopoietic growth factor that is responsible for proliferation and maturation of bone-marrow stem cells to PMNs [9, 10]. In addition, elevated systemic and local concentrations of G-CSF are found in patients with infections [11–13]. G-CSF is a cytokine that is produced by monocytes and macrophages at the site of infection [14]. Within the pulmonary compartment, alveolar macrophages produce G-CSF after stimulation with lipopolysaccharide (LPS), interleukin (IL)–1, or tumor necrosis factor (TNF)–α [15]. Exogenously administered G-CSF has been demonstrated to enhance
the recruitment of PMNs in response to pulmonary infection and inflammation in vivo [16, 17]. In addition to this increase in chemotaxis [18], improved phagocytic activity, bactericidal function [19], and respiratory burst [20] have also been attributed to G-CSF in in vitro systems with isolated human PMNs. G-CSF is thought to act both locally, contributing to the recruitment and performance of PMNs at the site of infection, and systemically, stimulating the formation of additional PMNs, thus reinforcing the host response until the infection is resolved [21].

Of note, uncontrolled stimulation of proinflammatory pathways and recruitment of PMNs is associated with tissue injury [22]. Prolonged attraction of PMNs, caused by release of pulmonary chemokines, is a known risk factor for the development of acute lung injury [23, 24]. G-CSF has been shown to exert anti-inflammatory effects on the cytokine network, adding to the favorable properties of this cytokine [25]. G-CSF does so by stimulating the production of anti-inflammatory mediators—including IL-1 receptor antagonist (IL-1ra) [26–28], soluble TNF receptor (sTNF-R) I and II [26, 28], and IL-10 [29]—and by attenuating the release of IL-8 [27], macrophage inflammatory protein (MIP)–2 [16], TNF-α, IL-1β, IL-18, and interferon-γ [26, 30, 31]. This attractive profile of G-CSF and the concomitant availability of recombinant G-CSF have led numerous investigators to study the effect of recombinant G-CSF on the course of infections. Indeed, the administration of G-CSF improved survival rates in endotoxemia and various animal models of sepsis [32], which encouraged several groups to investigate the usefulness of G-CSF in treating pneumonia. In pneumococcal pneumonia, a survival benefit has been described in G-CSF–treated rats and splenectomized mice [33–35]. Another study found improved survival only in mice infected with a low inoculum of S. pneumoniae [36]. However, 2 recent studies of patients with multilobar pneumonia or pneumonia complicated by severe sepsis did not reveal beneficial effects of adjunct treatment with G-CSF [37, 38].

Despite the fact that many studies have investigated the utility of adjunct treatment with G-CSF in pneumonia, the role of endogenous G-CSF in the pulmonary host defense in vivo has not been examined thus far. Therefore, in the present study, we investigated local concentrations of G-CSF at the site of the infection in patients with unilateral CAP and in mice with experimentally induced pneumococcal pneumonia and studied the effect of passive immunization against G-CSF in murine pneumonia.

PATIENTS, MATERIALS, AND METHODS

Patient Study

Patients. Four patients (3 men and 1 woman; mean ± SE age, 41 ± 5 years) with unilateral CAP were enrolled in the study. Samples obtained from these patients were used previously to investigate local alterations in fibrinolysis during CAP [39]. All patients fulfilled the following criteria: fever (temperature >37.7°C), new unilateral infiltrate on chest radiograph within 2 days after admission, no antibiotic pretreatment, and arterial partial pressure of oxygen >7.5 kilopascals while breathing room air. Exclusion criteria were hospitalization within 2 weeks before admission and receipt of any immunosuppressive therapy. Ten healthy volunteers (mean ± SE age, 32 ± 8 years) not taking any medication served as control subjects. The protocol was reviewed and approved by the Medical Ethics Committee of the University of Amsterdam (Amsterdam, The Netherlands), and written, informed consent was obtained from all subjects.

Bronchoalveolar lavage (BAL). Within 12 h after admission, BAL was performed in a standardized fashion, according to the guidelines of the European Society of Pneumology [40], by use of a flexible fiberoptic video-bronchoscope. Seven successive 20-mL aliquots of prewarmed isotonic saline were instilled in a subsegment of the lung and aspirated immediately with low suction. BAL was first performed at the uninfected side of the middle lobe or lingula, followed by lavaging a subsegment of the infected lobe. In general, 10–15 mL of the instilled 20 mL was recovered. There was no difference between the recovered volumes from the infected or uninfected side.

Specimen processing. BAL fluid (BALF) was kept at 4°C until processing, which was performed within 30 min. The samples were centrifuged at 200 g for 15 min at 4°C. The first 3 recoveries were sent to the microbiology department of the University of Amsterdam for culture, and the remaining supernatant was stored at −80°C until concentrations of G-CSF were assessed. Concentrations of G-CSF were measured by use of a commercially available ELISA kit (R & D Systems), according to the instructions of the manufacturer. The limit of detection of the assay was 31 pg/mL.

Mouse Studies

Mice. Pathogen-free 6–8-week-old female BALB/c mice were obtained from Harlan Sprague-Dawley. The Animal Care and Use Committee of the University of Amsterdam (Amsterdam, The Netherlands) approved all experiments, and the protocol was reviewed and approved by the Medical Ethics Committee of the University of Amsterdam.

Anti–G-CSF antibody (Ab). Sheep polyclonal immunoglobulin (Ig) Ab to mouse G-CSF was generated as described elsewhere [41]. Administration of this Ab to mice in a dose also used in the present study resulted in a complete neutralization of endogenous G-CSF for 2–3 days, which was associated with a ~50% reduction of PMN counts in peripheral blood 2 days after inoculation [41]. The biological activity of the anti–G-CSF batch used in the present study was confirmed by demonstrating a similar 50% decrease in circulating PMNs.
24 h after treating healthy mice with anti–G-CSF, relative to mice that received sheep Ig (Sigma) (data not shown).

Induction of pneumonia and design. Pneumonia was induced as described elsewhere [42, 43]. In brief, S. pneumoniae serotype 3 was obtained from American Type Culture Collection (ATCC 6303). Pneumococci were grown to midlogarithmic phase for 6 h at 37°C by use of Todd-Hewitt broth (Difco), harvested by centrifugation at 1500 g for 15 min, and washed twice in sterile isotonic saline. Bacteria were then resuspended in sterile isotonic saline at a concentration of ∼10^8 cfu/50 μL, as determined by plating serial 10-fold dilutions on sheep-blood agar plates. Mice were lightly anesthetized by inhalation of isoflurane (Upjohn), and 50 μL (∼10^5 cfu) of S. pneumoniae was inoculated intranasally (inl). Anti–G-CSF Ig or sheep Ig (Sigma), respectively, was administered intraperitoneally in 200 μL of sterile saline 1 h before and 24 h after induction of pneumonia. In preliminary experiments, we established that sheep Ig did not influence the host response to pneumococcal pneumonia, compared with saline. At 24 and 48 h after inoculation, mice were anesthetized with Hypnorm (Janssen Pharmaceutica) and midazolam (Roche) and were killed by exsanguination of the vena cava inferior. Blood was collected in EDTA-containing tubes.

Myeloperoxidase (MPO) assay. Levels of MPO were measured as described elsewhere [42, 44]. Lung tissue was homogenized in potassium phosphate buffer (PPB), by use of a tissue homogenizer (Biospec Products), and was pelleted at 300 g for 20 min. Pelleted cells were lysed in PPB (pH 6.0) supplemented with hexadecyltrimethyl ammoniumbromide and 10 mmol/L EDTA. MPO levels were determined by measuring the H2O2-dependent oxidation of 3,3′,5,5′tetramethylbenzidine. In brief, serial dilutions of PPB were mixed with tetramethylbenzidine substrate N,N′dimethylformamide. The reaction was stopped with glacial acetic acid followed by reading of optical density at 655 nm. MPO activity is expressed as activity per gram of lung tissue per reaction time. All reagents were purchased from Sigma.

Histologic examination. Lungs for histologic examination were harvested at 24 and 48 h after inoculation, fixed in formalin, and embedded in paraffin. Sections (4 μm thick) were stained with hematoxylin-eosin and analyzed by a pathologist who was blinded to group identity. To score lung inflammation and damage, the lung sample was screened for the following parameters: interstitial inflammation, intra-alveolar inflammation, edema, endothelialitis, bronchitis, pleuritis, and thrombus formation. Each parameter was graded on a scale of 0 to 3 (0, absent; 1, mild; 2, moderate; and 3, severe). The total injury score was expressed as the sum of the score for all parameters, with a maximum of 21.

Lung cell counts and fluorescence-activated cell sorter (FACS) analysis. For FACS analysis and cell counts, whole lungs were flushed with sterile saline via the pulmonary artery, to eliminate blood leukocytes. Pulmonary cell suspensions were then obtained from whole lungs by use of an automated disaggregation device (Medimachine System; Dako) and resuspended in FACS buffer (PBS supplemented with 0.5% bovine serum albumin, 0.01% NaN3, and 100 mmol/L EDTA), exactly as described elsewhere [42, 45]. Total numbers of cells were counted from each sample by use of a hemocytometer (Türck chamber), and differential cell counts in lungs were performed on cytospin preparations stained with Giemsa. For analysis of PMN activation, lung cells were stained with anti-CD11b–fluorescein isothiocyanate and anti–Gr-1–phycoerythrin for 30 min at 4°C, washed twice in FACS buffer, and analyzed by flow cytometry using a FACSScan (Becton Dickinson). To correct for aspecific staining, appropriate isotype controls were used. All reagents were purchased from BD Pharmingen and were used in concentrations recommended by the manufacturer.

Preparation of lung tissue for cytokine measurements. At the time of death, whole lungs were harvested and homogenized at 4°C in 4 volumes of sterile saline by use of a tissue homogenizer (Biospec Products). For cytokine measurements, lung homogenates were diluted 1:2 in lysis buffer containing 300 mmol/L NaCl, 30 mmol/L Tris, 2 mmol/L MgCl2, 2 mmol/L CaCl2, 1% Triton X-100, and pepstatin A, leupeptin, and aprotonin (all at concentrations of 20 ng/mL; pH 7.4) and were incubated for 30 min at 4°C. Homogenates were centrifuged at 1500 g for 15 min at 4°C, and supernatants were stored at −20°C until assays were performed. All reagents were purchased from Sigma.

Cytokine and chemokine assays. Cytokines and chemokines (G-CSF, TNF-α, IL-1β, and cytokine-induced neutrophil chemoattractant [KC]) were measured by use of specific ELISAs

<table>
<thead>
<tr>
<th>Patient (sex)</th>
<th>BALF culture for Streptococcus pneumoniae</th>
<th>G-CSF in BALF, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected side</td>
<td>Healthy side</td>
</tr>
<tr>
<td>1 (M)</td>
<td>Positive</td>
<td>465</td>
</tr>
<tr>
<td>2 (F)</td>
<td>Negative</td>
<td>12,239</td>
</tr>
<tr>
<td>3 (M)</td>
<td>Positive</td>
<td>84</td>
</tr>
<tr>
<td>4 (M)</td>
<td>Negative</td>
<td>ND</td>
</tr>
</tbody>
</table>

NOTE. Concentrations of G-CSF were measured in BALF samples obtained from patients with community-acquired pneumonia (CAP).
Elevated concentrations of G-CSF in BALF samples from mice with Streptococcus pneumoniae pneumonia, by time after inoculation.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Below LOD</td>
<td>Below LOD</td>
</tr>
<tr>
<td>Control</td>
<td>199 ± 55</td>
<td>176 ± 82</td>
</tr>
<tr>
<td>Anti–G-CSF</td>
<td>Below LOD</td>
<td>Below LOD</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SE (pg/mL) of 6–8 mice/group. Mice were treated intraperitoneally with anti–G-CSF or control Ig 1 h before and 24 h after intranasal (inl) inoculation with 10⁶ cfu of S. pneumoniae, and concentrations of G-CSF in plasma were measured 24 and 48 h after induction of pneumonia. Mice inoculated inl with sterile saline served as uninfected controls. LOD, limit of detection.

**Results**

**Elevated concentrations of G-CSF in BALF samples from patients with CAP.** To obtain insight into local concentrations of G-CSF during pneumonia, 4 patients with unilateral CAP underwent bilateral BAL within 12 h after admission, first at the uninfected site and then in the area with the infiltrate on the chest radiograph. Concentrations of G-CSF in BALF and plasma were measured by use of an ELISA; BALF samples obtained from healthy subjects served as controls (table 1). Concentrations of G-CSF were increased in BALF samples obtained from infected lungs of 3 of 4 patients but were close to or below the limits of detection in uninfected lungs. Plasma concentrations of G-CSF were below the limit of detection in all patients with CAP, as were concentrations of G-CSF in plasma and BALF samples obtained from healthy control subjects. Hence, G-CSF was detectable locally at the site of infection in patients with CAP.

**Elevated concentrations of endogenous G-CSF during murine pneumococcal pneumonia.** We then investigated whether concentrations of endogenous G-CSF increase in mice after inoculation with S. pneumoniae. For this purpose, concentrations of G-CSF were measured in plasma and lung homogenates obtained from mice treated with sheep Ig or anti–G-CSF before infection with S. pneumoniae and were compared with concentrations in mice inoculated inl with sterile saline only. Induction of pneumonia was associated with moderately elevated concentrations of G-CSF in plasma of mice treated with sheep Ig but not in anti–G-CSF–treated mice (table 2). G-CSF could not be detected in plasma of healthy, uninfected mice (table 2). At 24 and 48 h after inoculation with S. pneumoniae, concentrations of G-CSF were increased in the lungs of up to 50% of sheep Ig–treated mice (630–770 pg/mL at 24 h in 3/8 mice and 660–4630 pg/mL at 48 h in 4/8 mice) but were below the limit of detection in all anti–G-CSF–treated mice and in mice without pneumonia.

**No major effect of anti–G-CSF on recruitment of pulmonary PMNs.** Because G-CSF is thought to contribute primarily to the enhanced recruitment of PMNs to the site of infection, we first examined whether endogenous G-CSF had an effect on the influx of pulmonary PMNs in pneumonia. Levels of MPO in the lungs and the extent of lung inflammation (as assessed by histopathologic examination) were indistinguishable between control and anti–G-CSF–treated mice 24 h after induction of pneumonia (figure 1 and table 3). However, 48 h after inoculation, levels of MPO were significantly higher in the lungs of control mice (figure 1). Despite a similar degree of lung inflammation in both groups of mice, the inflammatory infiltrate was predominantly composed of PMNs in control mice but was predominantly composed of mononuclear cells in anti–G-CSF–treated mice (figure 2 and table 3). At 48 h, the numbers of circulating PMNs were decreased in anti–G-CSF–treated mice, compared with those in control mice (726 ± 120 vs. 2516 ± 427 PMNs/μL; P<.05), as opposed to the number of PMNs in blood at baseline (1418 ± 88 PMNs/μL for anti–G-CSF–treated mice vs. 1781 ± 186 PMNs/μL for control mice;
Table 3. Histologic scores of lung inflammation in mice with *Streptococcus pneumoniae* pneumonia, by time after inoculation.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.9 ± 0.4</td>
<td>6.3 ± 1.1</td>
</tr>
<tr>
<td>Anti–G-CSF</td>
<td>6.9 ± 0.7</td>
<td>5.8 ± 1.3</td>
</tr>
</tbody>
</table>

**NOTE.** Data are lung inflammation mean ± SE score of 8 mice/group. Mice were treated intraperitoneally with anti–granulocyte colony-stimulating factor (G-CSF) or control Ig at 1 h before and 24 h after intranasal inoculation with 10^5 cfu of *S. pneumoniae*. Lung inflammation at 24 and 48 h after inoculation was scored as described in Patients, Materials, and Methods.

P, not significant [NS]). This result prompted us to more precisely investigate the cellular composition of lungs 48 h after inoculation with *S. pneumoniae*. To avoid the possibility that contaminating blood leukocytes influenced pulmonary cell counts, lungs were flushed thoroughly via the pulmonary artery until they appeared to be macroscopically pale. As shown in table 4, total counts of lung cells and PMNs were slightly, but not statistically significantly, lower in anti–G-CSF–treated mice. Surprisingly, the number of macrophages was reduced in lungs of anti–G-CSF–treated mice, whereas the number of lymphocytes was increased in lungs of anti–G-CSF–treated mice (both P < .05 vs. controls). At 48 h, the numbers of blood lymphocytes and monocytes were unaltered in anti–G-CSF–treated mice, compared with those in control mice (lymphocytes, 1854 ± 164 vs. 1570 ± 132 cells/μL; monocytes, 3379 ± 516 vs. 3261 ± 448 cells/μL; P NS). Thus, treatment with anti–G-CSF did not significantly impair the pulmonary influx of PMNs but affected the recruitment of macrophages and lymphocytes to the lungs.

**Activation of PMNs by endogenous G-CSF.** In addition to exerting chemotactic activities, G-CSF has been reported to be involved in the activation of PMNs, including the up-regulation of CD11b [28]. We therefore investigated the activation state of recruited lung PMNs by measuring expression of CD11b on Gr-1^+^ cells in whole-lung cell suspension 48 h after induction of pneumonia. Expression of CD11b was significantly elevated on Gr-1^+^ PMNs from control mice, compared with those from mice treated with anti–G-CSF (mean fluorescence intensity, 332.3 ± 57.0 vs. 202.1 ± 38.0; P < .05).

**Bacterial clearance unimpaired by anti–G-CSF.** Having shown that G-CSF contributes to the activation of PMNs, we next studied whether one of the main biological tasks of PMNs (i.e., the elimination of bacteria) was impaired in anti–G-CSF–treated mice. Lung colony-forming unit counts were slightly higher in anti–G-CSF–treated mice at 24 and 48 h after inoculation, but the differences with control mice did not reach significance (figure 3). Similarly, the number of bacteremic mice was identical in anti–G-CSF–treated and control mice 48 h after induction of pneumonia (3/8 mice with positive blood cultures in both groups), whereas blood cultures were negative in all mice at 24 h. Hence, endogenous G-CSF activity does not have a major effect on bacterial clearance in pneumococcal pneumonia.

**Increased proinflammatory cytokines and CXC chemokines in anti–G-CSF–treated mice.** Among the proinflammatory cytokines, TNF-α and IL-1β are key mediators in host defense against *S. pneumoniae* pneumonia [43, 46], and anti-inflam-
S. pneumoniae.

Cell counts and differentials were performed on cell suspensions obtained from the site of infection from patients and lung homogenates from mice at that time point (P \(<\) .05, vs. controls; figure 4). These chemokine KC was markedly increased in anti–G-CSF–treated controls; figure 4). In addition, the important murine CXC chemokine KC was markedly increased in anti–G-CSF–treated mice at that time point (P \(<\) .05, vs. controls; figure 4). These differences disappeared 48 h after induction of pneumonia (data not shown). Hence, endogenous G-CSF attenuated the early proinflammatory cytokine and chemokine response in pneumococcal pneumonia.

Influence of anti–G-CSF on survival in S. pneumoniae pneumonia. Finally, we examined the effect of endogenous G-CSF on survival. After inh inoculation with 10^5 cfu of S. pneumoniae, 50% of control mice (6/12) and 69% of anti–G-CSF–treated mice (9/13) survived (P \(<\) .05, vs. controls; figure 4). These differences disappeared 48 h after induction of pneumonia (data not shown). Hence, endogenous G-CSF attenuated the early proinflammatory cytokine and chemokine response in pneumococcal pneumonia.

**DISCUSSION**

*S. pneumoniae* is the leading causative pathogen of CAP, and infection with pneumococci is associated with high morbidity and mortality rates worldwide. Although G-CSF has gained much attention as an adjunct treatment modality in pneumonia [37, 38], the role of endogenous G-CSF in host defense against pneumococcal pneumonia in vivo has thus far never been investigated. We therefore decided to determine the biological significance of G-CSF production triggered by infection. We have demonstrated here increased levels of G-CSF in BALF samples obtained from the site of infection from patients with unilateral CAP. By neutralizing endogenous G-CSF immediately before induction of pneumonia in mice, we have observed that the role of endogenous G-CSF in the host response to *S. pneumoniae* pneumonia is 2-sided. On the one hand, G-CSF was involved in the activation of pulmonary PMNs, whereas, on the other hand, treatment with G-CSF reduced concentrations of TNF-\(\alpha\), IL-1\(\beta\), and KC in the lungs during pneumonia. However, G-CSF did not influence local antibacterial defense or the dissemination of bacteria and did not modify the clinical outcome of the infection.

Our findings in patients with CAP confirm and extend those of previous studies. Mice with *Escherichia coli* infection have elevated levels of G-CSF in their BALF [14]. Ex vivo, alveolar macrophages from patients with respiratory tract infections have been shown to release G-CSF [15]. We have extended these findings and pinpointed production of G-CSF to the site of infection by studying 4 patients with unilateral CAP, 2 of whom were infected with *S. pneumoniae*. Similar to other researchers, we observed a wide range of concentrations of G-CSF in patients with infection [13], but we were unable to detect G-CSF in plasma samples obtained from patients with CAP. We also observed a wide range of levels of G-CSF in lungs of mice infected with *S. pneumoniae*. Although the sensitivity of the G-CSF assay was reduced because of dilution of both BALF specimens from patients and lung homogenates from mice, our results clearly indicate that G-CSF is produced locally after infection of the lower respiratory tract.

It is generally accepted that endogenous G-CSF is important for maintaining adequate numbers of circulating PMNs in healthy subjects and during infections. Indeed, G-CSF–deficient mice are neutropenic and have a diminished increase in PMNs after infection, which limits their utility in infectious disease models [48]. Similarly, in a model of peritonitis, neutralizing G-CSF activity 3–5 days before infection rendered mice neutropenic and led to decreased recruitment of PMNs and impaired bacterial clearance with increased lethality [41]. Despite many studies of the effect of exogenously administered G-CSF, reports about the role of endogenous G-CSF during infections are confined to a few observations. One study observed an unaltered host response when endogenous G-CSF was neutralized immediately before induction of peritonitis [41], whereas, more recently, Noursadeghi et al. demonstrated that an acute-phase response–induced increase in levels of endogenous G-CSF before induction of *E. coli* sepsis exerts beneficial

![Figure 3](image329x138 to 538x238)
Figure 4. Higher concentrations of tumor necrosis factor (TNF)–α, interleukin (IL)–1β, and cytokine-induced neutrophil chemoattractant (KC) in lungs, caused by treatment with anti–granulocyte colony-stimulating factor (anti–G-CSF). Mice were treated intraperitoneally with anti–G-CSF or control immunoglobulin (Ig) 1 h before intranasal inoculation with 10⁵ cfu of Streptococcus pneumoniae, and pulmonary cytokines/chemokines were measured 24 h after induction of pneumonia. Data are mean ± SE of 8 mice/group. *P < .05, vs. control.

Figure 5. Unimpaired survival and blocking of endogenous granulocyte colony-stimulating factor (G-CSF). Mice were treated intraperitoneally with anti–G-CSF or control immunoglobulin (Ig) 1 h before and 24 h after intranasal inoculation with 10⁵ cfu of Streptococcus pneumoniae, and survival was monitored for 7 days. Data are mean ± SE of 12 mice/group.

In the present study, we pretreated mice with anti–G-CSF 1 h before intranasal inoculation with S. pneumoniae. This approach was chosen to inhibit the endogenous G-CSF generated in response to the infection. Earlier administration of anti–G-CSF (i.e., several days before infection) renders mice neutropenic [41], which is expected to impair host defense against pneumococcal pneumonia by mechanisms not directly related to G-CSF produced during respiratory tract infection. By doing so, we observed only a modest reduction in recruitment of PMNs to the site of infection in anti–G-CSF–treated mice, as indicated by slightly lower PMN counts in lungs of these mice (statistically not significantly different from control mice), together with reduced levels of MPO in lungs 48 h after induction of pneumonia. Of interest, anti–G-CSF significantly decreased the number of macrophages attracted to the pulmonary compartment, which might be responsible, in part, for the more-profound effects of this Ig on levels of MPO [50]. Decreased monocyte attraction was reported earlier in G-CSF–deficient mice infected with Listeria monocytogenes [48, 51], suggesting that G-CSF plays a broad role in multilineage cell recruitment. In addition, administration of exogenous G-CSF to volunteers challenged with LPS resulted in an increase in monocyte counts in the circulation [30]. To our surprise, we observed an increased recruitment of cells with morphologic features of lymphocytes to lungs of mice pretreated with anti–G-CSF. Zhan et al. reported a similar observation in G-CSF–deficient mice with L. monocytogenes peritonitis [52] and disclosed that these cells exhibit some characteristics of poorly differentiated macrophages, although their precise origin remains to be elucidated.

The key event in host defense against pneumococcal pneumonia is the elimination of bacteria from the lungs and the prevention of systemic bacterial dissemination. Previous investigations reported improved bacterial clearance in G-CSF–treated mice with pneumococcal pneumonia [35, 36]. Despite the fact that G-CSF is known to improve bactericidal properties of neutrophils and macrophages [19, 20, 53], we did not observe impaired bacterial clearance in anti–G-CSF–treated mice. This phenomenon might be explained, in part, by the fact that concentrations of protective mediators, such as TNF-α and IL-1β, were significantly higher in the lungs of these mice. Indeed, TNF-α and IL-1, in particular, are associated with improved bacterial clearance and synergistically enhance survival in S. pneumoniae pneumonia [43, 46]. Of note, Dallaire et al. did not find any effect of administration of recombinant G-CSF on concentrations of TNF-α or IL-1β in lungs during pneumococcal pneumonia [36]. However, our present findings are in agreement with those of earlier reports on diminished release of TNF-α and IL-1β from whole blood and from isolated monocytes, respectively, stimulated in the presence of G-CSF [30, 31] and with in vivo findings obtained in a pneumococcal meningitis model [54]. In the present study, we have also revealed an increase in pulmonary concentrations of KC after administration of anti–G-CSF, which might have compensated, in part, for the loss of the chemotactic activity of G-CSF on PMNs in mice injected with the Ab. Dallaire et al. reported decreased levels of KC in mice with pneumococcal pneumonia treated with recombinant G-CSF, which is in line with these findings [36].
In summary, our results show that (1) endogenous G-CSF inhibits the proinflammatory arm of the cytokine network in vivo, reducing the concentrations of TNF-α, IL-1β, and KC at the site of infection during pneumonia; (2) endogenous G-CSF contributes to activation of PMNs recruited to the lung; and (3) the net effect of endogenous G-CSF on antibacterial defense and the clinical outcome of pneumococcal pneumonia is limited. Further studies are warranted to investigate the role of endogenous G-CSF in other types of pneumonias, such as those caused by Klebsiella pneumoniae, in which detrimental effects of exogenous G-CSF have been reported in animal models [55].

Acknowledgments

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