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Bacterial Clearance and Survival Are Dependent on CXC Chemokine Receptor-2 Ligands in a Murine Model of Pulmonary *Nocardia asteroides* Infection

Thomas A. Moore,* Michael W. Newstead,* Robert M. Strieter,* Borina Mehrad,* Blaine L. Beaman,† and Theodore J. Standiford*

Survival from murine pulmonary nocardiosis is highly dependent on CXC chemokine receptor-2 (CXCR2) ligand-mediated neutrophil chemotaxis and subsequent clearance of the infectious agent *Nocardia asteroides*. Intratracheal inoculation of *N. asteroides* rapidly up-regulated the CXC chemokines macrophage inflammatory protein-2 (MIP-2) and KC within 24 h, with levels remaining elevated through day 3 before returning to near baseline levels by day 7. Coinciding with elevated MIP-2 and KC were the rapid recruitment of neutrophils and clearance of the organism. Anti-Ly-6G Ab-mediated neutrophil depletion before bacterial challenge resulted in strikingly increased mortality to *N. asteroides* infection. The relative contribution of MIP-2 in neutrophil recruitment was examined by anti-MIP-2 Ab treatment before nocardial infection. MIP-2 neutralization had no detrimental effects on survival, neutrophil recruitment, or bacterial clearance, suggesting the usage of additional or alternative CXCR2-binding ligands. The importance of the CXC family of chemokines was determined by the administration of an anti-CXCR2 Ab capable of blocking ligand binding in vivo. Anti-CXCR2 treatment greatly increased mortality by preventing neutrophil migration into the lung. Paralleling this impaired neutrophil recruitment was a 100-fold increase in lung bacterial burden. Combined, these observations indicate a critical role for neutrophils and CXC chemokines during nocardial pneumonia. These data directly link CXCR2 ligands and neutrophil recruitment and lend further support to the concept of CXC chemokine redundancy. For infections highly dependent on neutrophils, such as nocardial pneumonia, this is of critical importance. *The Journal of Immunology*, 2000, 164: 908–915.

Pulmonary nocardiosis can be an acute or chronic infection characterized by a supplicative response with pathology ranging from mild, diffuse peribronchial infiltration to lobar or multilobar consolidation (1, 2). Granulomatous lesions may form with or without central necrosis. Lesions usually consist of a mixed cellular response of neutrophils, macrophages, and lymphocytes. Dissemination beyond the primary pulmonary site of infection may occur, with nearly one-half of the patients with systemic nocardiosis having CNS involvement. Whereas mortality is low (<20%) in patients with no predisposing underlying illness, mortality rates of >50% exist in patients with disseminated disease. Although *Nocardia asteroides* is considered an opportunistic pathogen, 30–40% of published patient cases have no predisposing underlying illness.

The rigorous characterization of >50 strains of *N. asteroides* has greatly facilitated the study of bacterial-host interactions (reviewed in Ref. 2). Virulent strains of *N. asteroides* have evolved unique mechanisms of evading host macrophage-mediated killing. Specifically, virulent strains can inhibit phagosome-lysosome fusion, decrease lysosomal enzyme activity, and neutralize phagosomal acidification (3–7). These evade mechanisms result in the ability of the organism to grow intracellularly within alveolar macrophages. During experimental pulmonary nocardiosis, the inflammatory response is predominantly neutrophilic, subsequently replaced by a mononuclear infiltration by day 7. However, the precise role of neutrophils during infection is unresolved. In vitro data indicate that neutrophils and monocytes only marginally kill phagocytized *N. asteroides* (8–11). In contrast, in vivo depletion of neutrophils using a polyclonal anti-neutrophil Ab or monocytes using silica resulted in increased nocardial burden (12, 13).

Chemokines are a growing collection of chemotactic cytokines divided into four families (14). The CXC chemokine family can be further subdivided based on the presence or absence of a three-amino acid motif termed ELR (glutamic acid-leucine-arginine). ELR + CXC chemokines, including IL-8, epithelial neutrophil-activating protein-78, macrophage inflammatory protein-2 (MIP-2),3 and KC, have potent neutrophil chemotactic activity (15–17). Two receptors for ELR + CXC chemokines have been identified in humans, CXC chemokine receptor-1 and -2 (CXCR1 and CXCR2) (18, 19). Murine CXCR2, like its human counterpart, binds all

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3 Abbreviations used in this paper: MIP-2, macrophage inflammatory protein-2; CXCR1 and 2, CXC chemokine receptor-1 and -2; LIX, LPS-induced CXC chemokine; BHI, brain-heart infusion; MPO, myeloperoxidase; H&E, hematoxylin and eosin; GMS, Gomori methenamine-silver.
ELR⁺ CXC chemokines (20–23). Interestingly, mice lack expression of CXCR1 (20). These receptors, like all chemokine receptors identified to date, are seven-transmembrane spanning, G-protein-coupled receptors. Neutrophils from CXCR2 knockout mice fail to migrate in response to MIP-2 and KC in vitro (24), confirming the exclusive utilization by CXC chemokines of this receptor on neutrophils.

Here, we detail the dependence of CXCR2 ligand-mediated neutrophil recruitment for effective host defense in pulmonary infections due to N. asteroides. These data directly link CXCR2-binding chemokines and neutrophil recruitment during N. asteroides pulmonary infection and indicate a critical role for neutrophils and CXC chemokines during the initial phases of pulmonary nocardiosis.

Materials and Methods

Mice

Female BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were used between the ages of 5–8 wk, being age-matched within a given experiment. All mice were housed under specific-pathogen-free condition within the animal care facility at the University of Michigan.

N. asteroides growth and intratracheal inoculation

N. asteroides strain GUH-2 was originally isolated from a fatal kidney infection in a renal transplant patient. It is a highly virulent strain of Nocardia with growth characteristics that have been well documented (25–27). Briefly, brain heart infusion (BHI) broth (Difco, Detroit, MI) starter cultures were inoculated from late stationary phase frozen stocks. Cultures were grown for 4–6 days on an orbital shaker at 37°C. Overnight cultures were then inoculated by transferring 10–30 μl of a late stationary phase starter culture into 50 ml BHI broth and shaken for 16–18 h. The bacterial concentration of early to mid-log phase cultures was then determined by measuring absorbance at 580 nm and compared with a predetermined standard curve. The filamentous, branching morphology characteristic of early log phase growth was confirmed for each culture by Gram stain (Difco). Bacteria were then diluted to the desired concentration for intratracheal inoculation. BALB/c mice were anesthetized with pentobarbital (diluted 1:7 in saline). The trachea was exposed, and 30 μl inoculum or saline administered via a sterile 26-gauge needle. An aliquot of the inoculated nocardial suspension was serially diluted onto blood agar plates to determine actual dose of intratracheally injected bacteria.

In vivo Ab administration

Neutrophils were depleted in vivo utilizing the pan-granulocytic Ab RB6-8C5 (28), directed against Ly-6G. Anti-Ly-6G was produced as an ascites in SCID mice by TSD BioServices (Germantown, NY) and used at a dilution determined to deplete both peripheral blood and resident lung neutrophils. The Ab was injected in a 0.5-ml volume i.p. 18 h before nocardial infection and again 1 day postinfection. This treatment scheme has been used to deplete neutrophils in vivo in a variety of bacterial and fungal models (29–31). Reagent control animals for anti-Ly-6G received normal rabbit serum i.p. in our hands, treatment did not alter the number of circulating neutrophils in BALB/c mice. Additionally, i.p. injection of anti-CXCR2 Ab abrogated neutrophil recruitment into the peritoneum in response to exogenous KC (data not shown). Reagent control animals for anti-CXCR2 received normal goat serum, and control mice in anti-MIP-2 experiments received normal goat serum and control mice in anti-MIP-2 experiments received normal goat serum and control mice in anti-MIP-2 experiments received normal goat serum and control mice in anti-MIP-2 experiments received normal goat serum and control mice in anti-MIP-2 experiments received normal goat serum. In our hands, infected mice receiving control reagents for anti-Ly-6G, anti-CXCR2, and anti-MIP-2 had no detrimental effects when compared with infected animals alone.

Whole lung homogenization for CFU, myeloperoxidase (MPO), and cytokine analysis

At designated time points, the mice were euthanized by inhalation of CO₂. The lungs were perfused with 1 ml PBS, 5 mM EDTA and removed for analyses as previously described (34). Briefly, lungs were homogenized with a tissue homogenizer (Biospec Products, Bartlesville, OK) in 1 ml PBS-complete protease inhibitor mixture (Boehringer Mannheim Biochemical, Chicago, IL). For lung CFU determination, a small aliquot of lung homogenate was serially diluted and plated on blood agar plates and incubated at 37°C, and colonies were counted.

Lung MPO activity, as an indirect measurement of total neutrophil numbers, was quantitated by a method described previously (34). Briefly, 100 μl lung homogenate were mixed with 100 μl MPO homocytogenization buffer (0.5% hexadecyltrimethylammonium bromide and 5 mM EDTA) and vortexed. The mixture was sonicated and centrifuged at 14,000 rpm for 15 min. The supernatant was then mixed 1:15 with assay buffer and read at 490 nm. MPO units were calculated as the change in absorbance over time.

For total lung cytokine ELISA analyses, lung homogenates were sonicated briefly to ensure complete cellular disruption, then centrifuged at 2500 rpm for 10 min. The supernatants were collected and assessed for cytokine levels by ELISA. Murine MIP-2 and KC were quantitated by a modification of a sandwich ELISA method. This methodology allows detection of MIP-2 and KC at concentrations of 20 pg/ml and higher. Additionally, assays have been shown to be specific for the indicated murine chemokine and show no cross-reactivity with any other murine cytokines tested (34).

Total lung leukocyte preparation

Total lung leukocytes were isolated from N. asteroides-infected mice on day 1 postinfection. Lungs were removed from euthanized animals, and leukocytes were prepared as previously described (35). Briefly, lungs were minced with scissors to a fine slurry in 15 ml ligation digestion buffer (RPML 1640, 5% FCS, 1 mg/ml collagenase (Boehringer Mannheim Biochemical), 30 μg/ml DNase (Sigma, St. Louis, MO)). Lungs slurries were enzymatically digested for 30 min at 37°C. Any undigested fragments were further dispersed by drawing the solution up and down through the bore of a 10-ml syringe. The total lung cell suspension was pelleted, resuspended, and spun through a 20% Percoll gradient to enrich for leukocytes before further analysis. Cell counts and viability were determined by trypan blue exclusion counting on a hemacytometer. Cytospin slides were prepared and stained with a modified Wright-Giemsa stain to determine percent and total lung neutrophil numbers.

Tissue harvesting for histological examination

Lungs for histochemistry were perfused with 4% paraformaldehyde in PBS, then inflated with 4% paraformaldehyde to improve resolution of anatomic relationships, and excised at the hilum. Lungs sections were then stained with hematoxylin and eosin (H&E) to determine inflammatory responses during infection and Gomori methenamine-silver (GMS) to determine the presence of N. asteroides.

Statistical analysis

Statistical significance was determined using the unpaired, two-tailed alternate Welsh t test and the nonparametric Mann-Whitney test. Calculations were performed using InStat For Macintosh (GraphPad Software, San Diego, CA).

Results

Dose-dependent mortality from nocardial pneumonia induced by N. asteroides strain GUH-2

Murine nocardial pneumonia was induced by the intratracheal injection of log growth phase cultures of the highly virulent strain GUH-2 of N. asteroides into BALB/c mice. To determine susceptibility in our model, various doses of GUH-2 were injected and animals were observed for signs of illness and mortality. Inoculum doses of less than 2 × 10⁶ CFU resulted in clinical signs of illness within one day post infection, which subsequently resolved without any mortality observed (Fig. 1). Doses of GUH-2 ranging between 3–4 × 10⁶ CFU induced significant signs of overt illness within one day which progressively worsened over the next 1–2 days, resulting in significant mortality. Mice surviving the first 3–4
inflammation within the lumen of distal airways 1 day after inoculation with GUH-2. Branching *Nocardia* organisms were still observed on day 3, although fewer in number. By day 7, few *Nocardia* were seen, in agreement with the paucity of recoverable CFU at this time point (~0.5% of the total inoculated dose of *N. asteroides*, data not shown).

**Effect of neutrophil depletion on nocardial pneumonia mortality**

The rapid increase in lung MPO activity suggested an important role for neutrophils in the resolution of *N. asteroides* pulmonary infection. To test this hypothesis, mice were depleted of neutrophils in vivo before intratracheal GUH-2 inoculation utilizing a mAb directed against the pan-granulocytic marker Ly-6G. Mice depleted of their neutrophils before infection required 100-fold fewer inoculated *N. asteroides* bacteria to achieve an equivalent lethal dose seen in non-neutrophil-depleted mice (Fig. 4, LD<sub>20</sub> 3 × 10<sup>6</sup> CFU in neutrophil-depleted animals vs 3 × 10<sup>6</sup> in control mice).

**Chemokine production after intratracheal inoculation with *N. asteroides***

Data thus far indicated a vigorous and rapid host response to nocardial pulmonary challenge, resulting in a neutrophil dominant inflammatory response. Given that ELR<sup>+</sup> chemokines exhibit potent neutrophil chemotactic activity in vitro and in vivo, production of the murine ELR<sup>+</sup> chemokines MIP-2 and KC was determined in lung homogenates after *N. asteroides* challenge (Table I). MIP-2 production was rapidly up-regulated within 24 h after nocardial challenge, with levels increasing through day 3 followed by a significant reduction by day 7 postchallenge. KC secretion was also rapidly induced within 24 h. Unlike MIP-2 production, KC levels increased only slightly by day 3, followed by a rapid decrease by day 7. The return to near baseline levels of both MIP-2 and KC by day 7 after infection is in concordance with both the reduction in lung MPO activity and histological examination, indicating the replacement of neutrophils within the sites of inflammation with mononuclear cells.

**Effect of anti-CXCR2 treatment on nocardial pneumonia mortality**

The rapid and sustained production of high levels of MIP-2 suggested that this chemokine may be an important component in neutrophil recruitment during *N. asteroides* infection. To test this hypothesis, mice were treated with an anti-MIP-2 Ab capable of inhibiting the bioactivity of MIP-2 in vivo (17). Interestingly, inhibition of MIP-2 activity before infection had no detrimental effect on survival after *N. asteroides* challenge (data not shown). Furthermore, anti-MIP-2 administration had no effect on the total number of pulmonary neutrophils recruited following nocardial infection or on pulmonary bacterial clearance (data not shown).

This anti-MIP-2 data suggested the dependence on alternative or additional chemokines for neutrophil recruitment during pulmonary nocardiosis. Examining the role of individual CXC chemokines as an entire family, a ligand-blocking polyclonal goat anti-mouse CXCR2 Ab was utilized. This Ab blocks the binding of MIP-2, KC, and other ELR<sup>+</sup> CXC chemokines to the CXCR2 receptor, thereby inhibiting neutrophil chemotaxis in vitro and in vivo. Importantly, mice treated with anti-CXCR2 were significantly more susceptible to nocardial infection (Fig. 5), analogous to that observed after depletion of neutrophils with anti-Ly-6G.
Effect of anti-CXCR2 or anti-Ly-6G treatment on neutrophil recruitment after N. asteroides infection

To assess the effect of anti-CXCR2 or anti-Ly-6G treatment on neutrophil recruitment during N. asteroides infection, total lung neutrophil numbers were examined after infection and Ab treatment. Total pulmonary neutrophils were determined by enzymatic dissociation of lung leukocytes to observe directly any effect on neutrophil recruitment by either anti-CXCR2 or anti-Ly-6G treatment. Anti-CXCR2 and anti-Ly-6G treated mice were highly susceptible to nocardial infection; therefore the inoculum dose of GUH-2 was reduced to 1–2 × 10^{5} CFU from 2–3 × 10^{6} to ensure mouse survival 24 h postinfection. This decreased dose resulted in 100% mortality in neutrophil-depleted mice by day 2 while still presenting a significant challenge to nondepleted mice. Intratracheal challenge with N. asteroides resulted in a 4-fold increase in total lung neutrophil numbers 1 day postinfection (Fig. 6). Anti-CXCR2 treatment did not decrease the baseline number of lung neutrophils below that of mice challenged with intratracheal saline. However, pretreatment of mice with anti-CXCR2 Ab prevented
neutrophil recruitment into the lung after bacterial challenge. In contrast, mice treated with anti-Ly-6G followed by bacterial challenge had a dramatic decrease in total lung neutrophils compared with saline administration or after GUH-2 infection.

Histological examination of lung sections from GUH-2-infected mice pretreated with anti-CXCR2 or anti-Ly-6G Abs confirmed both the extensive growth of branching chains of *N. asteroides* localized predominantly in the distal airways and the paucity of inflammatory cell influx 24 h postinfection (Fig. 7). Filamentous *N. asteroides* extended into pulmonary airspaces in anti-CXCR2 or anti-Ly-6G-treated mice. In comparison, airspace invasion was only rarely seen in control infected mice.

**Effect of anti-CXCR2 or anti-Ly-6G on pulmonary bacterial burden**

The effect of anti-CXCR2 or anti-Ly-6G treatment on bacterial clearance was determined 24 h after inoculation. Treatment of mice with anti-CXCR2 or anti-Ly-6G before bacterial inoculation resulted in a >100 fold increase in total lung bacterial burdens when compared with control Ab-treated mice (Fig. 8).

**Effect of anti-CXCR2 or anti-Ly-6G treatment on pulmonary MIP-2 and KC production**

MIP-2 and KC are both rapidly produced at high levels during the normal course of nocardial infection. Because anti-CXCR2- or anti-Ly-6G-treated mice developed invasive disease in response to *N. asteroides* challenge, we reasoned that both MIP-2 and KC would be strongly up-regulated in these mice. MIP-2 (2.7-fold) and KC (3.6-fold) were induced in control mice infected with a sublethal dose (1–2 $\times 10^5$ CFU) of GUH-2, albeit at lower levels than seen with higher inoculum doses (see Table I). Both anti-CXCR2- and anti-Ly-6G-treated mice had greatly enhanced production of MIP-2 (anti-CXCR2, 63 fold; anti-Ly-6G, 59 fold) and KC (anti-CXCR2, 347-fold; anti-Ly-6G, 205-fold) compared with saline-treated mice. These elevated levels are in part likely due to the increased pulmonary bacterial burden seen in these mice (Fig. 8).

**Discussion**

We have established a murine model of acute pulmonary nocardiosis via intratracheal inoculation of the highly virulent strain

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**Table I. Kinetics of MIP-2 and KC production during *N. asteroides* pulmonary infection**

<table>
<thead>
<tr>
<th>Group</th>
<th>MIP-2</th>
<th>KC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
</tr>
<tr>
<td>Saline</td>
<td>0.10 (0.01)</td>
<td>0.09 (0.014)</td>
</tr>
<tr>
<td>GUH-2</td>
<td>14.88 (3.07)</td>
<td>155.02 (56.27)</td>
</tr>
</tbody>
</table>

* BALB/c mice were intratracheally inoculated with 2–3 $\times 10^7$ CFU GUH-2 bacteria. Lungs were then harvested on the indicated time points, homogenized, and then assayed for MIP-2 and KC protein by ELISA as described in Materials and Methods. Data are presented as mean (SEM) and were generated from 2–3 independent experiments with a total of 15–20 mice.

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**FIGURE 5.** Effect of CXCR2 receptor blockade on nocardial pneumonia mortality. Mice were injected i.p. 2 h before nocardial infection with 0.5-ml volume of a polyclonal goat anti-mouse CXCR2 Ab and again 36 h postinfection. Mice were infected with the indicated doses of nocardial strain GUH-2 bacteria and effects on mortality were determined. Curves were generated from 2 independent experiments per bacterial dose with a total of 10–15 mice per dose.

**FIGURE 6.** Total lung neutrophil numbers in anti-CXCR2- or anti-Ly-6G-treated mice 1 day post-pulmonary nocardial infection. Because anti-Ly-6G- or anti-CXCR2-treated mice are significantly more susceptible to infection (see also Figs. 4 and 5), the dose of GUH-2 was decreased to 1–2 $\times 10^5$ CFU to ensure survival 1 day after infection. Lungs were harvested 24 h postinfection, and total neutrophils were determined from enzymatically digested lung leukocyte preparations. Even at this lowered inoculum dose, there is a 4-fold increase in total neutrophils in infected Ab control mice. Anti-CXCR2 treatment prevented the recruitment of neutrophils in response to infection ($p < 0.005$ vs Ab control group) without altering the total number of resident lung neutrophils seen in saline control animals. Anti-Ly-6G treatment dramatically reduced the total number of lung neutrophils compared with saline treatment alone ($p < 0.001$). Statistical significance was determined by the unpaired, two-tailed Alternate Welsh t test and nonparametric Mann-Whitney test. Both tests resulted in nearly identical significant $p$ values. Ab control group is a composite of mice receiving either normal goat serum (control for anti-CXCR2) or normal mouse serum (control for anti-Ly-6G ascites). Results are from three independent experiments consisting of three mice per group per experiment.
GUH-2 of *N. asteroides*, thereby allowing the detailed examination of early events during host innate immune responses to this organism. Intratracheal infection rapidly up-regulated pulmonary MIP-2 and KC production, with levels remaining elevated for several days before returning to near baseline levels by day 7. Coincident with rapid CXC chemokine production was neutrophil recruitment into sites of active infection. As MIP-2 and KC levels decreased, neutrophils were replaced by mononuclear cells in sites of inflammatory response. Lung bacterial burden also decreased over time such that by day 7 fewer than 0.5% of inoculated CFU were recovered from infected lungs.

The rapid up-regulation of the ELR<sup>+</sup>CXC chemokines MIP-2 and KC suggests an important role for this family of chemokines in neutrophil recruitment during pulmonary nocardiosis. When MIP-2 bioactivity was neutralized in vivo before infection, no detrimental effects on survival, neutrophil recruitment, or bacterial clearance were seen. CXC chemokines display functional redundancy and overlapping neutrophil chemotactic activity between numerous members of this chemokine family. Our data using a ligand blocking anti-CXCR2 Ab clearly indicates a critical role for CXCR2 ligands as a family in the recruitment of neutrophils to sites of nocardial infection. Of the murine CXCR2 ligands, MIP-2 and KC are the best studied. MIP-2 and KC have been shown to play important roles in neutrophil dependent pulmonary host defenses in response to other bacterial pathogens (17, 36). The inability of neutralizing anti-MIP-2 Ab to alter neutrophil recruitment during *N. asteroides* infection suggests additional or alternative CXCR2 ligand usage. The rapid and sustained production of KC during the first 3 days post inoculation would suggest an important role for this chemokine during nocardiosis. We are currently attempting to generate in vivo neutralizing anti-KC Abs which will allow us to systematically determine the effects of anti-KC alone or in combination with anti-MIP-2. Other intriguing ELR<sup>+</sup>CXC chemokine candidates include LPS-induced CXC chemokine (LIX) and the recently described lungkine. LIX has significant structural homology with human epithelial neutrophil-activating protein-78 and granulocyte chemotactic protein-2 (37, 38). During murine endotoxemia, prominent LIX expression was...
observed in heart tissue with lesser amounts seen in lung (39). Lung kinase of particular interest in that it is selectively produced by bronchoepithelial cells and induces neutrophil migration in vivo and in vitro (40). Although not formally shown to utilize the CXCR2 receptor, similarities in biological activity of lung kinase with known CXCR2 ligands suggest usage of this receptor. Current studies are ongoing examining the role of lung kinase and LIX in the host response to *N. asteroides* pulmonary infection.

Our observations point to a critical role for neutrophils in host survival and eventual resolution of pulmonary nocardiosis. Mice treated with anti-CXCR2 or anti-Ly-6G are exquisitely sensitive to nocardial infection, requiring 100 fold fewer inoculated bacteria to achieve an equivalent lethal dose seen in control mice. These mice are also severely impaired in their ability to clear the pulmonary infection, displaying a 100 fold increase in bacterial burden 24 h after inoculation. These data extend a previous report indicating increased pulmonary nodular burden in neutrophil depleted mice (12). Histological sections confirm the extensive growth of branching chains of *N. asteroides* in the absence of neutrophils (Fig. 7). Nocardial filaments were also seen extending from masses of *Nocardia* into pulmonary airspaces. This type of airspace invasion was rarely observed in nondepleted animals. Additionally, epithelial cells lining the airspaces of nocardial infected, neutrophil depleted mice appear enlarged and possibly disrupted. The strain of *N. asteroides* used in these studies (GIU-2) has been shown to specifically bind to and penetrate pulmonary epithelial cells in vivo and in vitro (27, 41). It is possible that in the absence of neutrophils, enhanced pulmonary epithelial cell invasion occurs, resulting in cellular injury and a likely worsening pathology.

In addition to inducing neutrophil migration, ELR+ CXC chemokines have been shown to activate neutrophils. Mice receiving anti-CXCR2 treatment before infection still contained significantly more neutrophils than did anti-Ly-6G treated mice (Fig. 6). It is likely that anti-CXCR2 treatment, in addition to blocking neutrophil recruitment, also prevented these resident cells from becoming activated by the high levels of CXC chemokines produced locally in the lung. This may in part explain why anti-CXCR2 treated mice displayed identical mortality rates and pulmonary bacterial burden as anti-Ly-6G mice while containing significantly more neutrophils.

The CXCR2 blockade studies clearly indicate the necessity of CXC chemokines for neutrophil recruitment in response to *N. asteroides* pulmonary challenge and that in their absence mortality rates are greatly elevated. Receptor blockade or ligand neutralization studies are useful for determining which cytokines are required for the clearance of an infectious agent. A logical extension of these studies would be to determine the therapeutic benefits of specific cytokine overexpression during infection. We have recently shown that transgenic mice expressing the chemokine KC driven by a lung specific promoter have increased resistance to *Klebsiella pneumoniae* infection (36). Preliminary studies using these mice indicate that lung specific overexpression of KC confers enhanced resistance to *N. asteroides* infection. Further studies are ongoing examining the role of KC in pulmonary nocardiosis.

We observed enhanced production of MIP-2 and KC in anti-CXCR2 or anti-Ly-6G treated mice suggesting that CXC chemokine production is independent of total lung neutrophil numbers. Elevated levels of MIP-2 and KC in these treated mice is in part likely due to the increased pulmonary bacterial burden in these mice (Fig. 8). Alternatively, the absence of recruited neutrophils into the lung may result in impaired binding and uptake of CXCR2 ligands via receptor internalization.

Our data provide direct evidence linking CXCR2 ligands and neutrophil recruitment during nocardial pneumonia. Furthermore, the inability of anti-MIP-2 treatment to negatively alter the outcome of infection lends further support to the concept of CXC chemokine redundancy, resulting in the increased potential for neutrophil recruitment in the absence of any single CXC chemokine. For infections highly dependent on neutrophils, such as pulmonary nocardiosis, this is of critical importance.

References


