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Lung-Specific Transgenic Expression of KC Enhances Resistance to *Klebsiella pneumoniae* in Mice[1]

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A vigorous host response is required to effectively clear pathogenic bacteria from the lungs and is dependent upon the recruitment and activation of neutrophils and macrophages. A family of chemotactic cytokines, referred to as chemokines, have been shown to participate in this complex protective response. In this study, we assessed the role of the C-X-C chemokine KC in lung antibacterial host defense using wild-type (wt) B6D2 mice or transgenic mice that had been bred on a B6D2 background expressing KC under the control of a Clara cell-specific promoter within the lung. The administration of *Klebsiella pneumoniae* to both wt and KC-transgenic mice resulted in a time-dependent expression of KC protein within the lung that peaked at 24 to 48 h postinoculation. When infected with *K. pneumoniae*, the KC-transgenic mice showed a striking improvement in survival compared with wt control mice. This improved survival was due to an increase in bacterial clearance, which occurred in association with a vigorous recruitment of neutrophils in the KC-transgenic mice compared with their wt control counterparts. No differences in the lung levels of the specific cytokines TNF-α, IFN-γ, IL-12, and IL-10 were noted. However, inducible macrophage inflammatory protein-2 levels were significantly decreased in the KC-transgenic mice compared with the wt mice. This study indicates that the compartmentalized overexpression of KC in vivo results in increased lung bacterial clearance and improved survival, which occurs in association with enhanced polymorphonuclear leukocyte influx to the lung. *The Journal of Immunology,* **1998, 161: 2435–2440.**

Despite the development of new broad spectrum antibiotics, the morbidity and mortality from pneumonia remains substantial (1). The clinical management of patients continues to be challenging, particularly in the face of multidrug-resistant Gram-negative bacteria such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (2, 3). Effective host defense against lung bacterial infection is primarily dependent upon the rapid clearance of the organism from the respiratory tract. This clearance is known to be mediated by neutrophils and macrophages (Mϕ)[3] that are vigorously recruited and/or activated at the site of infection (4). Leukocyte recruitment is an inherently integral component of the host defense cascade and is mediated in part by the production of chemotactic cytokines (5).

Four closely related polypeptide chemotactic cytokine or chemokine families, C-C, C-X-C, C, and C-X-X-X-C chemokines, are now known to exist in humans; most have murine homologues (6–9). The C-C and C-X-C chemokines, in particular, have been recognized as crucial mediators in several inflammatory disease states (8, 9). More importantly, both families of chemokines have been shown to be critical participants in host defense against infection (10–13). In *Cryptococcus neoformans* pneumonia, the neutralization of Mϕ inflammatory protein (MIP)-1α and monocyte chemoattractant protein-1 (MCP-1/JE), which are members of the C-C chemokine family, resulted in a significant decrease in Mϕ and lymphocyte influx and a consequential increase in fungal burden and mortality (11, 12). In a murine *Klebsiella* pneumonia model, the C-X-C chemokine MIP-2 has been shown to be an important contributor of neutrophil recruitment and bacterial clearance (13). Specifically, neutralization with anti-MIP-2 Abs significantly attenuated neutrophil influx, resulting in increased bacterial burden and early mortality (13).

KC is a member of the C-X-C chemokine family. Like other members of the family, KC is broadly produced by numerous cells, including fibroblasts, endothelial cells, and peritoneal and alveolar Mϕ (14–17). In vitro, these cells express KC in response to inflammatory signals, such as LPS and the host-derived cytokines IFN-γ and platelet-derived growth factor, suggesting a prominent role for KC in the inflammatory cascade (18, 19). Similar to other members of the C-X-C chemokine family, KC induces neutrophil chemotaxis and weakly stimulates respiratory burst to levels that are comparable with those of its human homologue, growth-regulated gene-α (20). In vivo, KC is expressed in the rat models of acute pulmonary inflammation that are induced by endotoxin, sulfur dioxide-mediated chronic bronchitis, and vanadium exposure (20–23), while the inhibition of KC has been shown to substantially attenuate the accumulation of neutrophils in the lungs after the intratracheal (i.t.) administration of LPS (20). Furthermore, the intrapulmonary administration of KC induces a dose-dependent neutrophilic influx that is compartmentalized to the lungs (20). Finally, KC and other chemokines have been implicated in extrapulmonary inflammatory responses, including the thrombogenesis that occurs in injured endothelium (24).

Several KC-transgenic mouse models have been developed and have been shown to promote controlled neutrophilic recruitment in

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[3] Abbreviations used in this paper: Mϕ, macrophage(s); MIP, macrophage inflammatory protein; MCP-1/JE, monocyte chemoattractant protein-1; i.t., intratracheal; PMN, polymorphonuclear leukocyte; wt, wild-type; BAL, bronchoalveolar lavage; MPO, myeloperoxidase.

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selectively targeted compartments (25–28). The programmed expression of the KC transgene using a thymus-specific or a human keratin promoter resulted in neutrophilic aggregates in the thymic cortical and juxtamedullary regions or epidermis, respectively (25). Moreover, transgenic mice that were generated under control of a myelin basic protein promoter expressed the KC transgene only in the central nervous system, resulting in site-specific polymorphonuclear leukocyte (PMN) sequestration (26). More recently, Lira and colleagues have constitutively expressed KC within the lungs using the transgenic KC gene fused to a Clara cell-specific promoter (27). The increased KC expression resulted in enhanced neutrophil migration within the lungs of transgenic mice (27). The lung overexpression of KC did not result in a substantial activation of recruited neutrophils, as evidenced by the lack of tissue damage histologically. This transgenic model allowed us the unique opportunity to examine the role of KC in lung host defense against Gram-negative bacterial organisms.

In this study, we describe the time-dependent expression of KC protein within the lungs after i.t. *K. pneumoniae* administration and correlate the changes in KC expression with an influx of neutrophils. In addition, transgenic mice were employed to determine whether overexpression of the KC chemokine could have a beneficial effect on bacterial clearance and survival in the setting of Gram-negative bacterial pneumonia.

Materials and Methods

Animals

Specific pathogen-free transgenic KC mice, $CC51^{+/+}$ (2–3-mo-old males and females), on a B6D2 background were generated from a microinjection of fertilized eggs carrying the KC transgene in their genome. The expression of the KC transgene was localized to the lung by incorporating regulating elements from the CC10 gene, which encodes for a 10-kDa protein of unknown function that is produced by nonciliated bronchial epithelial cells (Clara cells) (25–27). Age- and gender-matched wild-type (wt) B6D2 mice of the same species as the transgenic mice served as control animals. All mice were housed under specific pathogen-free conditions within the animal care facility at the University of Michigan until the day of sacrifice.

K. pneumoniae *inoculation*

We chose to use *K. pneumoniae* strain 43816, serotype 2 (American Type Culture Collection, Manassas, VA) in our studies, as this strain has been shown to induce an impressive inflammatory response in mice (13, 28, 29). *K. pneumoniae* was grown in tryptic soy broth (Difco, Detroit, MI) for 18 h at 37°C. The concentration of bacteria in the broth was determined by measuring the amount of absorbance at 600 nm. A standard of absorbancies based on known CFU was used to calculate the inoculum concentration. A dose of 3×10^3 organisms per transgenic or wt mouse was chosen, since this dose allowed for the development of substantial inflammation by 36 to 48 h without excessive mortality at that timepoint. Animals were anesthetized i.p. with ~1.8 to 2 mg of pentobarbital per animal. The trachea was exposed, and 30 μl of inoculum or saline was administered via a sterile 26-gauge needle. The skin incision was closed with surgical staples.

Bronchoalveolar lavage (BAL)

Bronchoalveolar lavage was performed to obtain BAL cells. The trachea was exposed and intubated using a 1.7-mm OD polyethylene catheter. BAL was performed by instilling PBS containing 5 mM EDTA in 1-ml aliquots. Approximately 5 ml of lavage fluid was retrieved per mouse. Cytospins were subsequently prepared from BAL cells and stained with Diff-Quick (Baxter, McGaw Park, IL); next, differential counts were determined.

Myeloperoxidase (MPO) assay

Lung MPO activity (as a measure of neutrophil quantity) was determined using a method that has been described previously (13). Briefly, lungs were homogenized in 2 ml of a solution containing 50 mM potassium phosphate (pH 6.0) with 5% hexadecyltrimethylammonium bromide and 5 mM EDTA. The resultant homogenate was sonicated and centrifuged at $12{,}000 \times g$ for 15 min. The supernatant was then mixed with assay buffer to a 1:15 ratio and read at 490 nm. MPO units were calculated as the change in absorbance over time.

Lung histologic evaluation

Mice were sacrificed by carbon dioxide inhalation. The pulmonary vasculature was perfused with 4% paraformaldehyde in PBS via the right ventricle. Lungs were then excised en bloc and inflation-fixed in 4% paraformaldehyde in PBS. The lungs were then embedded in paraffin, and sections were cut and stained with hematoxylin and eosin using standard techniques.

Lung harvesting for cytokine analysis

Mice were sacrificed by carbon dioxide inhalation at designated timepoints, and blood was collected by orbital bleeding or direct cardiac puncture. Whole lungs were then harvested for assessment of the various cytokine protein levels. Before lung removal, the pulmonary vasculature was perfused via the right ventricle with 1 ml of PBS containing 5 mM EDTA. After removal, whole lungs were homogenized in 1.5 ml of complete protease inhibitor lysis buffer (Boehringer Mannheim, Indianapolis, IN). Homogenates were incubated on ice for 30 min and then centrifuged at 2500 rpm for 10 min. Supernatants were collected, passed through a 0.45-μ filter (Gelman Sciences, Ann Arbor, MI), and then stored at −20°C for an assessment of cytokine levels.

Determination of plasma and lung K. pneumoniae *CFU*

At the time of sacrifice, plasma was collected, the right ventricle was perfused with 1 ml PBS, and then the lungs were removed aseptically and placed in 3 ml of sterile saline. The tissues were subsequently homogenized with a tissue homogenizer under a vented hood. The lung homogenates were placed on ice, and serial 1/10 dilutions were made. A total of 10 μl of each dilution was plated on soy-based blood agar plates (Difco) and incubated for 18 h at 37°C; colonies were counted after the incubation.

Murine cytokine ELISA

Murine TNF-α, IFN-γ, IL-12, IL-10, MIP-2, MCP-1/JE, and KC were quantitated using a modification of a double ligand method as described previously (13). Briefly, flat-bottom, 96-well microtiter plates (Immuno-Plate I 96-F, Nunc, Fisher Scientific, Itasca, IL) were coated with 50 μl/well of rabbit Ab against the various cytokines (1 μg/ml in 0.6 M NaCl, 0.26 M H_3BO_4, and 0.08 M NaOH, pH 9.6) for 16 h at 4°C and then washed with PBS (pH 7.5) plus 0.05% Tween 20 (wash buffer). Microtiter plate nonspecific binding sites were blocked with 2% BSA in PBS and incubated for 90 min at 37°C. The plates were rinsed four times with wash buffer, and diluted (neat and 1/:10) cell-free supernatants (50 μl) in duplicate were added; next, the plates were incubated for 1 h at 37°C. The plates were washed four times, 50 μl/well of biotinylated rabbit Abs against the specific cytokines was added (3.5 μg/ml in PBS (pH 7.5) 0.05% Tween 20, and 2% FCS), and the plates were incubated for 30 min at 37°C. Next, the plates were washed four times, streptavidin-peroxidase conjugate (Bio-Rad, Richmond, CA) was added, and the plates were incubated for 30 min at 37°C. The plates were washed four additional times, and chromogen substrate (Bio-Rad) was added. We incubated the plates at room temperature to the desired extinction, and the reaction was terminated with 50 μl/well of 3 M H_2SO_4 solution. The plates were read at 490 nm in an ELISA reader. Standards were one-half log dilutions of recombinant murine cytokines from 1 pg/ml to 100 ng/ml. This ELISA method consistently detected murine cytokine concentrations that were >25 pg/ml. The ELISA did not cross-react with IL-1, IL-2, IL-4, or IL-6. In addition, the ELISA did not cross-react with other members of the murine chemokine family, including murine RANTES or epithelial neutrophil-activating protein-78.

Statistical analysis

Data were analyzed by a Macintosh computer using the Statview II statistical package (Abacus Concepts, Berkeley, CA). The survival data were compared using by χ^2 analysis. All other data were expressed as the mean ± SEM and compared using a two-tailed Student's *t* test. Data were considered statistically significant if *p* values were <0.05.

Results

Time-dependent production of KC after i.t. inoculation with K. pneumoniae

In uninfected mice at baseline, there was a significant quantity of KC detected within the lungs of KC-transgenic mice, whereas no KC was detected in the lungs of wt control animals (Fig. 1, $p < 0.001$). The i.t. administration of *K. pneumoniae* (3×10^3 CFU) resulted in a time-dependent production of KC in the lungs of both

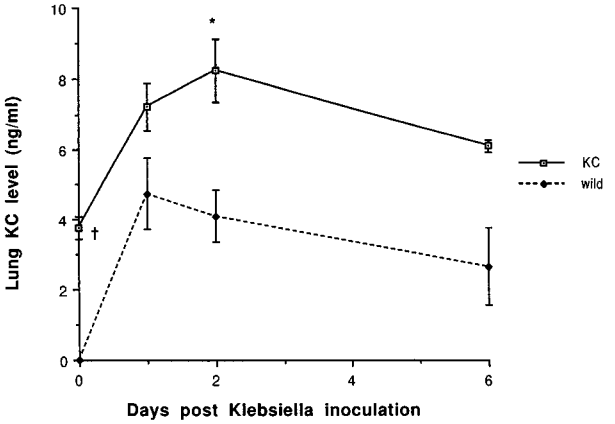

FIGURE 1. Time-dependent production of KC in lung homogenates and plasma after inoculation with *K. pneumoniae* (3×10^3 CFU). KC = transgenic mice; wild = wt control mice. *$p < 0.05$ compared with wt mice. n = five to six animals per group.

KC-transgenic and wt mice as determined by a specific ELISA. Maximal lung KC levels peaked at 48 h following bacterial inoculation; the levels of KC were significantly greater in transgenic mice compared with the levels observed in wt controls ($p = 0.01$). In both KC-transgenic mice and wt control mice, the levels of KC remained elevated at 6 days postinoculation compared with noninfected controls. No increase in KC was observed in the blood of either infected KC-transgenic or wt mice compared with noninfected counterparts (data not shown).

Effect of compartmentalized overexpression of KC on survival in Klebsiella *pneumonia*

Given the potent neutrophil chemotactic effects of KC, we next sought to determine whether compartmentalized overexpression of KC within the lung would provide protective effects in animals challenged with intrapulmonary *K. pneumoniae*. For these studies, KC-transgenic and wt mice were administered *K. pneumoniae* i.t. at 3×10^3 CFU, which us an approximately LD_{80-90} dose in wt mice. At 48 h after *K. pneumoniae* inoculation, both transgenic and wt animals developed signs of pneumonia, including lethargy and ruffled fur. However, survival was significantly greater in KC-transgenic mice that had been challenged with *K. pneumoniae*, with 80% long-term survival observed in this group compared with only 10% survival in the wt infected control mice (Fig. 2).

Effect of KC overexpression on bacterial clearance in Klebsiella *pneumonia*

To determine whether the observed improvement in survival in KC-transgenic mice was due to an enhancement in bacterial clearance, transgenic mice and wt control mice were administered *K. pneumoniae* (3×10^3 CFU) i.t., and lungs and plasma were harvested at 24 and 48 h postinoculation. At 24 h, wt animals had an approximately eightfold greater number of *K. pneumoniae* CFU in the lung compared with KC-transgenic animals that had been challenged with *K. pneumoniae* (data not shown). In addition, 66% of wt animals were bacteremic by 24 h, whereas no mice overexpressing KC were bacteremic at this timepoint after *K. pneumoniae* administration. By 48 h, a more than fourfold increase in *K. pneumoniae* CFU in blood was noted in wt control mice compared with transgenic KC mice ($p < 0.05$). An even more striking 13-fold increase in *K. pneumoniae* CFU was noted in the lungs ($p < 0.005$) of wt animals compared with mice overexpressing KC at the latter timepoint (Table I). These results indicate that KC overexpression significantly augmented effective bacterial clearance in the lung and limited the dissemination of the organism to the bloodstream.

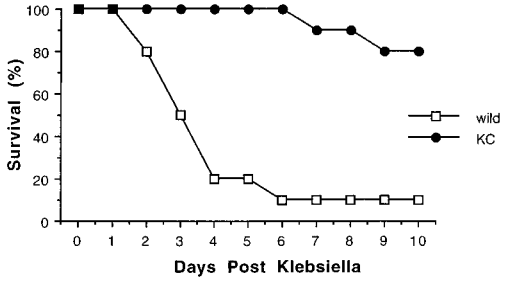

FIGURE 2. Effect of KC overexpression on the survival of mice after inoculation with *K. pneumoniae* (3×10^3 CFU). *$p < 0.05$ compared with wt mice. $n = 10$ mice per group. KC = transgenic mice; wild = wt control mice. Results are representative of two separate experiments.

Table I. *Effect of lung KC overexpression on bacterial clearance in* Klebsiella *pneumonia*[a]

Site	Treatment	*K. pneumoniae* CFU (48 h)
Plasma	wt	12.3 ± 1.9
	KC-transgenic	3.1 ± 1.7[b]
Lung	wt	$117.5 \pm 2.6 \times 10^4$
	KC-transgenic	$8.7 \pm 2.2 \times 10^4$[c]

[a] The effect of KC overexpression on *K. pneumoniae* bacterial clearance in lung homogenates and plasma at 48 h postinoculation is shown (3×10^3 CFU).
[b] $p < 0.02$;
[c] $p < 0.005$ (transgenic mice compared with wt control mice). $n = 16$ mice per group at 48 h. Data are means ± SEM.

Characterization of the lung inflammatory cell influx in KC-transgenic mice after i.t. inoculation with K. pneumoniae

To determine whether the improvement in bacterial clearance in transgenic KC mice was due to alterations in the recruitment of inflammatory cells to the airspace, KC-transgenic animals or wt controls were challenged with *K. pneumoniae*, and BAL was performed at 48 h postinoculation. This timepoint was chosen because a maximum influx of neutrophils in response to the i.t. administration of *K. pneumoniae* occurs at 48 to 72 h postchallenge (11, 17). In uninfected mice, no differences in the percent or absolute number of total BAL cells, alveolar Mϕ, or lymphocytes was noted in KC-transgenic mice compared with control animals. However, a significant increase in the percent neutrophils ($12.2 \pm 4.1\%$ KC-transgenic vs $0.17 \pm 0.17\%$ wt; $p < 0.05$) and total neutrophils ($10.8 \pm 3.1 \times 10^4$ KC-transgenic vs $0.67 \pm 0.67 \times 10^4$ wt; $p < 0.05$) was observed in KC-transgenic mice compared with wt mice. The intrapulmonary administration of *K. pneumoniae* in transgenic mice resulted in a marked increase in the total number of cells; this increase was largely due to a substantial increase in BAL fluid neutrophils ($p < 0.05$) (Fig. 3). Challenge with *K. pneumoniae* did not significantly alter the numbers of BAL fluid Mϕ or lymphocytes in either the transgenic or wt mice. To confirm the BAL findings, lungs were harvested at 48 h postinoculation with *K. pneumoniae* or saline and assayed for lung MPO activity. In animals that were challenged with *K. pneumoniae*, there was a significant increase in lung MPO activity in the KC-transgenic mice compared with wt controls (data not shown). These results indicate that the augmented host response observed in KC-transgenic mice is associated with an enhanced influx of neutrophils into the airspace.

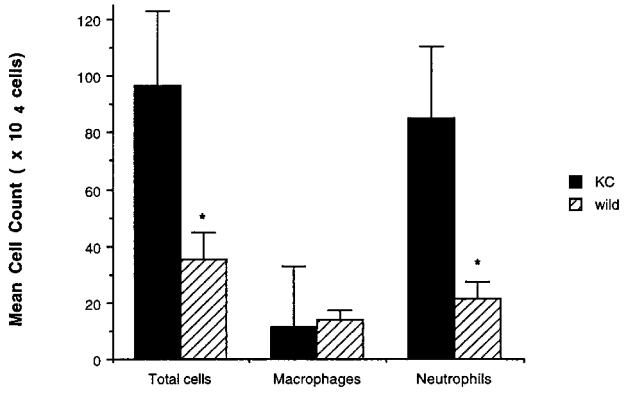

FIGURE 3. Effect of KC overexpression on BAL cell counts at 48 h following *K. pneumoniae* administration (3×10^3 CFU). *$p < 0.05$ compared with wt mice that had been challenged with *K. pneumoniae*. KC = transgenic mice; wild = wt control mice. $n = 14$ mice per group.

Anatomic distribution of inflammation in KC-transgenic and wt mice after i.t. inoculation with K. pneumoniae

The transgenic expression of KC using a Clara cell-specific promoter has been shown to result in a predominant expression of KC within the terminal bronchioles, with lesser quantities observed in larger airways, including bronchi (25). Given this anatomic distribution of KC transgene expression, we subsequently wanted to determine whether differences in the distribution of the inflammatory response to *Klebsiella* challenge were observed in KC-transgenic mice compared with wt control animals. Little to no pulmonary inflammation was observed in either KC or wt mice at 48 h or at 7 or 14 days after the i.t. administration of saline (data not shown). At 48 h following the administration of 3×10^3 CFU of *K. pneumoniae*, the lungs of infected wt mice showed focal consolidation with an infiltration of moderate numbers of neutrophils into the airspaces; some airspaces contained large numbers of extracellular *K. pneumoniae* (Fig. 4*A*). In contrast, the lungs of KC mice contained focal areas of dense airspace neutrophil accumulation and no visible extracellular bacteria. In addition, neutrophil aggregates were observed in the terminal airways that were contained and adjacent to areas of alveolar consolidation (Fig. 4*B*). By 7 days after *Klebsiella* administration, a partial resolution of the alveolar inflammatory cell infiltrates was observed in transgenic mice, although persistent neutrophil aggregates within distal airways were also observed (data not shown).

Effect of lung KC overexpression on the production of cytokines within the lung after i.t. challenge with K. pneumoniae

Subsequent experiments were performed to determine whether the compartmentalized overexpression of KC resulted in an altered production of important pro- and antiinflammatory cytokines. Several cytokines have been shown to be crucial to antibacterial host defense in Gram-negative infection, particularly TNF-α, T1 phenotype cytokines (IFN-γ and IL-12), the T2 phenotype cytokine IL-10, and members of the C-X-C and the C-C chemokine families (MIP-2 and MCP-1/JE, respectively). The baseline levels of TNF-α, IFN-γ, IL-12, IL-10, MIP-2, and MCP-1/JE were low or undetectable in the uninfected lungs of KC-transgenic animals, and no differences were seen in these levels compared with wt animals. The i.t. administration of *K. pneumoniae* resulted in a maximal expression of cytokines at 48 h in both KC-transgenic and wt mice, representing at least a fourfold increase in the protein levels of all cytokines assessed (TNF-α, IFN-γ, IL-12, IL-10, MCP-1/JE, and MIP-2), when compared with the expression seen in lung homogenates prepared from saline-treated control animals. There were no differences in the protein levels of TNF-α, IFN-γ, IL-12, IL-10, or MCP-1/JE in transgenic infected mice compared with wt infected mice (data not shown). However, the expression of MIP-2 in wt mice was delayed compared with the expression of KC; maximal lung MIP-2 levels were noted at 72 h after *K. pneumoniae* challenge, whereas maximal KC levels were noted at 24 h postchallenge and decreased toward baseline thereafter (Fig. 5). Furthermore, maximal MIP-2 levels tended to be lower or were significantly decreased in

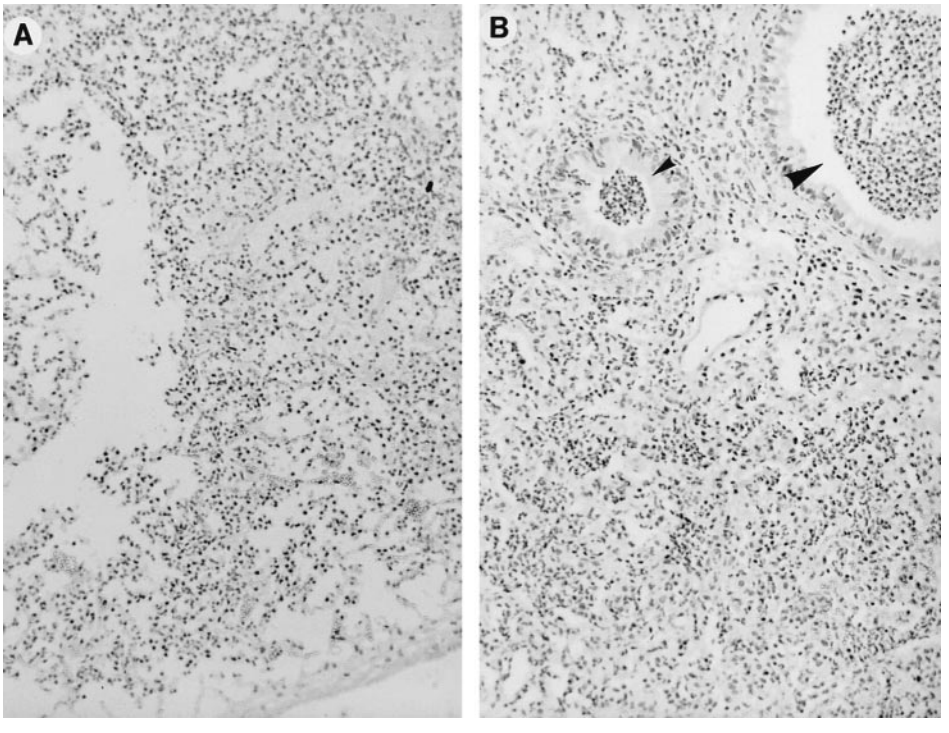

FIGURE 4. Effect of *K. pneumoniae* administration on the distribution of inflammatory cell influx in wt (*A*) and KC-transgenic (*B*) mice at 48 h. The *K. pneumoniae* dose used was 3×10^3 CFU. In KC-transgenic mice, neutrophils were observed in lung airspaces; neutrophil aggregates were also present in both terminal airways (small arrowhead) and larger airways (large arrowhead). Results are representative of three separate animals per group.

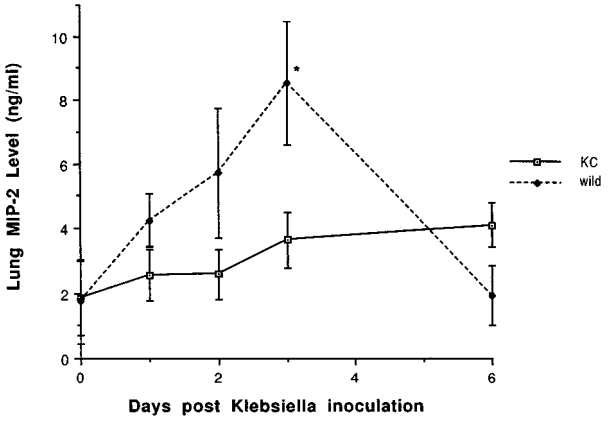

FIGURE 5. Time-dependent MIP-2 expression in the lungs of transgenic KC and wt control mice that were infected with *K. pneumoniae* (3×10^3 CFU). *$p = 0.01$ compared with wt mice that had been challenged with *K. pneumoniae*. KC = transgenic mice; wild = wt control mice. n = five mice per group.

KC-transgenic mice at 48 and 72 h after bacterial challenge, respectively, compared with wt infected mice ($p = 0.08$ and 0.01, respectively). These results suggest that KC does not appear to regulate the expression of the other relevant cytokines involved in host defense against *Klebsiella* pneumonia. However, the overabundance of KC in transgenic mice was associated with a reduction in the expression of another C-X-C chemokine, MIP-2.

Discussion

Effective host defense against bacterial pathogens requires the generation of a vigorous inflammatory response involving the recruitment and activation of neutrophils (2). MIP-2, a member of the C-X-C chemokine family has been recognized as a contributor to antibacterial host defense by effecting neutrophil trafficking and activation (13). In this study, we examined the contribution of KC, another member of the C-X-C chemokine family, to neutrophil recruitment and bacterial clearance in a murine model of *Klebsiella* pneumonia.

In this study, we used transgenic mice in which the KC gene was fused to a Clara cell-specific promoter (26, 27), which resulted in an enhanced constitutive and time-dependent expression of KC within the lungs during the course of *Klebsiella* pneumonia. This transgenic model allowed us to modulate a single chemokine, KC, in our in vivo bacterial pneumonia model to examine whether the overexpression of KC in these transgenic mice would affect overall antibacterial host defense. The enhancement of KC significantly improved survival when compared with wt control mice and was a result of increased bacterial clearance. The mechanism for enhanced bacterial clearance is likely attributable to significantly augmented neutrophil recruitment, as we observed a greater magnitude of neutrophil influx into the airspaces of KC-transgenic mice after *K. pneumoniae* challenge compared with wt controls. Given that the predominant site of KC transgene expression in transgenic mice is the terminal bronchioles (26, 27), it appears that the enhanced alveolar PMN influx results from the spillover of KC into the airspace. We also observed neutrophil aggregates in the terminal airways in proximity to the sites of airspace inflammation; this finding was distinctly unique to KC-transgenic animals and probably reflects site-directed PMN migration as a result of KC production from the airway epithelium. We cannot exclude a concomitant effect on neutrophil activation, as KC and other C-X-C chemokines have been shown to activate PMN effector cell activities, including respiratory burst and antimicrobial activity in vitro (8, 9, 20). There is also a modest but significant BAL neutrophilia in KC-transgenic animals at baseline, and it is plausible the these neutrophils facilitate the initial clearance of *K. pneumoniae* after intrapulmonary challenge. Although alveolar Mϕ and other Mϕ populations have been shown to play a critical role in the clearance of Gram-negative organisms from the lung (30), there is no evidence to suggest that KC or other C-X-C chemokines have direct stimulatory effects on Mϕ antimicrobial activity.

While the enhanced production of KC in KC-transgenic mice provided protective effects in murine *Klebsiella* pneumonia, we also observed a substantial early induction of KC in wt mice in response to the intrapulmonary delivery of *K. pneumoniae*. Indeed, the marked beneficial effect of compartmentalized KC overexpression taken together with the fact that KC is maximally expressed early (at 24 h) in the host response suggests that KC may play a more important role in antibacterial host defense than MIP-2, which is expressed in a delayed fashion (maximal at 72 h in wt mice); the neutralization of MIP-2 during the evolution of *Klebsiella* pneumonia resulted in relatively modest although significant reductions in bacterial clearance and survival (13). However, in vivo neutralization studies have not been possible due to the lack of an appropriate anti-murine KC-neutralizing Ab and of KC-deficient mice.

Unlike C-C chemokines (31, 32), there is no evidence to support a role of C-X-C chemokines in directly regulating the expression of other cytokines. Similarly, the compartmentalized overexpression of KC in infected mice did not alter the expression of the cytokines TNF-α, IFN-γ, IL-12, and IL-10 compared with wt animals that had been challenged with *K. pneumoniae*, suggesting that KC likely occupies a terminal and distal position in the overall cytokine network involved in the host defense cascade. However, we did observe a significant decrease in the inducible, but not the constitutive, expression of MIP-2 in KC transgenic mice that were administered *K. pneumoniae* compared with wt animals. This observation raises the possibility that a substantial redundancy in chemokine responses exists, such that the overexpression of a specific chemokine may obviate the need for (or directly inhibit) the production of another family member with similar biologic activities. The attenuated expression of one chemokine by the overexpression of other functionally and structurally related chemokines has not been reported previously.

The compartmentalized augmentation of KC within the lung improved the innate host defense system against *K. pneumoniae* in our murine model of bacterial pneumonia. Given the emergence of highly resistant bacterial pathogens and the increasing population of immunocompromised hosts (1–3, 33), the treatment of bacterial infection has and will continue to be quite difficult. Therefore, the immunomodulation of host responses, especially if delivered in a site-directed fashion, may prove to be an attractive adjuvant to conventional antibiotic therapy. Because C-X-C chemokines appear to occupy a distal position in the cytokine network and exhibit more neutrophil chemotactic than activating effects, these favorable characteristics suggest that the augmentation of chemokines such as KC may potentially improve the clinical outcome of patients with severe bacterial pneumonias while limiting the potentially serious adverse effects that are mediated by overzealous leukocyte activation and/or the regulation of other potentially injurious cytokines.

References

1. Doyle, R. 1997. U.S. deaths from pneumonia. *Sci. Am. 276:29.*
2. Davies, J. 1994. Inactivation of antibiotics and dissemination of resistance genes. *Science 264:375.*

3. Svarva, P. L., R. V. Lyng, and J. A. Maeland. 1985. Emergence of β-lactam multiresistant variants of gram-negative bacilli in the presence of cefotaxime. *Scand. J. Infect. Dis. 17:387.*
4. Toews, G. B., G. N. Gross, and A. K. Pierce. 1979. The relationship of inoculum size to lung bacterial clearance and phagocytic cell response in mice. *Am. Rev. Respir. Dis. 120:55.*
5. Standiford, T. J. 1997. Cytokines and Pulmonary Host Defenses. *Current Opinion in Pulm Medicine. Rapid Science Publishers. 3:81.*
6. Bazan, J. F., K. B. Bacon, G. Hardiman, W. Wang, K. Soo, D. Rossi, D. R. Graves, A. Zlotnik, and T. J. Schall. 1997. A new class of membrane-bound chemokine with a CX_3C motif. *Nature 385:640.*
7. Schall, T. J. 1997. Fratalkine–a strange attractor in the chemokine landscape. *Immunol. Today 18:147.*
8. Matsushima, K., and J. J. Oppenheim. 1989. Interleukin-8 and MCAF: novel inflammatory cytokines inducible by interleukin-1 and TNF. *Cytokine 1:2.*
9. Oppenheim, J. J., C. O. C. Zachariae, N. Mukaida, and K. Matsushima. 1991. Properties of the novel proinflammatory supergene intercrine cytokine family. *Annu. Rev. Immunol. 9:617.*
10. Huffnagle, G. B., R. M. Strieter, S. L. Kunkel, G. B. Toews, and T. J. Standiford. 1996. The role of chemokines in pneumonia. In *Chemokines in Disease.* A. Koch and R. Strieter, eds. R. G. Landis, Austin, Texas, p. 151.
11. Huffnagle, G. B., R. M. Strieter, T. J. Standiford, R. McDonald, M. Burdick, S. Kunkel, and G. Toews. 1995. The role of monocyte chemotactic protein-1 (MCP-1) in the recruitment of monocytes and $CD4^+$ T cells during a pulmonary *Cryptococcus neoformans* infection. *J. Immunol. 155:4790.*
12. Huffnagle, G., R. M. Strieter, T. J. Standiford, R. McDonald, L. K. McNeil, M. D. Burdick, S. L. Kunkel, and G. B. Toews. 1997. Macrophage inflammatory protein-1α (MIP-1α) is required for the efferent phase of pulmonary cell-mediated immunity to a *Cryptococcus neoformans* infection. *J. Immunol. 159:318.*
13. Greenberger, M. J., R. M. Strieter, S. L. Kunkel, J. M., Danforth, L. L. Laichalk, D. C. McGillicudy, and T. J. Standiford. 1996. Neutralization of MIP-2 attenuates neutrophil recruitment and bacterial clearance in murine *Klebsiella* pneumonia. *J. Infect. Dis. 173:159.*
14. Ryseck, R.-P., H. MacDonald-Bravo, M. G. Mattei, and R. Bravo. 1989. Cloning and sequence of a secretory protein induced by growth factors in mouse fibroblasts. *Exp. Cell Res. 180:266.*
15. Wen, D.Z., A. Rowland, and R. Derynck. 1989. Expression and secretion of GRO/MGSA by stimulated human endothelial cells. *EMBO J. 8:1761.*
16. Introna, M., R. C. Bast, Jr., C. S. Tannebaum, T. A. Hamilton, and D. O. Adams. 1987. The effect of LPS on expression of the early "competence" genes JE and KC in murine peritoneal macrophages. *J. Immunol. 138:3891.*
17. Huang, S., J. D. Paulauskis, J. Godleski, and L. Kobzik. 1992. Expression of macrophage inflammatory protein-2 and KC mRNA in pulmonary inflammation. *Am. J. Pathol. 141:981.*
18. Yu, S.-F., T. J. Koerner, and D. O. Adams. 1990. Gene regulation in macrophage activation: differential regulation of genes encoding for tumor necrosis factor, interleukin-1, JE, and KC by interferon-γ and lipopolysaccharide. *J. Leukocyte Biol. 48:412.*
19. Oquendo, P., J. Alberta, D. Wen, J. L. Graycar, R. Derynck, and C. D. Stiles. 1989. The platelet-derived growth factor-inducible KC gene encodes a secretory protein related to platelet α-granule proteins. *J. Biol. Chem. 264:4133.*
20. Frevert, C. W., S. Huang, H. Danaee, J. D. Paulauskis, and L. Kobzik. 1995. Functional characterization of the rat chemokine KC and its importance in neutrophil recruitment in a rat model of pulmonary inflammation. *J. Immunol. 154: 335.*
21. Farone, A., S. Huang, J. Paulauskis, and L. Kobzik. 1995. Airway neutrophilia and chemokine mRNA expression in sulfur dioxide-induced bronchitis. *Am. J. Respir. Cell Mol. Biol. 12:345.*
22. Pierce, L. M., F. Allessandrini, J. J. Godleski, and J. D. Paulauskis. 1996. Vanadium-induced chemokine mRNA expression and pulmonary inflammation. *Toxicol. Applied Pharmacol. 138:1.*
23. Schwartz, D., A. Andalibi, L. Chaverri-Almada, J. A. Berliner, T. Kirchgessner, Z. T. Fang, P. Tekamp-Olson, A. J. Lusis, C. Gallegos, and A. M. Fogelman. 1994. Role of the GRO family of chemokines in monocyte adhesion to MM-LDL-stimulated endothelium. *J. Clin. Invest. 94:1968.*
24. Wakefield, T. W., R. M. Strieter, M. R. Prince, L. J. Downing, and L. J. Greenfield. 1997. Pathogenesis of venous thrombosis: a new insight. *Cardiovasc. Surg. 5:6.*
25. Lira, S., P. Zalamea, J. Heinrich, M. Fuentes, D. Carrasco, A. Lewin, D. Barton, S. Durham, and R. Bravo. 1994. Expression of the chemokine N51/KC in the thymus and epidermis of transgenic mice results in marked infiltration of a single class of inflammatory cells. *J. Exp. Med. 180:2039.*
26. Lira, S. A., M. E. Fuentes, R. M. Strieter, and S. K. Durham. 1997. Transgenic methods to study chemokine function in lung and CNS. *Methods Enzymol. 287: 304.*
27. Lira, S. 1996. Genetic approaches to study chemokine function. *J. Leukocyte Biol. 59:45.*
28. Greenberger, M. J., R. M. Strieter, S. L. Kunkel, J. M., Danforth, R. E. Goodman, and T. J. Standiford. 1995. Neutralization of IL-10 increases survival in a murine model of *Klebsiella* pneumonia. *J. Immunol. 155:722.*
29. Broug-Holub, E., G. B. Toews, J. Freek Van Iwaarden, R. M. Strieter, S. L. Kunkel, R. Paine, III, and T. J. Standiford. 1997. Alveolar macrophages are required for protective pulmonary defenses in murine *Klebsiella* pneumonia: elimination of alveolar macrophages increases neutrophil recruitment but decreases bacterial clearance and survival. *Infect. Immun. 65:1139.*
30. Lukacs N. W., S. W. Chensue, W. L. Karpus, P. Lincoln, C. Keefer, R. M. Strieter, and S. L. Kunkel. 1997. C-C chemokines differentially alter interleukin-4 production from lymphocytes. *Am. J. Pathol. 150:1861.*
31. Zisman, D. A., S. L. Kunkel, R. M. Strieter, W. C. Tsai, K. Bucknell, J. Wilkowski, and T. J. Standiford. 1997. MCP-1 protects mice in lethal endotoxemia. *J. Clin. Invest. 99:2832.*
32. Centers for Disease Control and Prevention. 1997. Update: *Staphylococcus aureus* with reduced susceptibility to vancomycin–United States, 1997. *MMWR Morb. Mortal. Wkly. Rep. 46:813.*
33. Greenberger, M., S. L. Kunkel, R. M. Strieter, N. W. Lukacs, J. Bramson, J. Gauldie, F. Graham, M. Hitt, J. Danforth, and T. J. Standiford. 1996. IL-12 gene therapy protects mice in lethal *Klebsiella* pneumonia. *J. Immunol. 157:3006.*