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Cathelicidin-Related Antimicrobial Peptide Is Required for Effective Lung Mucosal Immunity in Gram-Negative Bacterial Pneumonia

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Cathelicidins are a family of endogenous antimicrobial peptides that exert diverse immune functions, including both direct bacterial killing and immunomodulatory effects. In this study, we examined the contribution of the murine cathelicidin, cathelicidin-related antimicrobial peptide (CRAMP), to innate mucosal immunity in a mouse model of Gram-negative pneumonia. CRAMP expression is induced in the lung in response to infection with Klebsiella pneumoniae. Mice deficient in the gene encoding CRAMP (Cnlp−/−) demonstrate impaired lung bacterial clearance, increased bacterial dissemination, and reduced survival in response to intratracheal K. pneumoniae administration. Neutrophil influx into the alveolar space during K. pneumoniae infection was delayed early but increased by 48 h in CRAMP-deficient mice, which was associated with enhanced expression of inflammatory cytokines and increased lung injury. Bone marrow chimera experiments indicated that CRAMP derived from bone marrow cells rather than structural cells was responsible for antimicrobial effects in the lung. Additionally, CRAMP exerted bactericidal activity in vitro. Similar defects in lung bacterial clearance and delayed early neutrophil influx were observed in CRAMP-deficient mice infected with Pseudomonas aeruginosa, although this did not result in increased bacterial dissemination, increased lung injury, or changes in lethality. Taken together, our findings demonstrate that CRAMP is an important contributor to effective host mucosal immunity in the lung in response to Gram-negative bacterial pneumonia. The Journal of Immunology, 2012, 189: 000–000.

Hospital-acquired pneumonia is a common nosocomial infection and is a leading cause of death among hospital-acquired infections (1–3). Mortality rates in patients with nosocomial pneumonia have been reported as high as 33–50%. Gram-negative pathogens, such as Pseudomonas aeruginosa, are a common cause of hospital-acquired pneumonia, particularly in mechanically ventilated patients. Klebsiella pneumoniae is another important Gram-negative bacterium with a rising prevalence as a nosocomial pathogen (4, 5). Nosocomial Klebsiella pneumonia now accounts for ~10% of all hospital-acquired pneumonias, including a growing number of carbapenemase-producing strains (5, 6). Because of the increasing rate of antibiotic-resistant pathogens, new therapeutic options to aid in the prevention and treatment of nosocomial infections are needed.

The lung is continuously bombarded by a vast array of inhaled microbial pathogens. To combat these infectious insults, the respiratory tract is armed with diverse mechanisms of innate mucosal immunity, including expression of antimicrobial peptides. Prominent among these antimicrobial peptides are the cathelicidin family. Cathelicidins are a family of cationic endogenous antimicrobial peptides that are synthesized as prepro-peptides (7, 8). These molecules are characterized by an N-terminal prepro region with a high degree of homology among diverse mammalian species, and a far more heterogeneous C-terminal antimicrobial domain (9). Upon stimulation, the C terminus is proteolytically cleaved. Cathelicidins are constitutively expressed by neutrophils (10), but are induced in epithelial cells, such as keratinocytes, intestinal epithelium, and lung epithelial cells (11–13). Additionally, cathelicidins can be expressed by other myeloid-derived cells, such as macrophages and lymphocytes (14, 15). The murine cathelicidin cathelicidin-related antimicrobial peptide (CRAMP) is encoded by the gene Cnlp on mouse chromosome 9 (16).

Cathelicidins exert antibacterial activity against both Gram-positive and Gram-negative bacteria via electrostatic interactions with the bacterial cell membrane (13, 17–21). Their activity is reduced in high salt concentrations, as well as in the presence of other anionic molecules such as F-actin, airway mucus, and DNA (22, 23). In addition to bactericidal activity, cathelicidins exert a number of immunomodulatory effects, including LPS binding and neutralization, chemotaxis of immune cells, and stimulating the release of inflammatory cytokines (24–29). It has also recently been discovered that they mediate lung epithelial cell stiffness and transepithelial permeability (19). In comparison with wild-type (WT) mice, Cnlp−/− mice demonstrate increased susceptibility to both Gram-positive and Gram-negative mucosal infections (30).

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Abbreviations used in this article: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; CRAMP, cathelicidin-related antimicrobial peptide; i.e., intratracheally loaded; PMN, polymorphonuclear neutrophil; WT, wild-type.

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The in vivo contribution of cathelicidins to lung mucosal immunity has not been characterized. However, transgenic expression of LL-37 restored the killing of P. aeruginosa and Staphylococcus aureus by bronchial epithelial cells isolated from patients with cystic fibrosis (31), and the in vivo pulmonary transgenic expression of LL-37 in mice challenged with P. aeruginosa simultaneously reduced lung bacterial burden and reduced inflammation (32).

In this study, we sought to characterize the effects of CRAMP in two models of Gram-negative bacterial pneumonia. Our findings indicate that CRAMP is required for protective immunity against both K. pneumoniae and P. aeruginosa, and that CRAMP may exert important immunomodulatory effects that regulate lung injury and bacterial dissemination.

Materials and Methods

Animals

Specific pathogen-free C57BL/6 mice (age- and sex-matched) were purchased from the Jackson Laboratory (Bar Harbor, ME). C57/6 mice breeding pairs (33) bred on a C57BL/6 genetic background were obtained from Richard Gallo (University of California, San Diego, CA). All mouse strains were housed in specific pathogen-free conditions within the animal care facility (Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI) until the day of sacrifice. Animal studies were reviewed and approved by the University Committee on the Use and Care of Animals (University of Michigan).

Intratracheal inoculation

For intratracheal (i.t.) injection, mice were anesthetized with an i.p. ketamine and xylazine mixture. Under sterile conditions, the trachea was exposed and a 30-μl inoculum was administered via a sterile 27-gauge needle. The skin incision was then closed with surgical staples.

Reagents

Anti-CRAMP Abs used in Western immunoblotting were purchased from Chi Scientific (Maynard, MA). Cathepsin B (Roche Applied Science, Indianapolis, IN). Homogenates were serially diluted 1:5 in PBS and plated on nutrient agar (BD Biosciences) to determine CFU. Whole blood was aspirated into heparinized syringes from the right ventricle. Whole lungs and spleen were removed, taking care to dissect away lymph nodes. The organs were then homogenized separately in 1 ml PBS with protease inhibitor (Boehringer Mannheim, Indianapolis, IN). Homogenates were serially diluted 1:5 in PBS and plated on nutrient agar (BD Biosciences) to determine CFU. Whole lungs were aspirated into heparinized syringes from the right ventricle at designated time points, serially diluted 1:5 with sterile PBS, and plated.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed for collection of BAL fluid (BALF) as previously described (35). Briefly, the trachea was exposed and intubated using a 1.7-mm outer diameter polyethylene tubing. PBS containing 5 mM EDTA was instilled into the trachea in three 1 ml aliquots and aspirated by syringe suctioning. Approximately 90% of BALF was retrieved.

BALF leukocyte analysis

BALF was centrifuged at 1800 rpm at 4°C for 10 min. Supernatants were removed and reserved for other separate experiments. Cell pellets were resuspended in 250 μl Life Technologies RPMI medium (Invitrogen, Carlsbad, CA). Cell counts and viability were determined using trypan blue exclusion counting on a hemacytometer. Cytospin slides were prepared and stained with a modified Wright-Giemsa stain.

Murine ELISAs for cytokine measurement

Cell-free BALF supernatants were analyzed for TNF-α, IL-17, MIP-2, and KC using mouse DuoSet ELISA kits (R&D Systems, Minneapolis, MN) employing a modified double ligand method.

Murine ELISA for albumin measurement

BALF albumin (albumin quantification kit; Bethyl Laboratories, Montgomery, TX) for lung permeability assessment was quantified using a modified double ligand method.

Bone marrow transplantation

Bone marrow transplantation was performed as previously described (36). Donor mice were euthanized via CO2 asphyxiation and the hind legs were removed. Bone marrow cells were harvested from donor mice and resuspended in serum-free medium (DMEM, 0.1% BSA, 1% penicillin-streptomycin, 1% l-glutamine, and 0.1% amphotericin B). Recipient mice received 13.5 Gy of total body irradiation (orthovoltage x-ray source) split in two fractions, 3 h apart. Bone marrow cells (5 × 10^6) were ad-
ministered by tail vein injection into total body irradiation recipient mice. All experiments with bone marrow transplantation mice were performed 5 wk after bone marrow transplantation when mice were fully donor cell reconstituted.

In vitro bacterial killing assay

A modified colony-counting assay was performed using *K. pneumoniae* culture (37). Briefly, bacteria were diluted to a concentration of 5 × 10^6 in 100 μl in 96-well plates. Cultures were incubated with various concentrations of synthetic murine CRAMP at 37°C for 2 h, then serially diluted 1:5 with sterile PBS and plated on nutrient agar plates. Each condition was carried out in triplicate.

Statistical analysis

Survival curves were compared using the log rank (Mantel–Cox) test. Statistical significance was determined using a two-tailed unpaired *t* test for Figs. 1, 2B, 3, 4, and 6–8. Fig. 5 was analyzed using one-way ANOVA. All calculations were performed using GraphPad Prism 5.0 software for Windows (GraphPad Software, San Diego, CA).

Results

**CRAMP is induced in response to Klebsiella infection of the respiratory tract**

To better understand the role of CRAMP during Gram-negative infection, we first sought to determine whether CRAMP was induced after i.t. challenge with *K. pneumoniae*. We examined whole lungs of infected mice (n = 3/time point) by real-time quantitative RT-PCR and Western blot at 6, 24, and 48 h postinfection (Fig. 1A). There was a time-dependent increase in CRAMP mRNA in lung, which was maximal by 48 h after *K. pneumoniae* administration (*p < 0.01*). There was also a corresponding induction of CRAMP protein in infected mice, as compared with whole lung from uninfected controls (*p < 0.001)*.

To define potential cellular sources of CRAMP expression by lung cells, we analyzed CRAMP induction in vitro in isolated primary lung macrophages and alveolar epithelial cells following exposure to heat-killed *Klebsiella* (Fig. 1B). Expression of CRAMP mRNA was significantly increased in both cell types, maximal by 24 h after exposure (*p < 0.01*).

**Survival and bacterial clearance are reduced in Cnlp^−/− mice after i.t. administration of K. pneumoniae**

Given that CRAMP was expressed during the evolution of *Klebsiella* pneumonia, we next examined the function of CRAMP in bacterial pneumonia. WT mice and mice lacking the gene encoding CRAMP (*Cnlp^−/−* mice) were inoculated with 5 × 10^3 CFU *K. pneumoniae* and survival was assessed to 9 d. As shown in Fig. 2A, survival was 50% in WT infected mice, whereas *Cnlp^−/−* mice died earlier and no mice survived past 6 d (*p < 0.01* compared with WT mice).

To determine whether reduced survival in *Cnlp^−/−* mice was associated with changes in bacterial clearance, *Klebsiella* CFU were quantified from whole lung, blood, and spleen at 48 h after i.t. infection (Fig. 2B). In lung, there was a 37-fold increase in bacterial CFU in *Cnlp^−/−* mice as compared with WT mice (*p < 0.001*). Likewise, bacterial counts were higher in blood (14-fold change, *p < 0.01*) and spleen homogenates (45-fold change, *p < 0.001*) in *Cnlp^−/−* mice.

**Enhanced lung inflammation/injury in Cnlp^−/− mice after i.t. *K. pneumoniae* administration**

Cathelicidins have been proposed to exert direct chemotactic effects on inflammatory leukocytes, including polymorphonuclear neutrophils (PMNs). To determine whether this effect contributes to the phenotypic difference observed in *Cnlp^−/−* mice, we examined inflammatory cell counts in WT and *Cnlp^−/−* mice following i.t. *K. pneumoniae* administration (Fig. 3). At 24 h postinfection, there was an increase in total BAL cells and BAL neutrophils in WT mice. Although there was no difference in total leukocyte counts, there was a small yet statistically significant decrease in neutrophils in the *Cnlp^−/−* mice at 24 h (*p < 0.05*). By 48 h postinfection, the increase in both total cells and numbers of clinical symptoms observed in WT mice was not seen in *Cnlp^−/−* mice.
8–10 animals per group. (WT. Survival curves are representative of two separate experiments with *\( \text{K. pneumoniae} \) were analyzed at 48 h after bacterial challenge (Fig. 4A). There compared with their WT counterparts (\( p \text{ } < 0.01 \text{ compared with WT. Survival curves are representative of two separate experiments with 8–10 animals per group. (B) WT or \( \text{Cnlp}^{−/−} \) mice were challenged with 5 \( \times \) 10³ CFU i.t. \( \text{K. pneumoniae} \). CFU were assessed at 48 h postinfection from whole blood, as well as lung and spleen homogenates. Values are expressed as means ± SEM and represent four to six animals per group, combined from two separate experiments. \( p \text{ } < 0.001 \text{ compared with WT; } \) \( \text{Cnlp}^{−/−} \text{ mice as a surrogate for injury to the alveolar capillary membrane (Fig. 4B). Using this inoculum of } \text{K. pneumoniae} \text{ in WT mice, we observed no increase in BAL albumin levels as compared with uninfected control mice. In comparison, we observed an increase in BAL albumin levels in infected \( \text{Cnlp}^{−/−} \text{ mice at 48 h after bacterial challenge, and there was significantly more albumin in } \text{Cnlp}^{−/−} \text{ mice compared with WT mice (} p < 0.05 \text{) at that time point.}

**Bone marrow cell-derived expression of CRAMP is responsible for the } \text{Cnlp}^{−/−} \text{ phenotype**}

We have shown that CRAMP is induced in response to \text{Klebsiella} \text{ infection, and that this protein is expressed by both cells of myeloid original and alveolar epithelial cells. To address which cell type is responsible for the expression of CRAMP required for effective bacterial clearance, we generated bone marrow chimeras of WT and \( \text{Cnlp}^{−/−} \text{ mice (Fig. 5). WT bone marrow cells transplanted into WT mice (WT → WT) and \( \text{Cnlp}^{−/−} \text{ marrow into } \text{Cnlp}^{−/−} \text{ mice (\( \text{Cnlp}^{−/−} \text{ → } \text{Cnlp}^{−/−} \)) resulted in lung bacterial clearance similar to nontransplanted controls (WT and \( \text{Cnlp}^{−/−} \text{ mice, respectively; data not shown). When WT marrow cells were transplanted into } \text{Cnlp}^{−/−} \text{ mice (WT → } \text{Cnlp}^{−/−} \text{), bacterial clearance in the lung was identical to that of transplanted WT → WT mice and was significantly less impaired than either \( \text{Cnlp}^{−/−} \text{ marrow transplanted into WT mice (\( \text{Cnlp}^{−/−} \text{ → } \text{WT} \)) or } \text{Cnlp}^{−/−} \text{ → } \text{Cnlp}^{−/−} \text{ mice (} p < 0.01 \text{ for both comparisons). However, \( \text{Cnlp}^{−/−} \text{ marrow transplanted into WT mice (\( \text{Cnlp}^{−/−} \text{ → } \text{WT} \)) demonstrated significantly impaired lung bacterial clearance, comparable to that of transplanted } \text{Cnlp}^{−/−} \text{ mice (\( \text{Cnlp}^{−/−} \text{ → } \text{Cnlp}^{−/−} \)).

**CRAMP exerts direct bactericidal effects against } \text{K. pneumoniae in vitro**

Among the many putative functions of cathelicidins, these molecules are known to exert direct bactericidal effects on a wide range of microbial pathogens. However, effects of CRAMP on heavily encapsulated bacteria are less well described. Similar to effects on other Gram-negative and Gram-positive organisms, we observed an increase in bacterial killing when \text{K. pneumoniae} was incubated with varying doses of synthetically generated CRAMP (Fig. 6), with bactericidal effects plateauing at a CRAMP concentration of ≥1 μM (\( p < 0.05 \text{).}

**CRAMP contributes to mucosal immunity in murine } \text{P. aeruginosa pneumonia**

We next assessed the importance of CRAMP in another relevant Gram-negative bacterial infection of the respiratory tract, \text{P. aeruginosa}. The i.t. administration of \text{P. aeruginosa} (10⁵ CFU) resulted in upregulation of CRAMP mRNA and protein (Fig. 7). As compared with \text{Klebsiella} administration, the increase in expression began earlier, with a progressive rise in both mRNA and protein at 6 and 24 h postinfection (\( p < 0.01 \text{ for mRNA and } p < 0.05 \text{ for protein at both time points compared with control). mRNA and protein dropped to basal levels of expression by 48 h postinfection. As compared with WT mice, \( \text{Cnlp}^{−/−} \text{ mice demonstrate impaired early lung bacterial clearance in response to } \text{P. aeruginosa} \text{ (Fig. 8B; } p < 0.05 \text{). Specifically, there was an ~35-fold increase in bacterial CFU at 24 h postinfection in } \text{Cnlp}^{−/−} \text{ mice compared with WT mice. However, statistically significant differences in lung bacterial clearance were not seen by 48 h postinfection.}
Moreover, bacterial dissemination did not differ between WT and Cnlp<sup>−/−</sup> mice when challenged with <i>P. aeruginosa</i>, as assessed by blood and spleen bacterial counts at any time point. Similar to Klebsiella-infected mice, we observed an early decrease in BAL PMNs at 24 h after i.t. <i>P. aeruginosa</i> administration in Cnlp<sup>−/−</sup> mice (Fig. 8A; *p < 0.01). Although PMNs were actually increased in Cnlp<sup>−/−</sup> mice at 48 h in Klebsiella-infected mice, there was no difference in BAL PMNs between WT and Cnlp<sup>−/−</sup> mice at 48 h in <i>Pseudomonas</i>-infected mice. We observed significantly increased levels of IL-17, MIP-2, and KC, but not TNF-α, in BALF of Cnlp<sup>−/−</sup> mice as compared with WT mice at 24 h postinfection (Fig. 8D; *p < 0.05). By 48 h postinfection, cytokine/chemokine levels were undetectable in BALF of WT mice, whereas levels of each of the measured cytokines and chemokines remained significantly elevated in Cnlp<sup>−/−</sup> mice. Despite the early differences in bacterial burden and cytokine/chemokine expression in lungs, we observed no survival difference between WT and Cnlp<sup>−/−</sup> mice when administered either an LD<sub>20</sub> or LD<sub>80</sub> inoculum of <i>P. aeruginosa</i> (Fig. 8C).

**Discussion**

Cathelicidins exert a variety of effects that can contribute to innate mucosal immunity. In this study, we have identified an important role for the murine cathelicidin CRAMP in a mouse model of Klebsiella pneumonia. Previous work in our laboratory has shown that CRAMP expression in the lung is significantly upregulated in response to intranasal bacterial flagellin administration (21). Additionally, i.t. <i>Pseudomonas</i> instillation also results in upregulation of CRAMP. In this study, we have demonstrated that CRAMP is also induced in the lung following infection with another Gram-negative bacteria, <i>K. pneumoniae</i> (Fig. 1). Whereas <i>Pseudomonas</i> is a flagellated bacterium, Klebsiella does not express flagellin. Furthermore, we have found that CRAMP is induced in response to both a standard isolate of <i>Pseudomonas</i> as well as an isogenic strain lacking flagellin production (T. Standiford, unpublished observations). Taken together, these observations suggest that although flagellin is one factor that triggers CRAMP expression,
multiple bacterial pathogen-associated molecular patterns certainly contribute to the induction of CRAMP in the lung.

We have shown that CRAMP can be expressed by both alveolar epithelial cells as well as pulmonary macrophages in vitro (Fig. 1A). This is consistent with previous studies showing CRAMP expression from dermal, intestinal, and bronchial epithelial cells, and from various hematopoietic cells sources, including macrophages and neutrophils (21, 38–40). Although in vitro studies indicate that both cell types express CRAMP upon exposure to Gram-negative bacteria, the relative contribution of myeloid versus epithelial cell expression to innate immune responses has not been elucidated in lung. In keratinocyte and gastrointestinal bacterial infection models, it appears that non-neutrophil sources of CRAMP were more relevant contributors to mucosal immunity, as the phenotype of Cnlp−/− mice was preserved when neutrophils were depleted. To directly address this question in the lung, we generated bone marrow chimeras of WT and Cnlp−/− mice and assessed clearance of K. pneumoniae from lung (Fig. 5). Importantly, Cnlp−/− hematopoietic cells transplanted into a WT mouse demonstrated clearance defects comparable to nontransplanted Cnlp−/− mice as well as Cnlp−/− marrow transplanted into Cnlp−/− mice. Conversely, WT hematopoietic cells transplanted into Cnlp−/− mice demonstrate similar bacterial clearance to both nontransplanted WT mice as well as WT marrow transplanted into WT mice. Our results indicate that CRAMP expression from bone marrow-derived cells, rather than structural cells (including epithelial cells), is primarily responsible for enhanced mucosal bacterial clearance.

Given the multiple reported functions of cathelicidins, there are several possible mechanisms to account for the impaired bacterial clearance and reduced survival observed in CRAMP-deficient mice. We have demonstrated that CRAMP has direct bactericidal effects on K. pneumoniae (Fig. 6). Prior studies have shown similar bactericidal activity of cathelicidins against a variety of bacteria, including P. aeruginosa (13, 17–21). Thus, direct bacterial killing is a likely mechanism for bacterial containment. However, cathelicidins are also known to exert other immunomodulatory effects, including binding and inhibition of LPS (26, 27) and stimulation of neutrophil and CD4+ T lymphocyte chemotaxis (28, 29). We observed a modest yet statistically significant impairment in early neutrophil influx into the alveolar space of Cnlp−/− mice infected with either K. pneumoniae (Fig. 3) or P. aeruginosa (Fig. 8A). Analysis of chemokines present in the alveolar space indicates that reduced PMN influx is not due to a deficiency in the neutrophil chemoattractants MIP-2 and KC (Figs. 4A, 8D). It is possible that this delay in neutrophil influx might contribute to impaired bacterial clearance and dissemination. Despite the early delay in neutrophil recruitment, inflammation and lung injury are overall increased in Cnlp−/− mice as compared with WT mice by 48 h postinfection in Klebsiella-infected mice (Figs. 3, 4). There are several potential explanations for enhanced inflammation at this later time point, including a higher bacterial burden driving inflammation, or alternatively the absence of CRAMP effects on LPS sequestration resulting in amplification of LPS-induced inflammatory cytokine/chemokine expression. Additionally, Byfield et al. (19) recently demonstrated that the human cathelicidin, LL-37, mediates increased lung epithelial cell stiffness and decreased transepithelial permeability in vitro. Thus, the enhanced epithelial permeability observed in Cnlp−/− mice 48 h after administration of Klebsiella could also be attributable to alterations in epithelial cell cytoprotection or other mechanical properties of the alveolar epithelium. Regardless of mechanisms, there is appreciable lung injury observed in Klebsiella-infected Cnlp−/− mice, whereas no alveolar leak was noted in WT mice infected with K. pneumoniae. Furthermore, this enhanced lung injury and increased bacterial dissemination in Cnlp−/− mice resulted in decreased survival in the setting of Klebsiella infection.

In this study, we show that CRAMP is required for effective lung mucosal immunity in the setting of Klebsiella pneumonia, as CRAMP-deficient mice displayed impaired bacterial clearance and increased dissemination, increased lung injury, and increased mortality (Figs. 2, 3). However, when we examine the role of CRAMP in a Pseudomonas pneumonia model, the effects are less robust. This does not appear to be attributable to a lack of bactericidal activity, as we and others have previously shown...
bactericidal activity against *P. aeruginosa* in vitro in a dose range comparable to that observed with *K. pneumoniae*. Moreover, we observed that the clearance of *P. aeruginosa* from lung is impaired in *Cnlp<sup>−/−</sup>* mice to a degree similar to that observed in the *Klebsiella* model at 24 h postinfection (Fig. 8). Nevertheless, there was no appreciable change in bacterial dissemination, and significant differences in lung bacterial clearance were not apparent at later time points (48 h). Despite early reductions in PMN influx in *Cnlp<sup>−/−</sup>* mice similar to that observed in our *Klebsiella* model, by 48 h postinfection there was no difference in BAL PMN counts between WT and *Cnlp<sup>−/−</sup>* mice. Additionally, there were no significant differences between WT and *Cnlp<sup>−/−</sup>* mice in epithelial permeability as assessed by BAL albumin levels in *Pseudomonas*-infected mice (data not shown). Potential explanations for these discrepancies include the fact that *Klebsiella* is a more virulent and invasive bacteria than *Pseudomonas*, and a fully intact innate mucosal system may be required for effective host defense against *K. pneumoniae*. An alternative explanation is that the fact that *P. aeruginosa* expresses several proteases in abundance. These proteases may degrade and inactivate CRAMP more efficiently than do *Klebsiella*-derived proteolytic enzymes, resulting in comparatively reduced biological activity in vivo.

In summary, we show that CRAMP is induced in response to Gram-negative bacterial infections in the lung, and this molecule is required for effective lung bacterial clearance. Although further studies are required to define the precise mechanisms of action, our findings suggest a role for cathelicidins that could be exploited therapeutically to improve outcomes in respiratory infections.
The authors have no financial conflicts of interest.

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