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*J Immunol* 2000; 165:5760-5766; doi: 10.4049/jimmunol.165.10.5760

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Bacterial Killing Is Enhanced by Expression of Lysozyme in the Lungs of Transgenic Mice

Henry T. Akinbi, Ralph Epaud, Hetal Bhatt, and Timothy E. Weaver

To assess the role of lysozyme in pulmonary host defense in vivo, transgenic mice expressing rat lysozyme cDNA in distal respiratory epithelial cells were generated. Two transgenic mouse lines were established in which the level of lysozyme protein in bronchoalveolar (BAL) lavage fluid was increased 2- or 4-fold relative to that in WT mice. Lung structure and cellular composition of BAL were not altered by the expression of lysozyme. Lysozyme activity in BAL was significantly increased (6.6- and 17-fold) in 5-wk-old animals from each transgenic line. To determine whether killing of bacteria was enhanced by expression of rat lysozyme, 5-wk-old transgenic mice and WT littermates were infected with 10^6 CFU of group B streptococci or 10^7 CFU of a mucoid strain of *Pseudomonas aeruginosa* by intratracheal injection. Killing of group B streptococci was significantly enhanced (2- and 3-fold) in the mouse transgenic lines at 6 h postinfection and was accompanied by a decrease in systemic dissemination of pathogen. Killing of *Pseudomonas aeruginosa* was also enhanced in the transgenic lines (5- and 30-fold). Twenty-four hours after administration of *Pseudomonas aeruginosa*, all transgenic mice survived, whereas 20% of the WT mice died. Increased production of lysozyme in respiratory epithelial cells of transgenic mice enhanced bacterial killing in the lung in vivo, and was associated with decreased systemic dissemination of pathogen and increased survival following infection. The Journal of Immunology, 2000, 165: 5760–5766.

Clearence of pathogens in the respiratory tract is mediated at least in part by the synthesis and secretion of host defense molecules into the airway lumen. One of the most abundant antimicrobial proteins in the lung, lysozyme, is synthesized and secreted by glandular serous cells, surface epithelial cells, and macrophages in the human airway (1). Human lysozyme (muramidase, N-acetyl muramidase glycanohydrolase, EC 3.2.1.17) is a cationic protein of 148 aa that cleaves glycosidic bonds of N-acetyl-muramic acid, damaging the bacterial cell wall and ultimately killing the organism by lysis (2). Bacteriolytic assays indicate that purified lysozyme is active against some Gram-positive bacteria, but has relatively little activity against Gram-negative bacteria, largely because the outer membrane of these organisms limits access of the enzyme to its substrate (3). The concentration of lysozyme in human airway surface liquid ranges from 20–100 μg/ml, a concentration sufficient to kill two important airway pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, in vitro (4). Despite numerous studies confirming the antibacterial properties of lysozyme in vitro, there are no studies that have directly assessed the role of lysozyme in the killing of lung pathogens in vivo. The current study was therefore undertaken to evaluate the impact of constitutively elevated levels of lysozyme on lung structure and pathogen clearance in transgenic mice.

The spatial expression of pulmonary lysozyme in rodents differs from that in humans in that the enzyme is primarily synthesized and secreted by type II alveolar epithelial cells (5, 6). Lysozyme is one of the most abundant proteins in rat bronchoalveolar lavage (BAL) fluid, accounting for as much as 6–7% of the total soluble protein (5). Although two lysozyme genes have been identified in the rat, only the type I gene is expressed at detectable levels (7); in contrast, two lysozyme genes are expressed in the mouse, lysozyme M and lysozyme P (8, 9). Lysozyme M is the major form expressed in the lung, and only very low levels of lysozyme P mRNA are detected in mouse lung (8). In the current study rat lysozyme type I, the homologue of mouse lysozyme M, was cloned under control of the human surfactant protein C (SP-C) promoter to direct expression of the transgene to the distal respiratory epithelium (10–12). Transgenic mice expressing rat lysozyme exhibited significantly enhanced antimicrobial activity, including enhanced bacterial killing, decreased systemic dissemination of pathogen, and increased survival following infection.

**Materials and Methods**

**Generation of transgenic mice expressing rat lysozyme**

The rat lysozyme cDNA was generated from rat type II cell RNA by RT-PCR using upstream primer 5'-GAA TTC ATG AAG GCT CTC CTA GTT CTC-3' and downstream primer 5'-GAA TTC TCA GAC TCC GCA GTT CGT GGA AA-3', which contained the 3.7-kb human SP-C promoter, rabbit β-globin intron 2, and bovine growth hormone polyadenylation signal (13, 14). The transgene DNA construct was excised from the PUC18 vector backbone by *Nod*I/*Ndel* digestion, gel purified using Qiagen resin (Qiagen, Germany), dialyzed for 48 h against 5 mM Tris (pH 7.4)/1 mM EDTA, and microinjected into fertilized FVB/N oocytes by the Children’s Hospital Transgenic Core facility. Founder mice were identified by a transgene-specific PCR with primers that amplified a 310-bp fragment spanning the junction of the rabbit β-globin intron and the rat lysozyme cDNA (upstream primer, 5'-AAT TCT GCC TGG CGT GGA AA; downstream primer, 5'-TTG GTA GGG ATC CCC AAG GCA TT-3'). PCR conditions were 25 cycles at 55°C annealing temperature with 0.5 μM transgene primers, 0.25 μM dNTPs, 1 U of AmpliTaq DNA polymerase (Perkin-Elmer/
littermates. BAL fluid containing 1 mM BAL fluid was obtained from 5-wk-old transgenic mice and control WT mice. To assess lysozyme enzyme activity, lysozyme enzyme activity assay.

Characterization of transgenic mice
RNA (RT-PCR) analysis. To identify lines that expressed rat lysozyme mRNA, lung tissues were collected from 5- to 6-wk-old transgenic mice and control WT littermates. Total cellular RNA was isolated using TRIzol reagent (Life Technologies, Grand Island, NY). One microgram of DNase I-treated RNA was amplified using the Superscript II kit (Life Technologies; downstream, 5'-AACA CAA GCA AGA AAC TAC AAAC; upstream, 5'-GAA TTC TCA GCA TCC GGT CCG AAC) and with the conditions described above. β-Actin was coamplified in the PCR reaction as an internal control.

Northern analysis. Ten micrograms of total RNA isolated from lung tissues of 5-wk-old transgenic mice and control WT littermates was fractionated by gel electrophoresis, blotted onto a nylon membrane, probed with biotin-labeled rat or mouse lysozyme cDNA (provided by Dr. Rainer Rennkawitz, Max Planck Institut für Biochemie, Martinsried, Germany), and detected by chemiluminescence (Pierce, Rockford, IL).

Analysis of protein expression. To identify transgenic mouse lines that expressed rat lysozyme protein, Western blotting was performed using a rabbit anti-human lysozyme Ab (Accurate Chemicals and Scientific Corp, Westbury, NY) that cross-reacts with both mouse and rat lysozyme (Sigma, St. Louis, MO). Protein concentration was determined by bicinchoninic acid protein assay (15). One-half microgram of total lung protein was analyzed by SDS-PAGE and Western blotting, as previously described (16). To assess the level of secreted lysozyme, BAL fluid was obtained from 5-wk-old transgenic mice and five control WT littermates as described below. One-half microgram of total protein of BAL fluid was resolved by SDS-PAGE and Western blotted with rabbit anti-human lysozyme Ab. Levels of lysozyme proteins in lung homogenates and BAL fluid were quantitated by scanning densitometry using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

BAL fluid cell count. Five 5-wk-old transgenic mice and five control WT littermates were exsanguinated by transecting the abdominal aorta and the inferior vena cava after a lethal i.p. injection of sodium pentobarbital. The lungs were lavaged three times with 1-ml aliquots of PBS. BAL fluid was centrifuged at 2000 × g for 10 min, and the pellet was resuspended in 0.5 ml of PBS. A 50-μl aliquot was stained with an equal volume of 0.4% trypan blue (Life Technologies) for total cell count on a hemocytometer. Differential cell counts were made on cytopsin preparations stained with Diff-Quick (Scientific Products, McGraw Park, IL).

Lysozyme enzyme activity assay. To assess lysozyme enzyme activity, BAL fluid obtained from 5-wk-old transgenic mice and control WT littermates. BAL fluid containing 1 μg of total protein was incubated with killed Micrococcus lysodeikticus suspended in 0.4 M phosphate buffer, pH 6.7, at an OD (450 nm) of 1 at 37°C. The change in OD was assessed after 1 h of incubation (17). Purified chicken egg lysozyme was used to generate a standard curve (1 U of enzyme activity equals a 0.001 change in OD).

Lung morphology and immunohistochemistry. To assess lung morphology and immunohistochemistry, mice from four 5-wk-old transgenic mice and four control WT littermates from transgenic line 3.5 were inflation-fixed for immunostaining and light microscopy as previously described (18). Immunostaining for lysozyme was performed using antisera directed against human lysozyme (Accurate Chemicals and Scientific Corp.). Parallel lung sections were incubated with normal rabbit serum to verify the specificity of immunostaining.

Bacteria killing assays
Group B streptococci (GBS). Stock cultures of GBS used in this study were clinical isolates provided by Dr. J. R. Wright (Duke University, Durham, NC). To minimize variation in virulence, all bacteria used in this study were selected from aliquots of the same passage that had been frozen at −70°C in 20% glycerol/PBS. For each experiment, an aliquot of bacteria was thawed, plated on tryptic soy/5% defibrinated sheep agar plates, and subsequently inoculated in 4 ml of Todd-Hewitt broth (Difco, Detroit, MI). Bacteria were grown for 14–16 h at 37°C with continuous shaking. The bacteria were harvested from the broth by centrifugation at 200 × g for 10 min, washed, and resuspended in sterile PBS at a concentration of 10^6 CFU/100 μl. The concentration of the inoculum was verified by quantitative culture on sheep blood agar plates. Pseudomonas aeruginosa. The strain of P. aeruginosa was a mucoid segregant isolated from the sputum of a cystic fibrosis patient (provided by Dr. D. J. Haslett, University of Cincinnati College of Medicine, Cincinnati, OH). P. aeruginosa obtained from a single passage was stored in aliquots at −70°C in 20% glycerol-Luria-Bertoni (LB) broth. For each experiment, an aliquot of bacteria was plated on LB agar followed by inoculation into 4 ml of LB broth. Preparation of the inoculum was conducted as described for GBS; the concentration (CFU) of the inoculum was verified by quantitative culture on LB plates.

Intratracheal infection. The dose of bacteria selected for these studies was based on the results of previous studies in the FVB/N mouse strain (19). Mice were anesthetized with isoflurane, the trachea was exposed through an anterior midline incision, and 10^5 CFU (GBS) or 10^6 CFU (P. aeruginosa), suspended in 100 μl of sterile PBS, was delivered just beneath the cricoid cartilage. The incision was sealed by applying one drop of surgical glue (Nexabrand, Veterinary Products Laboratories, Phoenix, AZ). As a control, 100 μl of nonpyrogenic PBS was similarly instilled into transgenic and WT mice. To assess bacterial load at 6 h (GBS) or 24 h (P. aeruginosa) postinfection, mice were anesthetized with i.p. pentobarbital and exsanguinated by transecting the abdominal aorta, and lung and splenic tissues were harvested, weighed, and subsequently homogenized in sterile PBS. Serial dilutions of homogenates were plated on blood agar (GBS) or LB (Pseudomonas) plates and incubated at 37°C overnight. Viable pathogen counts in the lung and spleen were estimated from the number of colonies after 24 h of quantitative culture and expressed as CFU per gram of tissue.

To determine survival following infection, 20 5- to 6-wk-old transgenic mice from the 3.5 line and 20 WT littermates were infected with 2 × 10^7 CFU P. aeruginosa intratracheally, as described above. Water and food were provided ad libitum during the period of observation. The number of surviving mice was counted every 12 h for up to 60 h, at which time all surviving mice were sacrificed.

Cytokine levels following infection with P. aeruginosa
Eight 5- to 6-wk-old transgenic mice and eight age-matched WT littermates were infected intratracheally with 10^5 CFU of P. aeruginosa suspended in 100 μl of PBS. Mice were sacrificed at either 6 or 24 h, lungs were lavaged as described above, and lung tissues were homogenized in 1 ml of PBS with 1% (v/v) protease inhibitor mixture; the total volume after homogenization was adjusted to 2 ml with PBS. Mouse TNF-α, IL-6, granulocyte inflammatory protein-2 (MIP-2), and RANTES were measured by sandwich enzyme immunoassay (R&D Systems, Minneapolis, MN). The limits of sensitivity for each cytokine are <5.1, <3.1, 1.5, and <2 pg/ml, respectively. All samples were assayed in duplicate, and values were normalized to total protein in the samples.

Statistical analyses
Differences between groups were assessed by one-way ANOVA, and differences between means were assessed by contrast comparisons and the Student-Newman-Keuls test (StatView, Abacus Concepts, Berkeley, CA). Data are expressed as the mean ± SD. Nonparametric survival distributions were estimated to examine differences in survival of transgenic mice and WT littermates. The differences between the two groups were analyzed using Kaplan-Meier curve statistics.

Results
Generation of transgenic mice
To assess the role of lysozyme in pulmonary host defense, transgenic mouse lines were generated in which rat lysozyme was targeted to the distal respiratory epithelium under the direction of the 3.7-kb human SP-C promoter. Seven of 21 offspring from fertilized oocyte injections were positive for the transgene, as assessed by PCR and confirmed by Southern blot analysis of tail DNA (not shown). Transgenic offspring were indistinct from WT littermates with respect to body weight, lung weight, longevity, and reproductive capability. Rat lysozyme mRNA was detected in the offspring of two of seven transgenic lines (lines 3.5 and 2.6) by RT-PCR, and only these two lines were further characterized.

Analysis of transgene expression
Expression of the lysozyme transgene was assessed by Northern blot analysis of total RNA isolated from the lungs of 5-wk-old
mice. A cDNA probe specific for the rat lysozyme transgene detected an approximately 1-kb transcript (Fig. 1A). When the same blot was probed with a mouse lysozyme cDNA, both the larger endogenous mouse lysozyme mRNA and rat lysozyme transgene mRNA were detected.

Because rat and mouse lysozyme have very similar m.w., it was not possible to distinguish the two proteins by SDS-PAGE. Total levels of lysozyme (rat and mouse) were estimated by Western blotting of equal amounts of protein from lung homogenates or BAL fluid from 5-wk-old transgenic mice and WT littermates. Total lysozyme protein in mice from transgenic line 2.6 was increased 2-fold in both lung homogenate (Fig. 1B) and BAL fluid (not shown); total lysozyme protein in mice from transgenic line 3.5 was increased 4-fold compared with that in control WT littermates (Fig. 1B). Lysozyme (\(M_r, 142\)) was not detected when lung homogenates and BAL were blotted with nonimmune rabbit serum.

To determine whether increased lysozyme protein levels were associated with increased enzyme activity, lysozyme enzyme activity in BAL fluid from 5-wk-old transgenic mice and wild-type littermate controls was assessed by a turbidimetric assay using purified chicken egg white lysozyme as a standard. Lysozyme enzyme activity in BAL fluid from 5-wk-old transgenic mice and WT littermates was assessed by a turbidimetric assay using purified chicken egg white lysozyme as a standard. Lysozyme enzyme activity in BAL fluid from 5-wk-old transgenic mice from lines 3.5 and 2.6 and WT littermates was assessed by a turbidimetric assay using purified chicken egg white lysozyme as a standard. Lysozyme enzyme activity in BAL fluid from 5-wk-old transgenic mice and WT littermates was assessed by a turbidimetric assay using purified chicken egg white lysozyme as a standard. Lysozyme enzyme activity in BAL fluid from 5-wk-old transgenic mice and WT littermates was assessed by a turbidimetric assay using purified chicken egg white lysozyme as a standard.

FIGURE 1. Expression of rat lysozyme in transgenic mice. A. Representative Northern blot analysis of 10 \(\mu\)g of total cellular RNA from lung tissue isolated from 5-wk-old transgenic mice from line 3.5 (lanes 4 and 5, left panel; lanes 3 and 4, right panel) and WT littermates (lanes 1–3, left panel; lanes 1, 2, and 5, right panel). Samples in the left panel were probed with the labeled mouse lysozyme cDNA, which detected both mouse lysozyme and the rat lysozyme transgene (~1 kb); samples in the right panel were probed with the rat lysozyme cDNA, which detected only rat lysozyme (+, samples from transgene-positive mice). B. Western blot analysis of 0.5 \(\mu\)g of total lung protein from 5-wk-old transgenic mice and WT littermates. Proteins were fractionated by SDS-PAGE, blotted onto nitrocellulose membrane, and incubated with anti-human lysozyme Ab, which detected both mouse and rat lysozyme, \(M_r, 15K\) (+, samples from transgene-positive mice).

Effect of transgene expression on killing of bacteria in infected mice

Killing of GBS. To determine whether increased lysozyme levels in the airway enhanced killing of bacteria in the lungs, quantitative cultures of lung homogenates from transgenic mice and WT littermate controls were compared following intratracheal injection

FIGURE 2. Lysozyme enzyme activity is significantly increased in the BAL fluid of transgenic mice. Lysozyme enzyme activity was assessed in BAL fluid from 5-wk-old transgenic mice from lines 3.5 and 2.6 and WT littermates. Ten nanograms of BAL fluid protein was incubated with *Micrococcus lysodeikticus* suspended at an OD of 1 at 450 nM. Following 1 h of incubation at 37°C, the change in OD of the suspension was determined. Purified chicken egg white lysozyme was used to generate a standard curve. Data are the mean ± SEM. n, number of animals per experiment.
of $10^6$ CFU of GBS. All mice survived until sacrifice at 6 h postinfection. Killing of GBS was enhanced 3.2-fold in mice from transgenic line 3.5 ($2.1 \pm 0.1 \times 10^6$ CFU/g protein vs $6.8 \pm 0.5 \times 10^6$ in WT mice; $p = 0.01$; Fig. 4). The incidence of systemic dissemination of infection, as assessed by growth of GBS on plates inoculated with splenic homogenates, was less in transgenic mice (27% vs 60%; $p = 0.04$). Killing of GBS was enhanced 1.7-fold in mice from transgenic line 2.6 ($4.2 \pm 0.8 \times 10^6$ CFU/g protein vs $7.1 \pm 0.6 \times 10^6$ in WT mice; $p = 0.04$).

**Killing of P. aeruginosa.** All transgenic mice survived until sacrifice at 24 h postinfection following intratracheal infection with $10^7$ CFU of *P. aeruginosa*; in contrast, 20% of infected WT mice died. Bacterial killing was enhanced 30.6-fold in mice from transgenic line 3.5 ($1.06 \pm 0.05 \times 10^6$ CFU/g protein compared with $32.4 \pm 0.41 \times 10^6$ in WT littermate controls; $p = 0.03$; Fig. 5). Bacterial killing was enhanced approximately 6.2-fold in mice from transgenic line 2.6 ($6.52 \pm 0.71 \times 10^6$ CFU/g protein; $p = 0.05$). Systemic dissemination was not detected in surviving mice at 24 h postinfection. Following intratracheal infection with $2 \times 10^7$ CFU of *P. aeruginosa*, the mean survival time for transgenic mice (49.0 ± 2.1 h) was significantly longer than that of WT littermates (36.0 ± 2.6 h; $p = 0.0047$; Fig. 6).

**Neutrophil recruitment following P. aeruginosa infection.** Following intratracheal infection with $10^7$ CFU of *Pseudomonas aeruginosa*, total cell count and neutrophil influx were assessed in the lungs of transgenic mice and WT littermate controls. Total cell counts were significantly increased in WT and transgenic mice compared with uninfected littermates at 6 and 24 h postinfection ($p < 0.0001$; Fig. 7A). The percentage of neutrophils in BAL fluid was significantly higher in transgenic mice than in WT littermates (61.5 ± 5.6 vs 29.9 ± 7.2; $p = 0.014$) at 6 h postinfection; however, at 24 h postinfection, the percentage of neutrophils in the BAL fluid was higher in WT mice (88.9 ± 1.9 vs 63.7 ± 8.0; $p = 0.04$; Fig. 7B).
Survival following infection with P. aeruginosa is enhanced in transgenic (Tg) mice. P. aeruginosa (2 × 10^7 CFU) was injected into the trachea of 20 5-wk-old transgenic mice and 20 age-matched WT littermates from line 3.5 as described in Materials and Methods. The number of surviving mice was counted every 12 h for up to 60 h. The mean survival time for transgenic mice was significantly longer than the survival time for WT littermates (49.0 ± 2.1 vs 36.0 ± 2.6 h), p = 0.0047. ■, WT mice; ○, transgenic mice.

Analysis of proinflammatory mediators following infection. The levels of four proinflammatory cytokines assessed following infection with P. aeruginosa were significantly elevated at both 6 and 24 h compared with those in uninfected littermates of either genotype (p < 0.02); uninfected mice had little or no detectable proinflammatory mediators in BAL fluid and lung tissue. Levels of mMIP-2 in BAL fluid and lung tissues were significantly higher in transgenic mice than in WT littermates 6 h after intratracheal infection with P. aeruginosa (p = 0.03 for lung homogenate; p = 0.04 for BAL fluid; Fig. 8); however, at 24 h postinfection there was no significant difference between transgenic mice and WT littermates. There were no significant differences in the levels of mouse (m) TNF-α, mIL-6, and mRANTES between transgenic mice and control littermates at 6 and 24 h postinfection (data not shown).

Discussion
Rat lysozyme was expressed in the respiratory epithelium of transgenic mice, increasing lysozyme protein levels and enzyme activity in BAL fluid without altering lung structure or function. Increased lysozyme was associated with enhanced bacterial killing and decreased mortality following infection with P. aeruginosa, a major human airway pathogen. Lysozyme also conferred protection against GBS, although the effect was not as pronounced as that against Pseudomonas. While other pathogens were not tested, the transgenic mice generated in this study should provide a useful animal model to begin to delineate the spectrum of lysozyme antimicrobial activity in the lung.

The spectrum of lysozyme antimicrobial activity in vitro appears to be relatively narrow, leading some investigators to conclude that exogenous lysozyme would be of little benefit in controlling bacterial infection (20). However, it is important to note that the results of in vitro studies are not necessarily predictive of lysozyme activity in vivo because of potential synergistic actions with other antimicrobial agents, including lactoferrin, secretory leukocyte protease, cathelicidins, and defensins. For example, the antibacterial activity of lysozyme and LL-37/hCAP-18, a cathelicidin, have been reported to be synergistic in vitro (21). Lysozyme alone is ineffective against Gram-negative bacteria, while lysozyme in combination with lactoferrin is bactericidal for several Gram-negative strains (22). In the current study the disproportional increase in enzyme activity relative to lysozyme transgene protein may also be the result of such synergy. These results underscore the importance of testing the antimicrobial activity of lysozyme in vivo where numerous synergistic interactions are possible.

Several mechanisms have been proposed for the microbicidal activity of lysozyme in vitro. Lysozyme is a muramidase whose enzymatic activity is typically assessed by hydrolysis of the glycosidic linkage between N-acetylmuramic acid and N-acetylgalactosamine in the cell wall of Micrococcus luteus and Escherichia coli. Muramidase activity in BAL of transgenic mice expressing rat lysozyme was increased 17.7-fold relative to that in WT mice, suggesting that elevated lysozyme enzyme activity may account for much of the enhanced clearance of P. aeruginosa. However, there is also evidence that lysozyme possesses antibacterial activity that is independent of muramidase activity. Enzymatically inactive lysozyme was shown to retain bactericidal activity (23, 24), and During et al. (25) recently demonstrated that small amphipathic helical peptides derived from T4 lysozyme were both bacteriostatic and fungistatic. In the present study, clearance of GBS and Escherichia coli (data not shown) in transgenic mice was enhanced only 3.2-fold, similar to the 4-fold increase in lysozyme protein levels but much less than the increase in lysozyme enzyme activity. These results are consistent with a nonenzymatically mediated bactericidal action of lysozyme against some bacteria.
FIGURE 8. Levels of mMIP-2 are significantly increased in transgenic mice 6 h postinfection. P. aeruginosa (10^7 CFU) was injected into the trachea of eight 5-week-old transgenic mice and eight age-matched control WT littermates from transgenic line 3.5. The levels of proinflammatory mediators were assessed in BAL fluid and lung tissues at 6 and 24 h postinfection and in an equal number of uninfected littermates as described in Materials and Methods. Levels of mTNF-α, mIL-6, and mRANTES were not significantly different between transgenic mice and control WT littermates. Levels of mMIP-2 were significantly higher in transgenic mice at 6 h in both BAL (p = 0.04; B) and lung homogenates (p = 0.03; A) in WT mice; transgenic mice.

Enhanced bacterial killing in transgenic mice may be partly due to increased recruitment of neutrophils early in the course of infection. Neutrophil influx into the airspaces was slightly, but significantly, higher in transgenic mice at 6 h postinfection; further, the level of mMIP-2, a neutrophil chemotactic factor, was significantly elevated in transgenic mice at this time point. In contrast, neutrophil numbers and mMIP-2 levels in unchallenged transgenic mice were not different from those in WT littermates. These data suggest that constitutive overexpression of lysozyme in the airways may enhance neutrophil recruitment following infection. Although it is reasonable to speculate that increased neutrophil influx may contribute to enhanced bacterial killing in these transgenic mice, this hypothesis has not been directly tested.

Lysozyme was previously identified in rat alveolar type II epithelial cells by immunohistochemistry (5, 6). In the present study lysozyme was amplified by PCR using cDNA generated from highly purified isolated rat type II epithelial cells; the deduced amino acid sequence of the amplified product was identical with the previously reported sequence for rat lysozyme (26) with the single exception of a substitution of glycine for tryptophan at position 46. Immunogold labeling and subcellular fraction of type II epithelial cells localized lysozyme to lamellar bodies, secretory granules in which pulmonary surfactant is stored (27–29). Following secretion, lysozyme was detected in association with tubular myelin, a lattice-like network of surfactant membranes (27, 28). Interestingly SP-A, another lamellar body protein involved in airway host defense (30, 31), was also associated with tubular myelin (28, 32), raising the possibility that this structure may serve as a scaffold for host defense proteins involved in alveolar surveillance.

Although the present study has not defined the precise mechanism by which lysozyme promotes bacterial killing in vivo, these results have important implications for enhancing airway host defense. Lysozyme levels in transgenic mice were constitutively elevated without affecting lung structure, suggesting that lysozyme could be safely administered for extended therapy. Most importantly, elevated levels of lysozyme significantly enhanced killing of P. aeruginosa, the most common pathogen associated with chronic colonization of the airway in cystic fibrosis patients. Lysozyme administration alone or in combination with other agents, such as elastase inhibitors (33), may provide an important adjunct to treatment of chronic lung infections with antibiotics.

Acknowledgments
We thank Dr. Jeffrey Whitsett for insightful suggestions and for review of the manuscript.

References


