The Role of Recombinant Human Elafin in the Resistance of A549 Cells against *Pseudomonas aeruginosa* Biofilm

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Key Words

Biofilm · Elafin · Epithelial cells · *Pseudomonas aeruginosa* · Respiratory pathogens

Abstract

Background: *Pseudomonas aeruginosa*, an opportunistic respiratory pathogen, often causes pulmonary infection in patients with chronic inflammatory airway diseases and forms drug-resistant biofilm. Elafin, a natural antibiotic, has antimicrobial properties against Gram-negative bacteria. **Objectives:** This study investigated the effects of elafin on the resistance of epithelial cells against *P. aeruginosa* biofilm. **Methods:** A549 epithelial cells transfected with pEGFP-N1-elafin or pEGFP-N1 were stimulated with tumor necrosis factor (TNF), *P. aeruginosa* supernatant and *Escherichia coli* supernatant followed by co-incubation for 24 h to form *P. aeruginosa* biofilms. **Results:** We found structural changes in the biofilms of all groups, especially the *P. aeruginosa* group. Bacterial counts in the TNF ($3.238 \pm 0.356 \times 10^6$ CFU/cm²) and *P. aeruginosa* ($3.317 \pm 0.247 \times 10^6$ CFU/cm²) groups were lower than in the control group ($5.946 \pm 0.453 \times 10^6$ CFU/cm²; $p < 0.01$). In the *P. aeruginosa* group, the bacterial count was lower than that of the *E. coli* group ($5.138 \pm 0.391 \times 10^6$ CFU/cm²; $p < 0.05$). Bacterial clearance rates were higher in *P. aeruginosa* (44.2%) and TNF groups (45.6%) than in the *E. coli* group (13.6%). Cell intactness rates in pEGFP-N1-elafin-transfected groups were consistently higher than those of

the control group ($p < 0.01$); the *P. aeruginosa* group rate at 24 h was the highest. Compared to the control group, the TNF, *P. aeruginosa* and *E. coli* groups showed increased elafin mRNA and protein in cells ($p < 0.05$), and contents in the TNF and *P. aeruginosa* group were higher than in the *E. coli* group ($p < 0.05$). **Conclusion:** Overexpression of elafin in epithelial cells attenuated the damage of *P. aeruginosa* biofilm on epithelia. *P. aeruginosa* has the potential to induce elafin expression.

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Introduction

Pseudomonas aeruginosa is an opportunistic respiratory pathogen. *P. aeruginosa* infections are common in patients with immune dysfunctions, especially chronic inflammatory airway diseases such as chronic bronchitis, bronchiectasis and cystic fibrosis. *P. aeruginosa* is difficult to clear from the airway, and is thus an important pathogen of intractable pulmonary inflammatory diseases [1–3]. Recent research has shown that this problem is mainly caused by the bacterial biofilm formed by *P. aeruginosa*. The biofilm is composed of bacterial colonies on the inner surface of the respiratory tract and produces copious amounts of sugary mucous coating [4, 5]. Research has demonstrated that various bacteria can form biofilm.

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Recently, elafin, a special proteinase inhibitor [6] with the potential to control the serine proteinase secreted in the airway, has been found to have multiple efficacy, including the reduction in airway mucus hypersecretion, decrease in inflammatory lung injury [7, 8] and antimicrobial activity [9]. Its expression is upregulated in response to early inflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-1 β .

In this study, we transfected the constructed eukaryotic expression vector pEGFP-N1-elafin into airway epithelial A549 cells. The effects of different treatments on the expression of recombinant human elafin and the potential protection of elafin from *P. aeruginosa* biofilm attacks on airway epithelial cells were investigated. Our work may provide some guidance for the treatment of chronic pulmonary *P. aeruginosa* infection.

Materials and Methods

Tissues

Normal human lung tissues were obtained from surgical specimens resected at the Department of Thoracic Surgery of the First Affiliated Hospital (Chongqing Medical University) between 2004 and 2005. Informed consent was obtained prior to sampling and there was no preoperative treatment. Approval was obtained from the Ethics Committee of the institution. The patient group consisted of 10 males aged 50–70 years. All specimens were taken from peripheral areas at least 5 cm away from the lung cancer focus and immediately stored in liquid nitrogen. The tissues were stained with hematoxylin and eosin to confirm that they were non-cancerous.

Reagents

The green fluorescent protein eukaryotic expression vector pEGFP-N1 was obtained from Clontech Laboratories, Palo Alto, Calif., USA. TriPure and diethylpyrocarbonate were obtained from Roche, Lewes, UK. A Moloney murine leukemia virus, first-strand cDNA kit was purchased from Sangon Biotechnology, Shanghai, China. Restriction enzymes, DNA ligase and *Taq* polymerase were purchased from Takara, Dalian, China. Gel extraction and plasmid extraction kits were from Watson Biotechnology, Shanghai, China. MTT, DMSO and TNF were from Sigma, St. Louis, Mo., USA. LipofectamineTM 2000 was from Invitrogen, Carlsbad, Calif., USA. Rabbit antibody against elafin was from Jingmei Biotechnology, Guangzhou, China.

Bacterial Strains and Growth Conditions

E. coli DH5 α was prepared and stored in our laboratory. *P. aeruginosa* standard strain ATCC27853 and quality-controlled *E. coli* strain ATCC25922 were obtained from ATCC, Manassas, Va., USA. Müller-Hinton broth (MHB) was from Difco Biotechnology, Detroit, Mich., USA. Stainless-steel filter nets with 200 wells (7 × 7 mm) were used as carriers for bacterial growth.

RNA Isolation and Reverse-Transcription Polymerase Chain Reaction (PCR)

Total RNA was extracted from the preserved lung tissue using TriPure according to the manufacturer's recommendations, and was used as the template for first-strand cDNA synthesis. According to Genbank NM_002638 (67–420 bp), the following primers designed with Primer Premier 5.0 software were used: elafin forward primer 5'-CGGAATTCATGTAGGAAACTCTTCGGA-CA-3' (with ATG start codon), and elafin reverse primer 5'-CGC-GGATCCGAATGAGATGGCAAAGAACA-3'. Total RNA was incubated with 0.25 μ g of oligo(dT), 1 μ l of 10× PCR buffer (Promega, Madison, Wisc., USA), 100 U of Moloney murine leukemia virus reverse transcriptase, 7 U of RNasin and 1.25 μ l of each deoxynucleoside triphosphate in a reaction mixture (total volume: 10 μ l). The reaction mixture was incubated at 37°C for 45 min, followed by incubation for 10 min at 95°C. Then, 4 μ l of 10× PCR buffer, 80 pmol each of the forward and reverse elafin primers, 2.5 U of *Taq* DNA polymerase and enough H₂O to amount to a total volume of 50 μ l were added to the reaction products. PCR was conducted on these in an automated research thermocycler for 30 cycles. PCR conditions included: denaturation for 45 s at 94°C; annealing for 45 s at 61°C; extension for 1 min at 72°C, and a final extension at 72°C for 10 min. PCR products were visualized after electrophoresis in 2% agarose gels.

Cloning Elafin cDNA into pEGFP-N1

The PCR products were cloned, using standard techniques, into the pUmC-T cloning vector. Restriction fragments (*Eco*RI and *Bam*HI) were subcloned into the corresponding sites in the pEGFP-N1 vector. The DNA products were sequenced by Sangon Biotechnology and were identical to those published previously. This construct was denoted as pEGFP-N1-elafin.

Establishment and Identification of the *P. aeruginosa* Biofilm Model in vitro

The biofilm model was established as previously described [10]. *P. aeruginosa* was separated from patient airways, grown in MacConkey plates and transferred to a 37°C/5% CO₂ incubator. The colonies were suspended in *P. aeruginosa* solution in 0.50 McFarland units, and then placed in 24-well culture dishes with 1.8 ml of MHB. A sterile 200-well stainless-steel net was placed into each well, and wells were incubated at 37°C for 7 days. Media were exchanged every other day. By performing a rapid silver nitrate staining procedure [11] on the net on days 1 and 7, we identified the formation of biofilm models by the existence of gray-black floc film.

Establishment of a Standard Curve with the MTT Technique

P. aeruginosa standard strain ATCC27853 was cultured overnight in MHB. Couples of bacterial colonies were extracted and were diluted to bacterial suspending solution with the concentration of 0.5 McFarland units using a nephelometer. The solution was then diluted 5 times with duplication dilution, followed by division into six different concentration solutions. After division, 200 μ l of the solution were poured into 24-well culture plates. Then 20 μ l MTT (5 mg/ml) were added to each well and the cells were transferred to the incubator for 2 h, and centrifuged at 1,000 rpm for 10 min. Once the supernatant was removed, 200 μ l of DMSO was added to each well and left for 5 min. Optical density (OD) values were subsequently determined at 570 nm on a micro-

plate reader. Standard curves were constructed with OD values on the x-axis and the live bacterial count on the y-axis.

Determination of Bacterial Counts Using the MTT Technique

MTT was used for the determination of bacterial counts as described by Gebran et al. [12]. The germ-free filtering nets with mature biofilm were washed with 0.9% sterile NaCl solution. Three groups of cells in 24-well plates were put into the net (6 wells per group) and incubated at 37°C for 8 h. After removing the net from the incubator and washing away floating bacteria, the net was rinsed with 1 ml 0.9% NaCl solution to completely remove the bacteria from the biofilm. The bacterial suspending solution was transferred to a 96-well plate (200 µl/well). OD values were determined using the above-mentioned technique, and live bacterial counts were obtained according to the above-established standard curve. Based on these numbers, we calculated the number of bacteria on each net (CFU/cm²).

Cell Line and Transfection

The pulmonary epithelial A549 cell lines derived from a human lung bronchioloalveolar carcinoma and representative of type II alveolar cells were maintained in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum, 1% L-glutamine and 1% penicillin/streptomycin at 37°C in 5% CO₂ atmosphere. A549 cells were transfected with pEGFP-N1-elafin using Lipofectamine 2000 according to the manufacturer's instructions. Cells were grown to 90–95% confluence in 6-well cell culture dishes with collagen on the bottom. Cells were starved in serum-free MEM overnight (24–48 h). The treatment consisted of the following: 10 µl of recombinant elafin DNA and 10 µl of Lipofectamine were diluted in 240 µl of serum-free MEM, respectively, and subsequently mixed for 5 min at room temperature. Following a rest period of 20 min, 500 µl of the solution were added to each well. Cells were maintained in a humidified 5% CO₂ incubator at 37°C for 6 h after removing any excess transfection medium, and were then incubated in 2 ml of medium with 10% fetal calf serum for 18 h. Cells transfected with pEGFP-N1 were used as negative controls.

Groups and Treatments

Transfected cells were given the following treatments during the 24-hour incubation: in the TNF group, cells were treated with 100 ng/ml TNF; the *P. aeruginosa* group was induced with *P. aeruginosa* (1.5×10^9 CFU/l) supernatant; the *E. coli* group with *E. coli* (1.5×10^9 CFU/l) supernatant, and the control group received no further treatment.

Real-Time Quantitative PCR

Total RNA was extracted from the treated A549 cells in each group using TriPure according to the manufacturer's recommendations. Elafin mRNA levels were determined by real-time quantitative PCR. This technique relates the amount of elafin mRNA present to levels of ribosomal 18S, controlling for the amount of RNA present. RNA samples were reverse transcribed using random primers. PCR reaction mixtures included *Taq* DNA polymerase (0.025 IU/ml), MgCl₂ (3.6 mM), deoxynucleotide triphosphates (all at 200 µM), specific forward and reverse primers (300 nM; Genebank) and probes (50 nM) for elafin. In addition, ribosomal 18S primers and probes (all at 50 nM) were added. Samples were measured in triplicate, and no template controls (containing

water instead of RNA) were included. The following primers designed with Primer Premier 5.0 software were used: elafin forward primer 5'-TGGCTCCTGCCCCATTATC-3'; elafin reverse primer 5'-CAGTATCTTTCAAGCAGCGGTTAG-3', and probe 5'-ATCCGGTGCGCCATGTTGAATCC-3'. A Taqman-specified protocol was used for the PCR reaction. The reaction began with incubation for 2 min at 50°C, followed by incubation for 10 min at 95°C. PCR was then conducted on reverse transcription (RT) reaction products in an automated thermocycler for 40 cycles. PCR conditions included: denaturation for 45 s at 94°C; annealing for 1 min at 60°C, and extension for 1 min at 60°C. The product melting temperature was 81°C. Primers and probes were validated at the above concentrations, and linearity of response was confirmed by serial dilution of cDNA. Within-assay variation of PCR measurements was calculated from six replicates to be 1.9%. Variability of the reverse transcription step was determined by reverse transcribing one RNA sample on eight separate occasions. The eight cDNA samples were then included within one PCR run, and variability (relative to SD) was calculated to be 3.65%.

Determination of Elafin Protein Content in Cell Supernatant by ELISA

To determine elafin protein content, 96-well plates were coated with polyclonal monospecific anti-elafin IgG (1:500) in carbonate buffer, pH 9.6, overnight at 4°C. After blocking, samples and standards (diluted in PBS gelatin) were applied for 2 h at room temperature followed by another 2-hour incubation with biotinylated anti-elafin IgG at 2 mg/ml. After addition of streptavidin-horseradish peroxidase for 2 h at room temperature, plates were washed and 100 µl of chromogenic substrate containing 0.001% H₂O₂ was applied to each well. The absorbance was read at 490 nm with a microplate reader. Within- and between-assay precision measurements were 8.12 and 12.74%, respectively.

Observation of the Cell Intactness Rate after Interaction between Bacteria and Cells

After incubation for 24 h following each group's treatment, cells were challenged with mature biofilm on the net for 8 h. Morphological cell changes were assessed under an inversion microscope after 4, 8, 12 and 24 h. Cell intactness rates were calculated using morphometric analysis. This method included random selection of 5 fields of high multiple vision, and took the mean from these fields as the rate of cell breakage. The cell intactness rate was calculated as the confluent rate minus the breakage rate.

Observation of the Effects of Elafin on Biofilm with Scanning Electron Microscopy (SEM)

The nets with mature biofilms were washed with sterile 0.9% NaCl solution, then divided into two groups of cells in 24-well culture plates at 37°C for 8 h. These were then observed with SEM after they were sufficiently washed, fixed, dehydrated in an ethanol series and dried. Finally, the nets were mounted on stubs with carbon adhesive disks and silver DAG (dodecyl diaminoethyl-glycinhydro-chloride, which is an aquadag material), sputter coated and viewed using an SEM.

Determination of the Ratio of Biofilm Area Integral to Net Well Area

The relevant region was outlined with computerized morphometric analyses. The ratio of the area integral to each net well area was obtained.

Fig. 1. Elafin mRNA expression in each group. * $p < 0.05$, ** $p < 0.01$, vs. control group.

Statistical Analysis

All data are expressed as means $\pm$ SD, and analyses were performed with SPSS 10.0 for Windows (SPSS, Chicago, Ill., USA). Pairs of groups were compared with two-tailed Student's *t* tests. $p < 0.05$ was considered statistically significant.

Results

Expression of Elafin mRNA in Cells

Elafin mRNA was detected in four cell groups. Dramatic increases in elafin mRNA were seen in cells of both the TNF and *P. aeruginosa* groups, and the expression of elafin mRNA was at least 5 times greater in these groups than that in the cells of the control group ($p < 0.01$). In addition, the expression of elafin mRNA in cells of the *E. coli* group was 2 times greater than that in cells of the control group ($p < 0.05$; fig. 1).

Protein Level of Elafin in Cell Supernatant

Elafin levels in both cell supernatant and cells were significantly higher than those of the control group as a result of the inducement of TNF, *P. aeruginosa* and *E. coli*. Furthermore, the above changes in the TNF and *P. aeruginosa* groups were more obvious than those in the *E. coli* group (table 1).

Rate of Cell Damage in Each Group

After cells interacted with the biofilms, we monitored cell confluence and breakage rates at different time points (4, 8, 12 and 24 h) with an inversion microscope. The cell breakage rate was calculated and compared to that of the control group at each time point. As shown in table 2, a

Table 1. Elafin levels in each group (means $\pm$ SD)

Group	Level of elafin secretion (OD)
Control	0.12 ± 0.05
TNF	$0.66 \pm 0.21^{*,***}$
<i>P. aeruginosa</i>	$0.62 \pm 0.26^{*,***}$
<i>E. coli</i>	$0.21 \pm 0.07^{**}$

* $p < 0.01$, ** $p < 0.05$, vs. control group; *** $p < 0.01$ vs. *E. coli* group.

significant decrease in cell damage was observed in the TNF and *P. aeruginosa* groups, and a lesser decrease was observed in the *E. coli* group. The most significant reduction in cell damage (in comparison to the control group) in the *P. aeruginosa* group was at the 24-hour time point.

Effect of Recombinant Elafin on the Number of Bacteria in Biofilms

The bacterial counts of the TNF and *P. aeruginosa* groups were 3.238 ± 0.356 and $3.317 \pm 0.247 \times 10^6$ CFU/cm², respectively. These counts were significantly different ($p < 0.01$) versus the control group ($5.946 \pm 0.452 \times 10^6$ CFU/cm²). The bacterial count in the *P. aeruginosa* group was lower than that in the *E. coli* group ($p < 0.05$; table 3).

Effect of Recombinant Elafin on the Biofilm Structure

After mature biofilm had acted on the three groups of cells with recombinant elafin and inducement for 8 h, the density of biofilm acting on cells in the TNF group significantly decreased, with some dissociation of bacteria (fig. 2). A similar change in structure occurred in cells in the *P. aeruginosa* group. The number of bacteria were significantly reduced (fig. 3) and were mostly dispersed. Little change was observed on the biofilm in the *E. coli* group (fig. 4).

Determination of the Biofilm Area Integral/Net Well Area Ratio

After co-incubation for 8 h with mature biofilm models, the highest ratio of biofilm area was observed in normal control cells without recombinant elafin. Elafin-recombinant cells treated with TNF and *P. aeruginosa* showed a significant reduction in the biofilm area in comparison to the control group ($p < 0.01$). The ratio of the biofilm area in the *E. coli* group also decreased modestly ($p < 0.05$ vs. control group; table 4).

Fig. 2. Biofilm of the TNF group. SEM (×3,000).

Fig. 3. Biofilm of the *P. aeruginosa* group. SEM (×7,000).

Table 2. Rate of cell damage in each group

Group	n	Rate of cell damage (means $\pm$ SD, %)			
		4 h	8 h	12 h	24 h
Control	12	86.5 $\pm$ 6.40	32.3 $\pm$ 3.72	56.2 $\pm$ 6.34	89.8 $\pm$ 10.2
TNF	12	1.5 $\pm$ 0.18*, ***	11.2 $\pm$ 1.56*, ***	25.1 $\pm$ 2.86*, ***	55.2 $\pm$ 6.92*, ***
<i>P. aeruginosa</i>	12	1.2 $\pm$ 0.12*, ***	12.6 $\pm$ 1.75*, ***	28.8 $\pm$ 3.16*, ***	41.2 $\pm$ 5.15*, ***, ****
<i>E. coli</i>	12	2.9 $\pm$ 0.31*	27.8 $\pm$ 3.23**	47.1 $\pm$ 4.12**	71.2 $\pm$ 7.16**

* $p < 0.01$, ** $p < 0.05$, vs. control group; *** $p < 0.01$, vs. *E. coli* group; **** $p < 0.05$, vs. TNF group.

Discussion

In many environments, bacteria form colonies called biofilms, which are considered to be a major source of infection. Biofilms are caused by various common bacteria such as *P. aeruginosa*, *E. coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* [13]. *P. aeruginosa* is the most common bacterium causing hospital-acquired infections, especially pulmonary infections [14, 15], and can increase neutrophil activation and neutrophil proteolytic activity in bronchoalveolar lavage [16]. Such infections usually affect patients with a history of chronic inflammatory airway diseases in large-scale hospitals. Biofilms also promote the emergence of drug-resistant bacteria, which increases morbidity and mortality of chronic airway diseases [17]. They can block the infiltration of antibiotics, and absorb inactive enzymes that promote the

hydrolyzation of antibiotics. In addition, bacteria under the biofilm coating remain in a hypometabolic condition and have a lower sensitivity to antibiotics [18, 19].

Elafin, the neutrophil elastase (NE) inhibitor, has a gene sequence about 2.3 kb long consisting of three exons and two introns [20]. It contains typical 5' TATA and CAAT boxes [21], and is synthesized by alveolar macrophages, Clara cells [22] and type II alveolar cells. The secretion of elafin is regulated by multiple factors, such as bacterial lipopolysaccharide [23], IL-1, TNF [24] and NE [25, 26]. Adenoviral A1E has the potential to suppress elafin release in human alveolar and bronchial epithelial cells [27]. This study demonstrates the expression of elafin protein and mRNA levels in A549 cells co-incubated with NE, *P. aeruginosa* and *E. coli*, respectively. *P. aeruginosa* had an elafin-inducing ability similar to NE, and both were stronger than that of *E. coli*. In addition, the

Fig. 4. Biofilm of the *E. coli* group. SEM ($\times 3,000$).

duration of the *P. aeruginosa*-induced elafin expression was longer, possibly related to the negative effect of elafin on NE [28–30]. In terms of its elafin-inducing ability, *P. aeruginosa* appears to be more specific than other bacteria, which partly explains why its local infection in airways is at least partially self-limited [31]. Elafin can also be secreted into mucosa because of its low-molecular-weight cationic peptide. In addition to its local anti-inflammatory effect, elafin has been shown to have antimicrobial properties against Gram-positive and -negative bacteria and fungi in vitro [32, 33], especially against bacteria responsible for hospital-acquired lower respiratory infections.

In our study, we observed a decrease in bacterial counts of biofilm on the surface of cells transfected with pEGFP-N1-elafin with SEM. Furthermore, the MTT technique showed that the bacterial structure became dissociated while the bacterial count significantly decreased. These results support the antibacterial activity of elafin against biofilm-forming bacteria. Moreover, the biofilm structure and the bacterial clearance rates changed with different inducements. Given the remarkable reduction in the intactness rate of cells transfected with recombinant elafin, we conclude that elafin can have a protective effect on airway epithelial cells to attenuate damage caused by *P. aeruginosa* biofilm. These data suggest that elafin may be a crucial endogenous defense molecule to resist localized airway infection [34, 35]. Studies have suggested that the anti-inflammatory and antibacterial properties of elafin may be independent of its antiprotease capability, but related to its cationic nature [36, 37]. Elafin is mainly

Table 3. Bacterial count and bacterial clearance ratio of each group (means $\pm$ SD)

Group	n	Bacteria, n $\times 10^6$ CFU/cm ²	Ratio of clearing, %
Control	24	5.946 $\pm$ 0.452	
TNF	24	3.238 $\pm$ 0.356*, ***	45.6
<i>P. aeruginosa</i>	24	3.317 $\pm$ 0.247*, ***	44.2
<i>E. coli</i>	24	5.138 $\pm$ 0.391**	13.6

* $p < 0.01$, ** $p < 0.05$, vs. control group, *** $p < 0.01$, vs. *E. coli* group.

Table 4. The proportion of biofilm in each group by silver nitrate staining (means $\pm$ SD)

Group	n	Ratio of biofilm area
Control	24	0.462 $\pm$ 0.135
TNF	24	0.243 $\pm$ 0.081*
<i>P. aeruginosa</i>	24	0.264 $\pm$ 0.108*
<i>E. coli</i>	24	0.373 $\pm$ 0.089**

* $p < 0.01$, ** $p < 0.05$, vs. control group.

secreted at mucosal sites. In respiratory tracts, bronchial epithelial cells, alveolar epithelial cells and alveolar macrophages contribute to elafin production, and neutrophils can promote elafin release. It has been shown to have multiple important roles, notably its inhibitory effect on human NE, porcine pancreatic elastase and proteinase-3 [38], all of which are beneficial in controlling NE-induced mucus hypersecretion. Elafin can also resist MMP8-induced hydrolyzation produced by neutrophils, thus increasing its potential in the treatment of neutrophil-mediated inflammatory lung diseases.

The main anti-inflammatory activities of elafin are as follows. First, it affects neutrophil function. It can restrain the release of inflammatory factors and suppress the outbreak of inflammatory cytokine cascades. The anti-inflammatory actions of elafin have been associated with reduced activation of transcription factor NF- κ B and concomitant increase in I κ B α protein [39]. Butler et al. [40] have reported that elafin suppressed the expression of TNF- α and IL-1 β in lipopolysaccharide-stimulated monocytes; it can also compete with lipopolysac-

charide-binding protein in binding to lipopolysaccharides [41]. Elafin can also restore apoptotic cell recognition by macrophages in inflammatory conditions [42]. It can increase the number and activation status of CD11c+/MHCII+ lung dendritic cells in vivo. Elafin overexpression protected mice against further challenges with Ad-LacZ. The pleiotropic molecule elafin was shown to have significant potential in modulating antigen-presenting cell numbers and activity, and could be beneficial in mucosal protective strategies [43–45]. Elafin may also play a role in maintaining epithelial integrity by protecting against NE-mediated inflammatory injuries [46].

In this study, we demonstrated that *P. aeruginosa* has the definite capability of inducing elafin production and

secretion. Elafin has natural antibacterial activity superior to that of artificially synthesized antibiotics, particularly considering the negative effects of the latter on the imbalance of airway bacterial colonies [47]. Further investigation is needed to improve the continual and high-level expression of elafin in human airways. In conclusion, gene augmentation of elafin may have considerable potential in the treatment of chronic pulmonary *P. aeruginosa* infection.

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