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Antimicrobial Activity of Murine Lung Cells against *Staphylococcus aureus* Is Increased In Vitro and In Vivo after Elafin Gene Transfer

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**Staphylococcus aureus** is a pathogen often found in pneumonia and sepsis. In the context of the resistance of this organism to conventional antibiotics, an understanding of the regulation of natural endogenous antimicrobial molecules is of paramount importance. Previous studies have shown that both human and mouse airways express a variety of these molecules, including defensins, cathelicidins, and the four-disulfide core protein secretory leukocyte protease inhibitor. We demonstrate here by culturing mouse tracheal epithelial cells at an air-liquid interface that, despite the production of Defb1, Defb14, and Defr1 in this system, these cells are unable to clear *S. aureus* when exposed to this respiratory pathogen. Using an adenovirus (Ad)-mediated gene transfer strategy, we show that overexpression of elafin, an anti-elastase/antimicrobial molecule (also a member of the four-disulfide core protein family), dramatically improves the clearance of *S. aureus*. In addition, we also demonstrate that this overexpression is efficient in vivo and that intratracheal instillation of Ad-elafin significantly reduced the lung bacterial load and demonstrates concomitant anti-inflammatory activity by reducing neutrophil numbers and markers of lung inflammation, such as bronchoalveolar lavage levels of tumor necrosis factor and myeloperoxidase. These findings show that an increased antimicrobial activity phenotype is provided by the elafin molecule and have implications for its use in *S. aureus*-associated local and systemic infections.

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**MATERIALS AND METHODS**

Recombinant replication-deficient Ad constructs. Two E1-partially E3-deleted type 5 recombinant replication-deficient adenoviruses were used. The Ad-LacZ.
construct was a gift from J. Gaudie and F. Graham (1), whereas the Ad-elafin construct was generated as described elsewhere (28).

**Bacteria.** *S. aureus* C1705 (a clinical strain [9, 18]) was grown initially as colonies on Colombia agar (Unipath, Basingstoke, United Kingdom) and then in 10 ml of tryptone soy broth (Unipath) overnight at 37°C in an orbital shaker (Gallenkamp; Fisher Scientific, Loughborough, United Kingdom) at 200 rpm at room temperature. The resulting suspension was centrifuged at 3,000 rpm for 15 min at room temperature. The supernatant was discarded, and the pellet resuspended in 10 ml of phosphate buffer (8 mM K2HPO4, 2 mM KH2PO4). Suspensions were adjusted with phosphate buffer to an A280 of 1.50, yielding an estimated bacterial concentration of 107 CFU/ml, in accordance with predetermined growth curves.

Serial dilutions were performed with phosphate buffer to obtain the desired concentration of viable bacteria (see below).

**Mouse epithelial cell culture.** Female C57BL/6 or BALB/c murine primary tracheal epithelial cells were generated and grown on semipermeable membranes at an ALI according to our previously published method (4). Briefly, tracheae were excised and severed at the proximal limit of the thyroid cartilage and at the junction of the main bronchi. The thyroid gland and other adherent tissues were removed before cutting trachea longitudinally. Dissociated cells from two tracheae (ca. 4 × 10^6 cells) were seeded onto one semipermeable support membrane, precoated with 0.5 mg of collagen/ml (24-well plate inserts, 0.4-μm pore size; Corning Costar) in 0.2 ml of culture medium (1:1 Dulbecco modified Eagle medium-Ham’s F-12 medium containing 100 U of penicillin and 100 μg of streptomycin/ml). Cells were incubated at 37°C in 6% CO2 for 3 days.

On day 4, medium bathing the apical cell surface was removed, along with any cell debris, and the medium on the outside of the insert replaced with 0.6 ml of tryptone soy broth (Unipath) overnight at 37°C in an orbital shaker construct was generated as described before (28).

**Ad infections.** Adfections were carried out in USG medium. Cells were incubated with these concentrations to allow for the generation of these precipitates (6). Alternatively, Ad infections were further diluted 10-fold with phosphate buffer–0.5% Triton X-100. These two washes were then pooled and centrifuged at 8,000 rpm for 10 min. Supernatants were used for elafin quantitation by enzyme-linked immunosorbent assay (ELISA) (27), whereas bacterial cell pellets were suspended in PBS and used for bacterial counts on Colombia agar. Agar plates were incubated overnight at 37°C prior to colony counting. Values represent the mean ± the standard error of three experiments, each performed in triplicate. (Significant differences [P < 0.05] compared to Ad-lacZ-infected cells are indicated in Table 1)

**In vivo studies.** Female C57BL/6 mice between 6 and 8 weeks old (Harlan Olac, Bicester, United Kingdom) were treated intratraehal (as described in reference 33) with 40 μl of Ad-LacZ (adenovirus control) or Ad-elafin (3 × 107 PFU in both cases). After 5 days, 4 × 107 CFU of S. aureus (in 40 μl) were administered via the same route. This bacterial dose was shown in previous pilot experiments to produce a reproducible inflammatory response in the lung. At 24 h after the bacterial administration, mice were euthanized and bronchoalveolar lavage (BAL) performed (two serial instillations of 0.4 ml of PBS). BAL fluid (BALF) was centrifuged 10 min at 4,000 × g; supernatants were stored at −20°C until further use, whereas each cell pellet was resuspended in 100 μl of PBS. Cell counting (with a hemocytometer) and cytospins (300 rpm for 3 min at room temperature) were then performed. BALF was recovered, the lungs were harvested for either RNA preparation (storage in RNA later [Sigma]), histology, or bacterial counts. For the latter, half lungs were homogenized in 2 ml of PBS by using a tissue homogenizer (OMNI μL International; Camlab), and homogenates were further diluted 10-fold with PBS. A total of 100 μl of the diluent was plated on Colombia agar plates, which were incubated at 37°C for 24 h.

**ELISAs.** ELISA analyses to detect murine tumor necrosis factor alpha (TNF-α) were performed by using the R&D Duoset kit (Abingdon, Oxon, United Kingdom) in accordance with the manufacturer’s instructions.

**Human elafin was measured by ELISA as described previously (27).**

**Protein quantification.** Protein was measured by using the bichinchoninic acid method (Pierce) with purified albumin (Pierce) as the standard.

**MP0 measurement.** A total of 40 μl of BAL fluid was incubated with 60 μl of TMB (3,3′,5,5′-tetramethylbenzidine substrate; 0.1 mg/ml in 0.1 M sodium acetate-citrate buffer [pH 4.9]) containing 0.15% H2O2 (final concentration). After 5 min, the reaction was stopped with concentrated H2SO4, and the absorbance was read at 450 nm (reference filter 560 nm) in a microtitre plate reader (MRX II microtiter plate; Dynex Technologies).

**Statistical analysis.** Statistical analyses used either Student t tests or Mann-Whitney tests.

**RESULTS**

**Murine defensein expression in murine primary epithelial cells.** RNA was prepared from C57BL/6 tracheal inserts as detailed in Materials and Methods, and RT-PCR was performed with primers for Hprt (housekeeping gene), as well as for the murine β-defensin genes relevant to antimicrobial activity against *S. aureus*. Figure 1 demonstrates that murine C57BL/6 tracheal cells express Defb1, the mouse orthologue of human *DEFB1*, and Defb14, the mouse orthologue of *DEFB103*. We also show for the first time that Defr1 is expressed in murine epithelial cells. There is no evidence for significant upregulation of these genes after LPS exposure.

**Innate antimicrobial activity of murine epithelial cells against *S. aureus*.** We were interested in examining whether any difference in bacterial killing was evident between BALB/c

3610 MCMICHAEL ET AL. INFECT. IMMUN.
and C57BL/6 mice since they have a polymorphism in one of their β-defensin genes. BALB/c has Defb8, a classic six-cysteine β-defensin, whereas C57BL/6 has a polymorphic allele with three nucleotide and amino acid changes that results in a five-cysteine β-defensin termed Defr1. This molecule has a novel structure, which when made synthetically has potent antibacterial activity (3). Despite the expression of β-defensins in C57BL/6 tracheal cells, neither these cells nor cells derived from BALB/c mice were able to inhibit the growth of an approximately 200 CFU S. aureus inoculum (20 nl of a 10^7-CFU/ml S. aureus suspension) (Fig. 2). This was considered already as a very small inoculum (identical to that used by Smith et al. [36]), and no attempt was made to further reduce that dose. Indeed, at 3 h postinoculation, an average of 3,077 and 2,050 CFU were recovered from C57BL/6 and BALB/c cells, respectively, compared to 329 in the original inoculum. This clearly demonstrates that, far from being bacteriostatic or bactericidal, the tracheal epithelium was able to promote S. aureus growth under these conditions.

With this observation in mind, we examined whether recom-

![FIG. 1. Expression of defensin genes in mouse primary tracheal epithelial cells. RNA was prepared from female C57BL/6 tracheal epithelial cells (unstimulated or stimulated with LPS for 30 min), and RT-PCR was performed as detailed in Materials and Methods with primers for three murine β-defensin genes (Defb1, Defb14, and Defr1) and for the housekeeping gene Hprt. In the negative control lane (−ve control), RNA was not included in the RT-PCR mixture. (a) Ethidium bromide analysis; (b) Southern blot analysis.](image)

![FIG. 2. Endogenous antimicrobial activity of murine C57BL/6- and BALB/c-derived tracheal cells. A 200-CFU inoculum of S. aureus (20 nl of either phosphate buffer or of a C1705 S. aureus suspension [10^7 CFU/ml]) was applied to inserts of either C57BL/6- or BALB/c-derived tracheal epithelial cells, as described in Materials and Methods. Inserts were washed with 105 μl of phosphate buffer and 105 μl of phosphate buffer–0.5% Triton X-100. Washes were pooled, followed by centrifugation at 8,000 rpm for 10 min, and the pellets were used for bacterial counts on nutrient agar plates at 37°C. Values represent means ± the standard errors of three experiments, each performed in triplicate (three inserts).](image)
binant adenovirus (Ad)-mediated overexpression of human elafin, an antimicrobial molecule with no ortholog in mice, was able to limit *S. aureus* growth in vitro and in vivo.

**Optimization of infection of murine C57BL/6 epithelial cells with Ad vectors.** We first tested the Ad expression system by using the *E. coli* β-galactosidase reporter gene (Ad-LacZ). Because others have reported poor adenoviral gene transfer to airway epithelia due to low apical expression of the coxsackie/adenovirus receptor (26), we used a method incorporating the use of CaPO₄ precipitates in the infection protocol in the present study, as previously investigated (6).

Indeed, we show here (Fig. 3) that generation of CaPO₄ precipitates in MEEM (a medium particularly well suited for the generation of the latter due to its high concentration of PO₄ ions) containing Ad-LacZ significantly increased (two- to threefold) the efficiency of infection of murine C57BL/6 tracheal cells compared to Ad-LacZ infection in the USG medium usually used for these cells (Fig. 3, compare panels D and C). This amended protocol, with MEEM as the medium of choice, was therefore used for all subsequent experiments.

Neither CaPO₄- nor Ad-mediated cytotoxicity was detected by trypan blue exclusion (not shown).

**In vitro antibacterial activity of Ad-elafin against *S. aureus.*** Having established the conditions for optimal Ad infection of murine C57BL/6 tracheal cells, we infected these cells with Ad-elafin and used Ad-LacZ as an Ad control. Figure 4 shows that, in accordance with all our previous studies (28, 30, 32), murine cells do not produce an elafin-related protein since our ELISA did not detect a cross-reacting species from untreated cells or cells treated with Ad-LacZ. In contrast, Ad-elafin infection induced the expression and secretion of very high levels of human elafin (right panel). This level correlated with an efficient reduction of *S. aureus* numbers (left panel, \( P = 0.005 \)).

**In vivo antibacterial activity of Ad-elafin against *S. aureus.*** When studying antimicrobial activity against *P. aeruginosa* (33), we have shown that Ad-LacZ was a useful Ad vector control which, by itself, did not induce a detectable increase in lung inflammation in comparison to PBS treatment. This lack of overt inflammation caused by Ad-LacZ is most probably due to the very low dose of Ad used in our study (\( 3 \times 10^7 \) PFU). In

![Image](https://example.com/image.png)
the present study we compared two experimental groups, Ad-LacZ (3 × 10⁷ PFU) plus S. aureus and Ad-elafin (3 × 10⁷ PFU) plus S. aureus, and one control group, PBS-PBS; the latter served as a control for potential background S. aureus commensals and contamination in the laboratory.

As expected and in keeping with our previous studies (and present in vitro data), no elafin mRNA was detected by RT-PCR in mice receiving Ad-LacZ or PBS, demonstrating that the band detected in the Ad-elafin–S. aureus group (Fig. 5, upper right) was specific to the human elafin transgene. This elafin expression was associated with a reduction in the number of recovered S. aureus in the BALF of Ad-elafin-treated mice, compared to mice given Ad-LacZ (Fig. 5, left panel; P = 0.038). As expected, no bacteria were recovered in the mice treated twice with PBS.

**Modulation of in vivo inflammatory responses by Ad-elafin gene transfer.** When inflammatory cell profiles in BALF were studied, compared to the PBS/PBS group, unsurprisingly, both the Ad-LacZ plus S. aureus and the Ad-elafin plus S. aureus groups showed increased numbers of total inflammatory cells, particularly neutrophils (P = 0.02 and 0.013, respectively). However, no statistically significant differences in cellular profiles were observed between the Ad-LacZ plus S. aureus and Ad-elafin plus S. aureus groups (data not shown). Interestingly, when the Ad-elafin plus S. aureus group was considered in isolation, there was a strong negative correlation between BAL elafin levels and neutrophil numbers (Fig. 6, r = −0.63, P = 0.069), suggesting that elafin may be associated in this model with a reduction in neutrophil numbers. Also, MPO levels, used as a marker of neutrophil activation, were lower in the Ad-elafin plus S. aureus group, although the difference did not reach statistical significance. A similar reduction (statistically significant) in the proinflammatory mediator TNF-α was noted in the Ad-elafin–S. aureus group, compared to the Ad-LacZ–S. aureus group (Table 1). When total protein levels were measured as an index of blood and/or alveolar barrier disruption, levels were lower in the PBS-PBS group compared to the Ad-groups, but within these there was a trend toward reduction of protein levels in the Ad-elafin–S. aureus group. Mirroring these data, blinded histological analysis showed neutrophilic inflammatory foci in the Ad-LacZ-treated animals, which were reduced in the Ad-elafin-treated mice. Quantification of neutrophils in whole lung sections is also given (representative sections are shown in Fig. 7).

**DISCUSSION**

*S. aureus* is a major human pathogen associated with localized and systemic infections. In order to facilitate and model the study of microbial infections in mice, cell culture systems have been developed where epithelial cells form a confluent, polarized, ciliated epithelium when grown at an air-liquid interface (4). Using this model, we have previously demonstrated that murine tracheal epithelial cells display many characteristics similar to those of murine tracheal epithelium in vivo and that these cells retain the ability ex vivo to produce important antimicrobial molecules such as β-defensins, which have been specifically demonstrated in the trachea (4). We have also shown previously that *Defb2* is expressed in these cells after LPS induction (4). However, we did not follow the in vivo fate of this gene in relation to *S. aureus* exposure because, in vitro, the *Defb2* peptide is a very poor antimicrobial against *S. aureus*.

![](image-url) FIG. 4. Ad-mediated elafin production and antibacterial activity in vitro. Female C57BL/6 mouse tracheal epithelial cells were either uninfected or infected with Ad-LacZ control or Ad-elafin (MOI = 100) in MEEM containing CaPO₄ precipitates. After 18 to 20 h at 37°C (see Fig. 3 legend), a 200-CFU inoculum of *S. aureus* was added (as for Fig. 2). Bacterial counts were performed as described in the same legend, and elafin quantification was performed by ELISA (21). Values represent mean ± the standard error of three experiments, each performed in triplicate (three inserts). **, Significant difference (Mann-Whitney, P = 0.005); ND, not detected.
strain C1705 (M. Rolfe et al., unpublished). Here, we have extended these findings by showing for the first time that two further important molecules with activity against *S. aureus* (*Defr1* and *Defb14*) are also expressed in this system. *Defr1* encodes a defensin related peptide (Defr1) that deviates from the canonical six cysteine defensin motif by having the first cysteine replaced with a tyrosine in C57BL/6 mice (21). Surprisingly, we have shown that the five cysteine Defr1 peptide is active in vitro as an antimicrobial and has salt sensitive activity against *S. aureus*. *Defb14* is the mouse orthologue of human *DEFB103*, which encodes a human peptide (hBD3), shown to have potent activity against *S. aureus*, including MRSA (17).

However, despite the ability of these cells to secrete these antibacterial molecules (Fig. 1), we have shown here that they are unable to control *S. aureus* growth in ALI cultures (Fig. 2). The mechanism for such resistance of *S. aureus* may be complex and could be due partly to its capacity to modify phosphatidylglycerol, the major phospholipid of *S. aureus*, with L-lysine, rendering the membrane more cationic and hence repulsive to cationic antimicrobial molecules such as defensins (25). A more specific mechanism could be the production of staphylokinase, which has been shown recently to inactivate human defensins (15). Although we have not demonstrated here the secretion of murine endogenous antimicrobial peptides (and only showed their transcripts [see Fig. 1]), it is likely that these were also produced as demonstrated for HBD-2 in the same ALI system (35). Since it is therefore likely that *S. aureus* was able to grow even in the presence of these endogenous peptides, we then tested whether overexpressing human

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**FIG. 5.** Ad-elafin expression and antibacterial activity in vivo. Female C57BL/6 mice were sequentially instilled intratracheally with either Ad-LacZ or Ad-elafin and then with *S. aureus* (S.a) (see Table 1). At 24 h after bacterial administration, BALF was obtained and centrifuged; the cell pellet was used for differential cell analysis, whereas supernatants were used for bacterial counts (Mann-Whitney analysis) and elafin quantification. In parallel, RNA was isolated from lungs and elafin RT-PCR analysis was performed as described in Materials and Methods. ND, not detected.

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**FIG. 6.** Correlation between neutrophil count and elafin concentration in BALF. BALF from mice given Ad-elafin and *S. aureus* (see Table 1) were analyzed for elafin levels (by ELISA) and neutrophil count (by using cytospins).
elafin, a molecule for which no ortholog exists in the mouse, would endow murine tracheal cells with defense against \textit{S. aureus}.

Here, using the Ad-overexpressing system described above, we show that murine tracheal epithelial cells producing human elafin were able to reduce \textit{S. aureus} numbers significantly (Fig. 4). Although the exact mechanism of action was not elucidated and was beyond the scope of the present study, the elafin molecule has a net positive charge of $+7$, suggesting that its activity against \textit{S. aureus} (34) may be directed at bacterial membranes.

We show in Fig. 4 that elafin secretion is associated with significant inhibition of growth of \textit{S. aureus}, although it is unclear whether it acts only to slow the growth of the organisms, directly kills a proportion of the organisms, or a combination of both, since the CFU counts of \textit{S. aureus} recovered after incubation with Ad-elafin-infected cells were not lower than those added at the outset (not shown). In addition, elafin

![FIG. 7. Ad-elafin treatment results in the reduction of neutrophil clusters after intratracheal administration of \textit{S. aureus} in mice. The histological appearance (magnification, $\times 400$) of representative 3-$\mu$m lung sections (fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin) is shown. (b and c) Ad-elafin--\textit{S. aureus} treatment reduced the numbers of neutrophil clusters (occasional “isolated” neutrophils were observed, see arrows) (c) compared to Ad-LacZ--\textit{S. aureus} treatment, where multiple clusters were present (b, arrows). (a) None were seen in PBS-PBS-treated animals. Corresponding neutrophil numbers from each section are shown in the relevant bar graph.](image-url)
may not be acting on its own since synergy between neutrophil defensins and cathelicidins has previously been demonstrated against *S. aureus* (23). In accordance with this, although a direct comparison between the present study and our previous study (34) describing the incubation of synthetic elafin with *S. aureus* in solution in the test tube is difficult, it seems that “cell-derived” elafin (the present study) is more efficient than synthetic elafin against *S. aureus*. Indeed, we show here that 3.5 μg/ml (350 nM) can restrict the growth of 200 bacteria by 40%, whereas 3.330 μM would be the extrapolated concentration of synthetic elafin required to kill the same number of bacteria to the same degree when incubated in solution in the test tube.

Having demonstrated a clear antimicrobial effect of elafin ex vivo, we set out to investigate whether it also had a similar effect in vivo. A total of 3 × 10^7 PFU of Ad-elafin or Ad-LacZ (a dose demonstrated to induce no obvious inflammatory effects per se [33]) were instilled intratracheally 5 days prior to the bacterial challenge. This protocol allowed for robust expression of elafin in the lungs (Fig. 5). Although this did not result in an apparent increase in the expression of the other β-defensins studied here (data not shown), Ad-elafin administration had a beneficial effect in this acute model. Indeed, mirroring the ex vivo data presented in Fig. 4, Ad-elafin instillation reduced the bacterial load in vivo (Fig. 5). Concomitantly, when the Ad-elafin group was considered on its own, there was a negative correlation between lung elafin levels and neutrophil numbers (Fig. 6). The histological analysis of lungs confirmed this finding and showed a reduction in the number of neutrophil pneumonia-like foci in the alveoli (Fig. 7). This is reminiscent of our previous study, in which *P. aeruginosa* was administered intratracheally in a protocol similar to the one used here (33). In that study, as here (Table 1), markers of inflammation such as BALF TNF-α and MPO levels were reduced in the Ad-elafin–plus–bacteria group. It could therefore be hypothesized that, in an infective situation, elafin may act directly as a bacteriostatic or bactericidal agent, thereby reducing the infective load. A consequence of this would be a reduction in the cellular inflammatory response, as exemplified by the reduction in cytokine levels, neutrophil activation, and neutrophil-associated “collateral lung damage,” as shown by a reduction in protein leakage in the alveolar compartment (Table 1). Elafin could also have a further direct anti-inflammatory function; indeed, we have shown recently that in vitro elafin inhibits NF-κB (11), an important proinflammatory transcription factor which has been shown to be activated by *S. aureus* protein A (7). Of note, the mode of action of elafin may be different in noninfective situations, for example, when LPS is used as an inflammatory stimulus, where elafin’s chemotactic functions may become prominent (30, 32).

In conclusion, we demonstrated here that elafin is able to confer an increased antimicrobial phenotype to primary murine tracheal epithelial cells which, despite the endogenous expression of at least three β-defensins (shown at the RNA level) with in vitro relevance to *S. aureus* killing, were otherwise unable to contain the growth of this pathogen in vitro.

This phenotype was replicated in vivo, in a model of acute inflammation in C57BL/6 mice. We believe that these data have implications both for the development of improved murine models of *S. aureus* lung infections and for the consideration of elafin as a potential therapeutic agent against *S. aureus*-associated local and systemic infections.

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**REFERENCES**


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