## Title

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ORIGINAL ARTICLE

# Keratinocyte Growth Factor Promotes Epithelial Survival and Resolution in a Human Model of Lung Injury

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## Abstract

**Rationale:** Increasing epithelial repair and regeneration may hasten resolution of lung injury in patients with the acute respiratory distress syndrome (ARDS). In animal models of ARDS, keratinocyte growth factor (KGF) reduces injury and increases epithelial proliferation and repair. The effect of KGF in the human alveolus is unknown.

**Objectives:** To test whether KGF can attenuate alveolar injury in a human model of ARDS.

**Methods:** Volunteers were randomized to intravenous KGF (60 μg/kg) or placebo for 3 days, before inhaling 50 μg LPS. Six hours later, subjects underwent bronchoalveolar lavage (BAL) to quantify markers of alveolar inflammation and cell-specific injury.

**Measurements and Main Results:** KGF did not alter leukocyte infiltration or markers of permeability in response to LPS. KGF increased BAL concentrations of surfactant protein D, matrix metalloproteinase (MMP)-9, IL-1Ra, granulocyte-macrophage colony–stimulating factor (GM-CSF), and C-reactive protein. *In vitro*, BAL fluid from KGF-treated subjects inhibited pulmonary fibroblast proliferation, but increased alveolar epithelial proliferation. Active MMP-9 increased alveolar epithelial wound repair. Finally, BAL from the KGF-pretreated group enhanced macrophage phagocytic uptake of apoptotic epithelial cells and bacteria compared with BAL from the placebo-treated group. This effect was blocked by inhibiting activation of the GM-CSF receptor.

**Conclusions:** KGF treatment increases BAL surfactant protein D, a marker of type II alveolar epithelial cell proliferation in a human model of acute lung injury. Additionally, KGF increases alveolar concentrations of the antiinflammatory cytokine IL-1Ra, and mediators that drive epithelial repair (MMP-9) and enhance macrophage clearance of dead cells and bacteria (GM-CSF). Clinical trial registered with ISRCTN 98813895.

**Keywords:** acute respiratory distress syndrome; acute lung injury; keratinocyte growth factor; lipopolysaccharide; clinical trial

The acute respiratory distress syndrome (ARDS) affects many critically ill patients (1). However, there is currently no specific pharmacotherapy to reduce injury or enhance resolution in ARDS (2). ARDS is characterized by neutrophilic infiltration to the alveolar space and resultant epithelial injury and denudation. Recovery requires regeneration of alveolar epithelial cells from their progenitor (type II) population.

Keratinocyte growth factor (KGF) is an epithelial growth factor produced by

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## At a Glance Commentary

**Scientific Knowledge on the Subject:** Keratinocyte growth factor (KGF) improves physiologic outcomes and markers of alveolar epithelial cell function in multiple animal models of acute respiratory distress syndrome (ARDS), leading to interest in its use as a treatment of ARDS in patients. The effect of exogenous KGF (palifermin) in the human lung *in vivo* has not been investigated.

**What This Study Adds to the Field:** In a human *in vivo* short-term model of acute lung injury and ARDS, KGF (palifermin) pretreatment did not reduce leukocyte infiltration or protein leak. However, palifermin increased bronchoalveolar lavage concentrations of surfactant protein D, a marker of type II cell proliferation, suggesting that it promotes epithelial cell survival. In addition, it increased concentrations of mediators that drive epithelial repair and improved macrophage phagocytosis of dead epithelial cells and bacteria, suggesting the induction of a proresolution environment. Taken together these data show for the first time that some of the beneficial effects of KGF demonstrated in animal models of ARDS can be reproduced in a human model, and support the hypothesis that KGF may have beneficial effects in ARDS. The role of KGF in treating ARDS should be investigated further.

fibroblasts (3) and other cells (4). Most epithelia express the KGF receptor (FGFR2-IIIb). *In vitro*, KGF has mitogenic effects on type II alveolar epithelial (ATII) cells (5, 6), inhibits apoptosis (7), and promotes migration and wound repair (8, 9). Additionally, KGF improves ATII cell barrier function (10), maintains sodium channel expression after epithelial injury (11), and supports surfactant production (12, 13).

Such observations led to consideration of KGF as an intervention to reduce epithelial injury and improve recovery in ARDS. In various rodent models of lung injury, pretreatment with recombinant human KGF or pulmonary-specific overexpression of KGF reduces alveolar leukocyte infiltration, hemorrhage, pulmonary edema, permeability, hypoxia, epithelial injury, and fibrosis, and increases compliance and type II alveolar/terminal bronchial epithelial cell proliferation (9, 14–19).

Furthermore, KGF may augment innate pulmonary immunity. KGF-treated mice cleared *Escherichia coli* and *Pseudomonas aeruginosa* more effectively, via granulocyte-macrophage colony–stimulating factor (GM-CSF) dependent macrophage activation (20). In a *P. aeruginosa* model of pneumonia and lung injury, KGF reduced bacterial translocation to the lung (21). Finally, KGF reduced bacterial translocation in *E. coli* lung injury in an *ex vivo* perfused human lung model (22)

In human *ex vivo* perfused lungs injured by LPS, KGF increased alveolar fluid clearance and lowered bronchoalveolar lavage (BAL) tumor necrosis factor (TNF)-α, IL-1β, and IL-8 concentrations (23). There are no published *in vivo* studies investigating the pulmonary effects of KGF in humans. Truncated recombinant human KGF (palifermin) is licensed to prevent and treat oral mucositis in hematology patients. Compared with endogenous human KGF, palifermin has the first 23 N-terminal amino acids deleted to improve stability, but has similar mitogenic activity (24). We hypothesized that systemically administered palifermin would reduce markers of alveolar injury in healthy human volunteers who had inhaled low-dose LPS. Low-dose LPS inhalation has been used in healthy human subjects as an *in vivo* model of pulmonary inflammation and alveolar epithelial cell activation without causing significant adverse effects (25). The model causes a transient injury, with inflammation detectable 6 hours post-LPS. We chose a pretreatment strategy to investigate if the antiinflammatory and proreparative effects identified in animals in pretreatment studies could be replicated in humans.

We show for the first time in humans that intravenous palifermin treatment increases BAL surfactant protein D (SP-D), suggesting reduced ATII cell injury, after LPS inhalation, although had no effect on a type I epithelial cell injury marker or epithelial permeability as measured by protein leak or protein permeability index. Furthermore, palifermin up-regulates alveolar concentrations of IL-1Ra, matrix metalloproteinase (MMP)-9, GM-CSF, and C-reactive protein (CRP). We demonstrate that BAL fluid from patients treated with palifermin before LPS challenge increases alveolar epithelial cell proliferation *in vitro*, and that the increased GM-CSF in this alveolar microenvironment drives macrophages to clear apoptotic epithelial cells and bacteria. Some of the results of this study have been previously reported in abstract form (26, 27).

## Methods

Further methodologic details are available in the online supplement.

### Trial

Healthy volunteers aged 18 or over were recruited. Exclusion criteria included smoking, history of respiratory disease, elevated serum amylase, pregnancy, lactation, or any female of childbearing age not using adequate contraception. Ethical approval was given by the Office of Research Ethics Committees, Northern Ireland.

In this randomized, double-blind, placebo-controlled, allocation-concealed clinical trial (ISRCTN 98813895) subjects were randomized to intravenous palifermin (60 μg/kg/day) or placebo for 3 days. On Day 3, 1 hour after study drug administration, 50 μg LPS were inhaled via an automatic inhalation–synchronized dosimeter nebulizer as previously described (25).

Blood was collected on Day 3 before the final dose of study drug and at 24 hours after LPS inhalation. BAL was performed 6 hours after LPS inhalation (*see* Figure E1 in the online supplement). Recruitment continued until 36 volunteers completed the study protocol.

### Inflammatory Mediators and Cell-Specific Biomarkers

Cytokines, cell-specific biomarkers, and MMPs were measured using commercially available kits (*see* online supplement for details). Values below the limit of detection of the assay were assigned the lowest standard value.

### Fibroblast Viability

Primary human lung fibroblasts (ATCC) were stimulated with pooled BAL fluid (1:5 dilution) from the 20 subjects treated with placebo (placebo BAL) or from the 16 subjects treated with palifermin (palifermin BAL). Cell proliferation was measured by CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega,

Southampton, UK) or by trypan blue exclusion (28).

### Wound Repair

A549 wound repair was measured using a standard scratch assay. Cells were treated with media or active MMP-9 (Calbiochem, Middlesex, UK). The percentage wound closure relative to the degree of closure of the "media control" wound was calculated at 24 hours.

### CRP Depletion in BAL

Depletion of CRP from BAL by immunoprecipitation was verified by Western blot and ELISA (R&D Systems, Abingdon, UK).

### Monocyte-derived Macrophages

Monocyte-derived macrophages (MDMs) were derived from blood as previously described (29). Blood donors were unrelated to the BAL study participants. At Day 5, media was replaced with RPMI 1% fetal calf serum and no GM-CSF. MDMs were used for experiments on Day 7.

### Phagocytosis of Apoptotic Epithelial Cells

A549 cells were stained with SE cell tracker dye before inducing apoptosis with camptothecin, and layering onto MDMs. Pooled palifermin BAL or placebo BAL (as defined above) was added to the cells at 1/10 dilution for 1 hour, before washing off nonphagocytosed epithelial cells. MDMs were suspended in crystal violet and incubated on ice to quench surface but not intracellular fluorescence (30). Cells were fixed with 2% paraformaldehyde, and the percentage macrophages containing cell tracker signal determined by flow cytometry. Experiments were repeated with anti–GM-CSF receptor antibody (MAB 1037; Merck Millipore, Middlesex, UK), and using macrophages from at least five donors.

### Phagocytosis of *Escherichia coli* Bioparticles

MDMs were incubated with palifermin BAL or placebo BAL at 1/10 dilution before incubation with inactivated *E. coli* bioparticles labeled with pHrodo Red dye (Life Technologies, Paisley, UK). MDMs were suspended in 2% paraformaldehyde and samples analyzed by flow cytometry. Experiments were performed using macrophages from five donors.

### Data Analysis

Categorical variables were compared using Fisher exact test. Continuous data were tested for normality using the D'Agostino and Pearson test, and compared using unpaired *t* or Mann-Whitney test as appropriate. For paired or sequential measurements on cells from the same donor, data were compared using paired *t* test, or repeated measures analysis of variance. A *P* value of 0.05 or less was considered significant. For all BAL analytes or measurements, results are for n = 20 in the placebo group and n = 16 in the palifermin group.

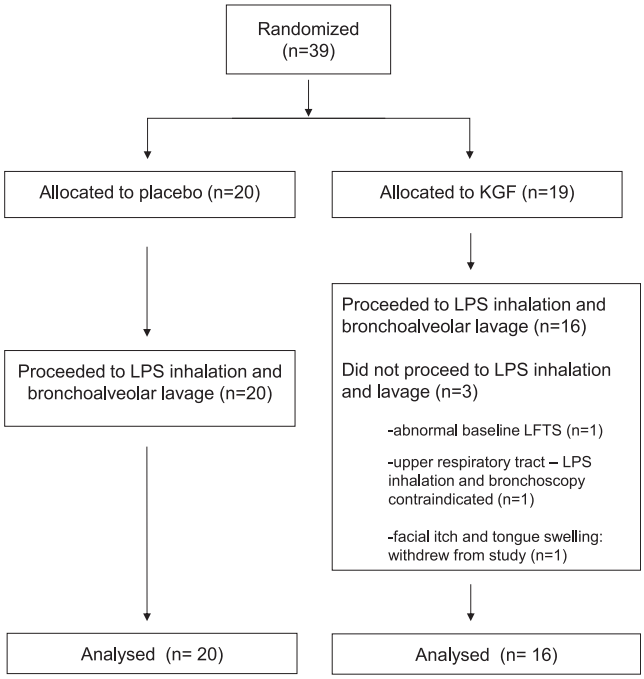


**Figure 1.** CONSORT diagram. KGF = keratinocyte growth factor; LFTS = liver function tests.

## Results

A total of 39 volunteers were recruited to receive placebo (n = 20) or palifermin (n = 19): three volunteers did not complete the study (did not undergo LPS inhalation). All three were in the palifermin group (CONSORT diagram, Figure 1). One subject withdrew from the study after receiving one dose of study drug when she experienced facial pruritus and a red tongue: symptoms resolved within 48 hours without intervention. One subject received two doses of study drug but developed an upper respiratory tract infection, and was withdrawn by the study team because LPS inhalation and bronchoscopy were not believed to be appropriate. The third subject was withdrawn on the basis of abnormal baseline liver function tests. The data presented are for those subjects who completed the study (placebo n = 20;

**Table 1.** Baseline Demographics of All Subjects Who Underwent LPS Inhalation and BAL

| | Placebo | Palifermin | *P* Value |
|---|---|---|---|
| Number of patients | 20 | 16 | — |
| % male | 45 | 45 | 1.00 |
| Age, yr | 26.1 (5.9) | 23.6 (5.2) | 0.2 |
| Height, cm | 170 (9.0) | 171 (10.4) | 0.98 |
| Weight, kg | 71 (11) | 68 (9) | 0.44 |
| Baseline $FEV_1$ | 3.8 (0.7) | 3.8 (0.8) | 0.93 |

*Definition of abbreviation*: BAL = bronchoalveolar lavage.
Data are mean (SD) except where indicated.
*P* value is for Fisher exact test for % male, and for unpaired *t* test for the remaining analyses.

KGF n = 16). Demographic characteristics including age, sex, height, weight, and lung function were similar in both groups (Table 1).

LPS was well-tolerated in both groups. There was no significant difference in $FEV_1$ 6 or 24 hours post-LPS inhalation (*see* Table E1) and no difference in incidence of $FEV_1$ decline greater than 10% post-BAL. Of the nine subjects in whom $FEV_1$ declined by more than 10% post-BAL, none were symptomatic and all had returned to baseline at follow-up. No serious adverse events occurred in either group. The palifermin-treated subjects had a higher incidence of facial flushing or erythematous rash and altered taste or tongue sensation than those randomized to placebo (*see* Table E1).

### Effect of Palifermin on Cell-Specific Markers of Injury in Response to LPS

Palifermin had no effect on BAL RAGE, a marker of type I cell injury (4.2 [SD 1.0] ng/ml in palifermin group vs. 3.5 [SD 1.5] ng/ml in placebo group; $P = 0.11$). However, BAL SP-D, a marker of type II cell proliferation, was significantly higher in the palifermin-treated group (Figure 2A). BAL vWF, a marker of endothelial dysfunction, was below the limit of detection of the assay in most samples.

### Effect of Palifermin on Alveolar Inflammation and Alveolar Capillary Leak

Pretreatment with palifermin had no effect on BAL total white blood cell count (Table 2) or differential white cell count (*see* Table E2). Palifermin did not alter protein permeability as measured by BAL albumin (Table 2), total protein, or protein-permeability index (Figures 2B and 2C). Palifermin pretreatment did not affect the levels of BAL TNF-α, IL-1β, IL-6, IL-8, vascular endothelial growth factor, or monocyte chemoattractant protein-1 (MCP-1) (Table 2). However, palifermin up-regulated IL-1Ra (Table 2), with a corresponding fall in IL-1β/IL-1Ra ratio (Table 2), and also increased BAL GM-CSF (Figure 2D). Palifermin did not alter pulmonary release of the alarmin HMGB1, or of calgranulin C (Table 2). Finally, BAL CRP was higher in the palifermin-treated group (Table 2).

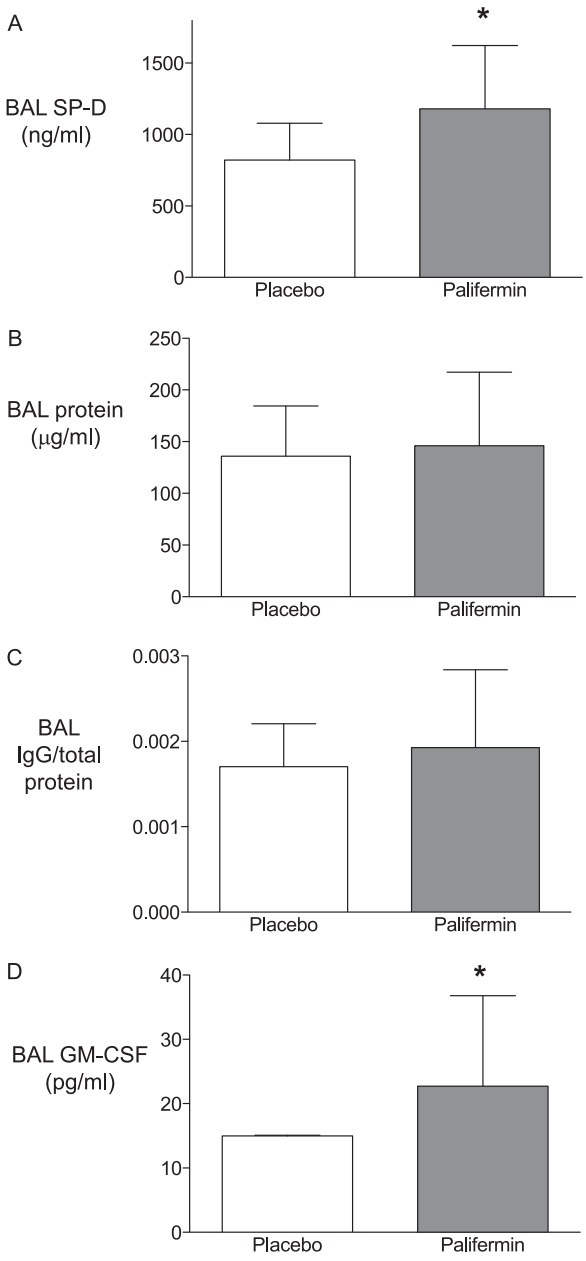


**Figure 2.** The effect of palifermin (keratinocyte growth factor) pretreatment on specific marker of type II alveolar epithelial cell injury, permeability, and inflammation in response to LPS inhalation. (*A*) Surfactant protein D (SP-D), a marker of alveolar type II cell proliferation, is increased in the group pretreated with palifermin. *$P = 0.005$, unpaired *t* test. (*B*) Palifermin had no effect on BAL total protein. (*C*) Palifermin had no effect on BAL IgG/total protein ratio. (*D*) Palifermin increases BAL granulocyte-macrophage colony–stimulating factor (GM-CSF), *$P = 0.003$, Mann-Whitney test. For all measurements n = 20 in placebo group, n = 16 in palifermin group. Data are mean + SD. BAL = bronchoalveolar lavage.

### Effect of Palifermin on MMP and Tissue Inhibitors of Metalloproteinase Concentrations

Palifermin increased concentrations of the gelatinolytic enzymes, MMP-2 and -9 (Figures 3A and 3B), but not the other MMPs (*see* Table E3). BAL MMP-9 concentrations were more than 1 log-fold higher than MMP-2 in both groups. Although concentration of the major MMP inhibitors (tissue inhibitors of metalloproteinase [TIMP]-1 and -2) was increased in the palifermin-pretreated group (Figures 3C and 3D), net active MMP-9 concentration was twofold higher in the palifermin-pretreated group than the placebo-treated group ($P = 0.005$) (*see* Figure E2).

**Table 2.** Inflammatory Profile of BAL Fluid from the Palifermin-treated Group versus the Placebo-treated Group

| | Placebo (*n* = 20) | Palifermin (*n* = 16) | *P* Value |
|---|---|---|---|
| WBC count ($\times 10^5$/ml) | 5.3 (4.0–7.6) | 5.5 (3.6–8.4) | ns |
| Albumin, mg/l | 58.7 (44.4–84.5) | 64.6 (49.2–111.6) | ns |
| IL-1Ra, ng/ml | 3.41 (1.73–5.42) | 5.02 (3.78–9.50) | 0.011* |
| IL-1β/IL-1Ra ratio | 0.009 (0.005–0.014) | 0.004 (0.003–0.007) | 0.009* |
| IL-1β, pg/ml | 29.0 (13.9–41.7) | 25.7 (15.1–48.6) | ns |
| TNF-α, pg/ml | 46.0 (20.9–65.6) | 63.5 (40–96) | ns |
| IL-6, pg/ml | 350 (175–512) | 527 (228–1,154) | ns |
| IL-8, pg/ml | 289 (197–457) | 336 (290–436) | ns |
| VEGF, pg/ml | 122 (93.5–213) | 140 (110–193) | ns |
| MCP-1, pg/ml | 347 (210–532) | 520 (366–684) | ns |
| HMGB1, pg/ml | 16.7 (9.9–34.8) | 21.8 (13.2–30.7) | ns |
| Calgranulin C, ng/ml | 28.6 (13.3–56.4) | 34.7 (18.5–49.5) | ns |
| CRP, pg/ml | 161 (62–392) | 491 (296–968) | 0.04* |

*Definition of abbreviations*: BAL = bronchoalveolar lavage; CRP = C-reactive protein; HMGB = high-mobility group box 1; MCP = monocyte chemoattractant protein; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor; WBC = white blood count.
Data are median (interquartile range).
For all analytes and measurements, n = 20 in the placebo group and n = 16 in the palifermin group.
*$P < 0.05$ for palifermin- versus placebo-treated group.

### Functional Effect of Palifermin on Alveolar Microenvironment

Having identified that palifermin increased SP-D, a type II epithelial cell proliferation marker, and gelatinolytic enzyme activity, factors that have been implicated in epithelial healing in other organs, we examined the potential functional effects of these on alveolar epithelial cells and fibroblasts *in vitro*.

Palifermin BAL increased the numbers of viable A549 cells compared with BAL from the placebo-treated group as measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay (Figure 4A) and total viable cell count (*see* Figure E3A). In contrast, palifermin BAL reduced proliferation of human fibroblasts compared with placebo BAL as assessed by similar methods (Figure 4B; *see* Figure E3B).

To assess the functional effects of increased MMP-9 in BAL fluid on alveolar epithelial wound healing, we treated wounded A549 cells with clinically relevant concentrations of the active form of this enzyme. Active MMP-9 significantly increased epithelial wound repair at 24 hours (Figure 5). Similar findings were obtained using primary small airway epithelial cells (*see* Figure E4).

CRP has been reported as an opsonizing factor in the pulmonary compartment for both bacteria and cellular material (31). GM-CSF also increases mononuclear cell phagocytosis (32, 33). We assessed the effect of palifermin-BAL (containing increased CRP and GM-CSF) on phagocytosis of apoptotic epithelial cells, and labeled bacterial particles. MDMs incubated with palifermin BAL showed increased phagocytic uptake of apoptotic A549 cells compared with MDMs treated with placebo BAL (Figure 6Ai), and increased uptake of fluorescently labeled *E. coli* bioparticles (Figure 6B). Blocking the GM-CSF receptor on macrophages before treatment with palifermin BAL reduced phagocytosis of both apoptotic epithelium and *E. coli* bioparticles (Figures 6Aii and 6B), whereas depletion of CRP in BAL had no effect (*see* Figure E5).

## Discussion

This study shows for the first time that intravenous palifermin (KGF) induces a proresolution and reparative microenvironment in the injured human alveolus in a clinically relevant model of ARDS.

Importantly, palifermin was well-tolerated in this study. There was an increased incidence in facial flushing, erythema, and sensation of tongue thickening in the palifermin-treated group, as described in patients being treated for mucositis, but this was minor and self-limiting. One rat study showed an increase in airway resistance after a single intratracheal administration of human KGF compared with vehicle control (34). In our study there were no symptomatic changes of cough or dyspnea reported by subjects in response to palifermin and there was no difference in $FEV_1$ in the palifermin and placebo groups.

LPS inhalation drives a reproducible mild alveolar injury with evidence of both inflammation and epithelial and endothelial activation, and therefore is a unique model to study human lung injury and its systemic effects. In this study palifermin pretreatment has no effect on the type I epithelial cell marker RAGE, suggesting no proliferative effect on the type I cell, but did inhibit the reduction in SP-D. A fall in SP-D occurs in response to LPS and in ARDS, in which lower SP-D concentrations in alveolar edema fluid are associated with a worse prognosis (35). SP-D is a marker of proliferating ATII cells and these are the first data indicating that KGF maintains this proliferating progenitor population in humans. Importantly, palifermin had no effect on permeability as measured by BAL total protein, albumin, and protein permeability index. This may reflect the fact that it had no effect on the type I epithelial cell injury marker (RAGE) in this transient model, and the type I epithelial cell accounts for most of the alveolar epithelial barrier. The protective effect of KGF in our and previous animal studies seems to be restricted to the type II cell, which acts as the progenitor cell to repopulate the alveolus after injury.

Although animal data suggest that potential protective effects of KGF are largely mediated by its effect on type II epithelial cells, the only previous data on KGF in an *ex vivo* human model of ARDS induced by LPS suggested that in addition to improving alveolar fluid clearance, a marker of epithelial cell function, recombinant KGF had an antiinflammatory role (23). Lee and coworkers (23) identified that recombinant human KGF can down-regulate CXCL8, IL-1β, and TNF-α in the *ex vivo* perfused lung model of ARDS post-LPS. In this human study, no down-regulatory effect was observed on these inflammatory cytokines or on cellular infiltrate, indicating that KGF is unlikely to down-regulate the initial inflammatory response in ARDS. The discrepancy between our findings and those of Lee and coworkers (23) may relate to a variety of factors: our model involves systemic, rather than endobronchial administration of KGF, administration for 3 days rather than a single dose as in the paper by Lee and

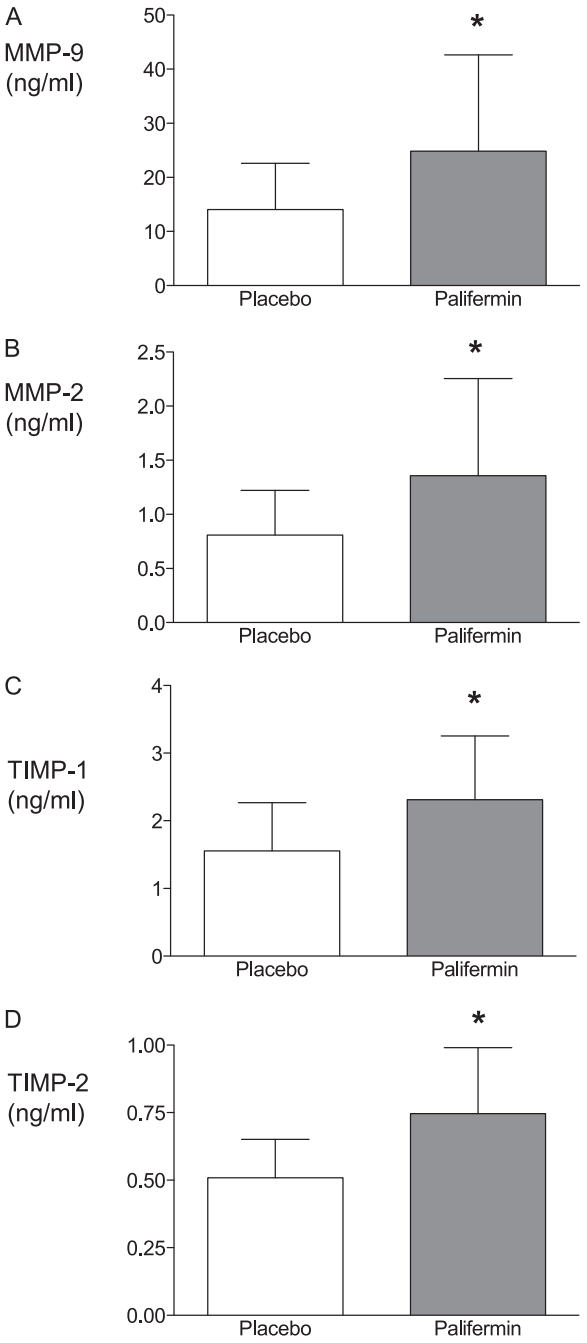


**Figure 3.** Palifermin (keratinocyte growth factor) pretreatment of healthy subjects before LPS inhalation increases matrix metalloproteinase (MMP)-2 and -9, but also tissue inhibitors of metalloproteinase (TIMP)-1 and -2. (*A*) MMP-9, **P* = 0.04, unpaired *t* test. (*B*) MMP-2, **P* = 0.03, unpaired *t* test. (*C*) TIMP-1, **P* = 0.01, Mann-Whitney test. (*D*) TIMP-2, **P* = 0.002, Mann-Whitney test. For all measurements n = 20 in placebo group, n = 16 in palifermin group. Data are mean + SD.

coworkers (23); a comparatively lower dose of LPS; and an intact whole body including excretory organs and immune response (not just the isolated human lung). Thus, we believe our model may be more clinically relevant in recapitulating the likely immune and inflammatory responses to LPS and its subsequent modulation by KGF.

The antiinflammatory cytokine IL-1Ra was increased in the palifermin group, with a corresponding fall in the IL-1β/IL-1Ra ratio. The role of IL-1Ra in lung injury is not well characterized, although a murine knockout study showed that IL-1Ra may have an antiinflammatory effect in, and promote resolution after, LPS-induced injury (36). Increased BAL IL-1Ra has been shown in patients with ARDS, and is proposed as a potential endogenous antiinflammatory mediator to limit damage in acute lung injury (ALI) (37). Mesenchymal stem cells, which reduce lung injury in animal models, are associated with increased pulmonary IL-1Ra concentrations (38), whereas aerosolized Anankinra, recombinant human IL-1Ra, reduced pulmonary arterial pressure and gene expression of IL-8 and IL-1β in a porcine model of lung injury, suggesting a reparative role for exogenous IL-1Ra (39). Finally, exogenous IL-1Ra reduced pulmonary edema and protein permeability in a rodent model of ventilator-induced lung injury (40).

Among the MMP family there was specific up-regulation of the gelatinolytic enzymes (MMP-2 and -9) in response to palifermin. MMP-9 was the predominant gelatinolytic enzyme (by >1 log) in the BAL fluid of these subjects, consistent with previous findings using the LPS model of ALI (25) and consistent with previous studies of ALI (41, 42). Although MMP-9 is implicated in basement membrane destruction, we and others have found that higher MMP-9 concentrations in BAL at Day 3 or 4 of ARDS is associated with a shorter duration of illness and improved survival (41, 42), suggesting it is involved in the repair and recovery process. We have previously reported that MMP-9 production by ATII cells is necessary for wound healing (41), and in this current study addition of the active recombinant enzyme to A549 cultures that themselves do not produce detectable MMP-9 increased wound repair. Given the potential limitations of using A549 cells to model alveolar epithelial cells, we repeated these experiments with small airway epithelial cells, commonly used as model for primary ATII cells, and found the same results. MMP-9 is associated with cellular motility and may be important in the degradation of intercellular junctional proteins allowing cells to detach and migrate (43). Others have identified MMP-9 as having a protective role in ventilator-induced lung injury in mice (44), supporting the idea that

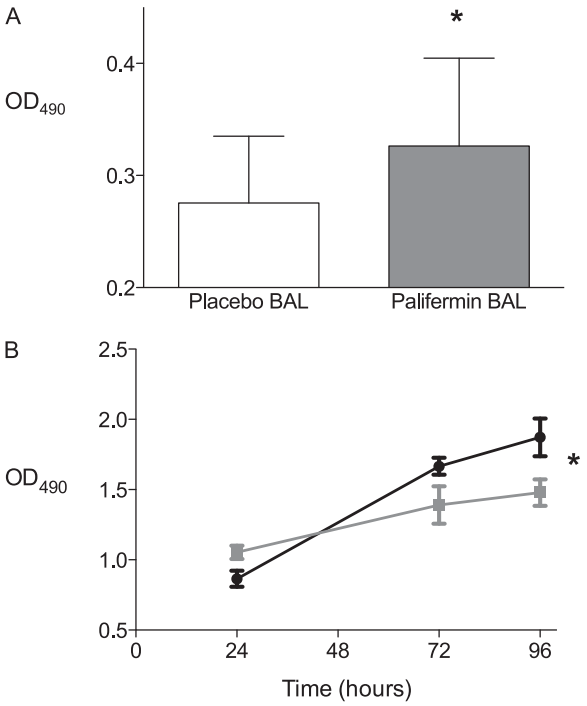


**Figure 4.** Palifermin (keratinocyte growth factor) bronchoalveolar lavage (BAL) accelerates epithelial cell growth but inhibits fibroblast growth. (*A*) Modified MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay of A549 cells treated with palifermin BAL or placebo BAL at 1/4 dilution for 24 hours, **P* = 0.03. (*B*) Modified MTT assay of normal human lung fibroblasts treated with palifermin BAL (*gray curve*) or placebo BAL (*black curve*) at 1/5 dilution over a 96-hour period. Two-way analysis of variance, **P* < 0.001 for change in OD450 with time: curves differ significantly, **P* = 0.04 (i.e., curve slope is significantly lower for palifermin BAL–treated group).

MMP-9 may have a reparative role in the injured alveolus.

Although TIMP-1 was increased in the BAL fluid of the subjects who had been treated with palifermin, the proportional increase was less than MMP-9, and the functional assay indicates a greater than twofold increase in net MMP-9 activity in the palifermin-pretreated group. Importantly, TIMP-1 is not specific to MMP-9 and also inhibits other MMPs present in BAL fluid (e.g., MMP-1, -2, -7, and -8).

Interestingly, despite the significant dilutional effect of lavage, palifermin BAL at the concentrations used increased total numbers and metabolic activity of epithelial cell cultures, reducing fibroblast numbers and metabolic activity, compared with placebo BAL, suggesting a milieu that induces epithelial proliferation but reduces fibroblast proliferation.

We identified increased phagocytic uptake of apoptotic epithelium and bacterial particles by macrophages preincubated with palifermin BAL compared with placebo BAL fluid. These data suggest that in addition to promoting type II epithelial cell proliferation, KGF may enhance clearance of dead cells and bacteria in the injured alveolus. Denudation of the alveolar epithelial layer is a key feature of ALI, and recovery requires clearance of dead cells in addition to epithelial proliferation and healing. GM-CSF, which is known to increase phagocytic activity of macrophages (33), was elevated in BAL fluid in the palifermin-pretreated group. Inhibition of GM-CSF in the BAL fluid with receptor-blocking antibody reduced apoptotic cell and bacterial particle uptake, suggesting that the increased phagocytosis is GM-CSF dependent. GM-CSF has been studied as a potential treatment for ARDS and to date has shown no benefit. A recent clinical trial showed no reduction in ventilator-free days, although the study was underpowered because it did not recruit the planned sample size. However, there was a nonsignificant trend toward reduction in 28-day mortality and an increase in organ failure–free days. Importantly, in this study, exogenous GM-CSF was safe (45).

Pulmonary CRP was also increased in the palifermin-treated group. Although predominantly considered a biomarker of inflammation, CRP is an acute phase protein that binds phosphocholine on the surface of dead cells (31) and bacteria (46) to activate the complement cascade and increase macrophage phagocytic uptake of cellular debris and bacteria. However, we did not identify a significant contribution of CRP to increased macrophage phagocytosis in this study.

Although our data suggest KGF may have a role in inducing type II cell survival and proliferation, with clearance of dead cells to restore epithelial integrity in patients with ARDS, the study has some limitations. The nature of the injury in this model is mild and transient, with no measurable physiologic deterioration to allow us to determine if the increase in SP-D in the palifermin-treated group had functional effects. Histologic assessment of epithelial proliferation is obviously not possible. Palifermin was administered preinsult (LPS), and whether the protective effect on the alveolar type II epithelium would persist if palifermin were administered after insult is unclear. Additionally, whether palifermin would maintain its protective effect in ARDS secondary to live bacterial infection

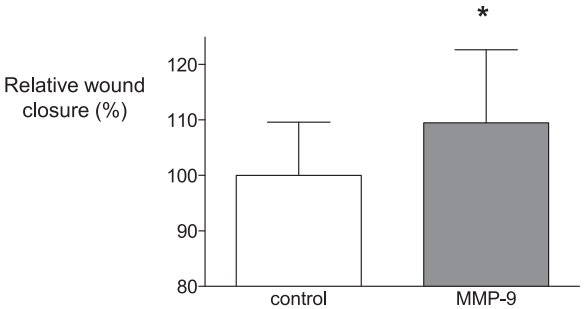


**Figure 5.** Epithelial wound repair is enhanced by active matrix metalloproteinase (MMP)-9. A549 cells were wounded with a pipette tip and treated with active MMP-9 (10 ng/ml) for 24 hours. MMP-9 improved wound closure, **P* = 0.007.

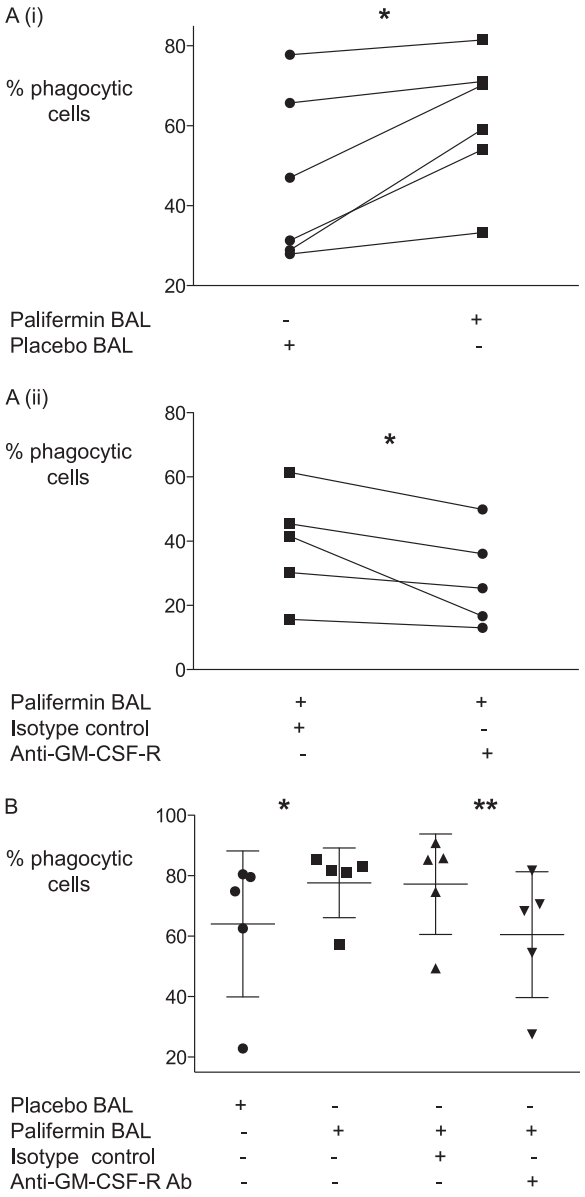


**Figure 6.** Treatment of macrophages with palifermin bronchoalveolar lavage (BAL) improves their phagocytic uptake of apoptotic epithelial cells and bacterial particles. (*A*) Monocyte-derived macrophages from healthy volunteers were incubated with fluorescently labeled, apoptotic A549 cells, and with palifermin BAL or placebo BAL. The percentage of macrophages containing labeled target epithelial cells as detected by flow cytometry after 1 hour is plotted. (*i*) Data are the paired observations from macrophages from each individual donor treated with either placebo BAL or palifermin BAL. **P* = 0.02, paired *t* test. (*ii*) Data are the paired observations of macrophages from each individual donor treated with palifermin BAL plus either isotype control IgG or anti–granulocyte-macrophage colony–stimulating factor (GM-CSF) receptor antibody (1 μg/ml), **P* = 0.05, paired *t* test. Different donors were used in *i* and *ii*. (*B*) Monocyte-derived macrophages were incubated with fluorescently labeled inactivated *Escherichia coli* ("bioparticles") in the presence of palifermin BAL and placebo BAL fluid. GM-CSF receptor activation on macrophages was inhibited using a neutralizing antibody. The percentage of macrophages with phagocytosed bioparticles is plotted. Data are the sequential observations for five different donors. **P* < 0.05 for palifermin BAL versus placebo BAL, ***P* < 0.05 for palifermin BAL + isotype control versus palifermin BAL + anti–GM-CSF receptor Ab, repeated measures analysis of variance with *post hoc* Bonferroni test.

or other noninfectious etiologies is unclear. Many therapeutic strategies tested for ARDS have the capacity to increase susceptibility to infection. Our data suggest KGF may increase bacterial clearance by enhancing macrophage phagocytosis via increased GM-CSF production.

Furthermore, this study investigated only a single dosing regimen for palifermin. The dose regimen was based on dose and duration known to effect a change in proliferation and epithelial repair in the oral mucosa in humans. Sequential dosing was required to achieve oral mucosal epithelial cell proliferation in human subjects. Clinical trials of palifermin showed a reduction in oral mucositis, with 40–60 μg/kg/day for 3 days before chemotherapy or radiotherapy, with higher doses being associated with increased adverse event incidence. We selected a dose that was known to deliver palifermin to an injured mucosa (oral) and achieve a biologically meaningful change, and which we knew to be safe in healthy human subjects, but it is possible that higher doses may have had additional effects not addressed by this study. Furthermore, whether intravenous administration is the optimal method is not addressed by the study. Many animal models have used targeted pulmonary delivery, or pulmonary overexpression, to test the effect of KGF specifically within the lung. Theoretically, targeted delivery in the lung in ARDS may be advantageous and avoid systemic adverse effects, but whether nebulized delivery in humans would actually reach the injured alveoli in the face of pulmonary edema and atelectasis requires investigation in patients.

Finally, the duration of effect of palifermin on alveolar epithelium or on consequent macrophage activation is not addressed by this study. Pretreatment for 3 days was associated with a marker of increased evidence of type II cell proliferation and up-regulation of factors required for alveolar epithelial repair and macrophage phagocytosis at 6 hours after injury, but how long that persists is unanswered by this study.

In summary, this study shows for the first time that systemic administration of KGF (palifermin) to human subjects before LPS inhalation reduces a marker of ATII cell injury *in vivo* and creates an alveolar microenvironment that supports epithelial proliferation, wound healing, and clearance of dead epithelial cells and bacteria by

macrophages. Together these findings, along with multiple previous animal studies showing a beneficial effect of KGF in models of ARDS, suggest systemic KGF administration may be a therapeutic strategy to increase type II epithelial cell survival, enhance epithelial healing, and promote resolution in ALI in patients with ARDS. Taken together, these data support the need for further investigation of KGF as a potential therapeutic strategy for ARDS. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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