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Human endogenous antibiotic LL-37 stimulates airway epithelial cell proliferation and wound closure

Renat Shaykhiev,[1] Christoph Beißwenger,[1] Kerstin Kändler,[1] Judith Senske,[1] Annette Püchner,[1] Thomas Damm,[1] Jürgen Behr,[2] and Robert Bals[1]

[1]*Hospital of the University of Marburg, Department of Internal Medicine, Division of Pulmonary Diseases, Philipps-Universtät Marburg, Marburg; and* [2]*Hospital of the University of Munich, Department of Internal Medicine I, Division of Pulmonary Diseases, Ludwig-Maximilians-Universtät Munich, Munich, Germany*

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Shaykhiev, Renat, Christoph Beißwenger, Kerstin Kändler, Judith Senske, Annette Püchner, Thomas Damm, Jürgen Behr, and Robert Bals. Human endogenous antibiotic LL-37 stimulates airway epithelial cell proliferation and wound closure. *Am J Physiol Lung Cell Mol Physiol* 289: L842–L848, 2005. First published June 17, 2005; doi:10.1152/ajplung.00286.2004.—Antimicrobial peptides are endogenous antibiotics that directly inactivate microorganisms and in addition have a variety of receptor-mediated functions. LL-37/hCAP-18 is the only cathelicidin found in humans and is involved in angiogenesis and regulation of the innate immune system. The aim of the present study was to characterize the role of the peptide LL-37 in the regulation of wound closure of the airway epithelium in the cell line NCI-H292 and primary airway epithelial cells. LL-37 stimulated healing of mechanically induced wounds in monolayers of the cell line and in differentiated primary airway epithelium. This effect was detectable at concentrations of 5 μg/ml in NCI-H292 and 1 μg/ml in primary cells. The effect of LL-37 on wound healing was dependent on the presence of serum. LL-37 induced cell proliferation and migration of NCI-H292 cells. Inhibitor studies in the wound closure and proliferation assays indicated that the effects caused by LL-37 are mediated through epidermal growth factor receptor, a G protein-coupled receptor, and MAP/extracellular regulated kinase. In conclusion, LL-37 induces wound healing, proliferation, and migration of airway epithelial cells. The peptide is likely involved in the regulation of tissue homeostasis in the airways.

airway epithelium; antimicrobial peptide; wound healing; cathelicidin; tissue repair

THE RESPIRATORY TRACT IS SHIELDED by a multicomponent host defense system (29). The conducting airways rely on an integral epithelial surface to support clearance, barrier function, and host defense. Airway epithelial cells represent an important structural and functional component of lung tissue homeostasis (23). Epithelial injury is a hallmark of several inflammatory lung diseases such as asthma, chronic obstructive pulmonary disease, or pulmonary fibrosis. In response to epithelial injury, repair processes are initiated that comprise cell migration, proliferation, and differentiation (19, 32). Inflammation is associated with epithelial injury and with increased expression of mediatory molecules. Some of these molecules such as keratinocyte growth factor (22) or epidermal growth factor (EGF) (24) are involved in the regulation of epithelial repair processes. Also antimicrobial peptides regulate epithelial reconstitution. Human neutrophil defensins induce airway epithelial proliferation and wound closure (1, 2).

Antimicrobial peptides are endogenous antibiotics that directly inactivate microorganisms (34). Cathelicidins are a family of antimicrobial peptides characterized by a highly conserved signal sequence and proregion ("cathelin") but show substantial heterogeneity in the COOH-terminal domain that encodes the mature peptide (8, 21). LL-37/hCAP-18 is the only cathelicidin in humans and is encoded by the gene *CAMP*. The cDNA was cloned from human bone marrow (12, 15). LL-37/hCAP-18 is expressed in myeloid cells and epithelial cells of the skin and the gastrointestinal, urinary, and respiratory tract (7, 14, 15). In addition to its direct antimicrobial function, LL-37 activates cells through receptors including the formyl peptide receptor-like 1 (FPRL1) (31) and the P2X7 receptor (13). Receptor-mediated activities include stimulation of angiogenesis (20), cutaneous wound healing (16), and chemoattraction of inflammatory and immune cells (3, 31). LL-37 activates airway epithelial cells involving the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and stimulates the release of IL-8 (27). Increased concentrations of LL-37 in the airways have been found in inflammatory (4) and infectious lung disease (25). On the basis of its activities, it appears that LL-37/hCAP-18 is involved in the pathogenesis of lung disease; however, it is unknown whether LL-37 influences the repair processes of airway epithelial cells.

It was the aim of the present study to characterize the role of the cathelicidin peptide LL-37/hCAP-18 in airway epithelial wound closure. We found that LL-37 induces the closure of epithelial wounds, stimulates epithelial proliferation, and chemoattracts epithelial cells.

MATERIALS AND METHODS

Cell types and culture conditions. The bronchial mucoepidermoid carcinoma-derived cell line NCI-H292 (ATCC, Manassas, VA) (9) was cultured at 37°C in 5% CO_2 in RPMI-1640 (GIBCO, Grand Island, NY) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (PAA Laboratories, Pasching, Austria) and containing 10% heat-inactivated fetal bovine serum (FBS, GIBCO). The culture medium was changed every 2 days. Cells were passaged every 5 days with 0.25% trypsin and 0.1% EDTA (PAA Laboratories). Human bronchial cells were isolated and cultivated as conventional submersed cultures or as air liquid interface

Address for reprint requests and other correspondence: R. Bals, Dept. of Internal Medicine, Div. of Pulmonary Diseases, Hospital of the Univ. of Marburg, Baldingerstr. 1, 35043 Marburg, Germany (e-mail: bals@mailer.uni-marburg.de).

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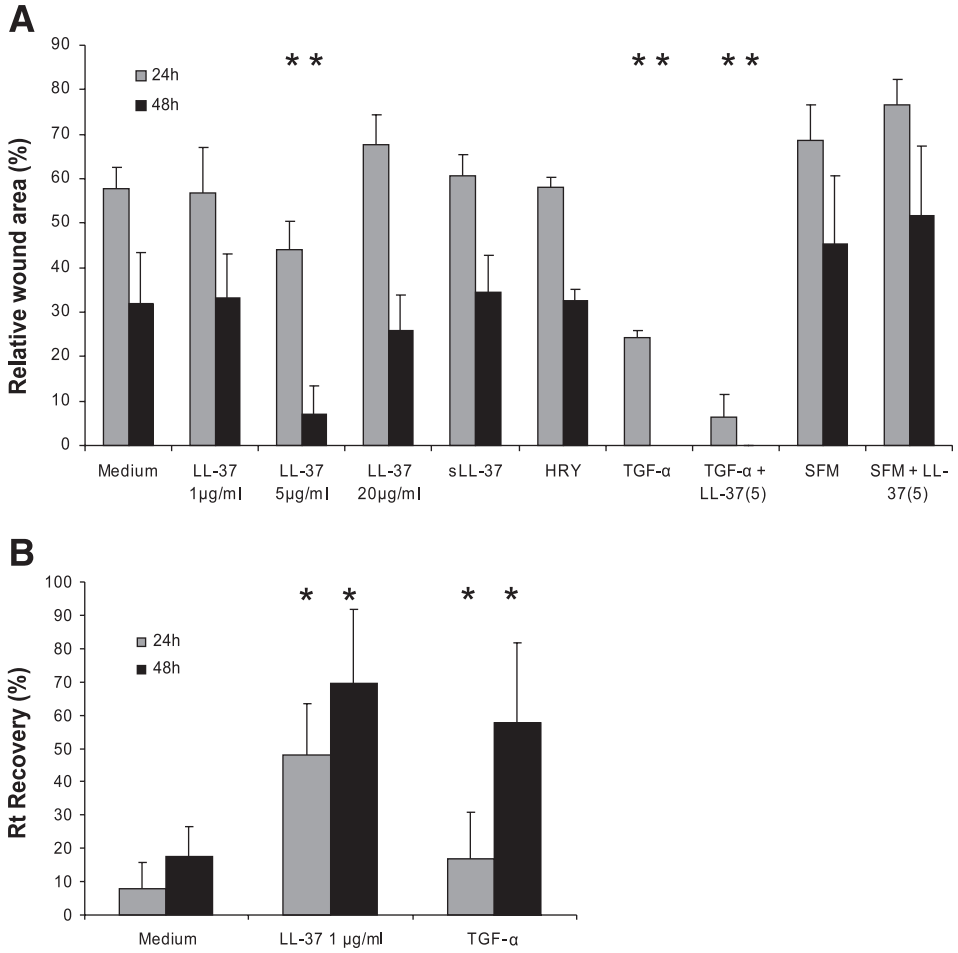

Fig. 1. Effect of LL-37 on bronchial epithelial wound closure. Mechanically wounded NCI-H292 (*A*) cells were incubated with different concentrations of LL-37, transforming growth factor (TGF)-α, HRY (FPRL agonistic peptide), and scrambled (s) LL-37. The remaining wound area (percentage of residual wound area compared with $t = 0$ h) was measured after 24 and 48 h. SFM, serum-free medium. $^*P < 0.05$ compared with the medium group ($n = 15$, per group). Differentiated airway epithelial cells in air-liquid interface culture (*B*) were wounded, and the wound closure was determined by measurement of transepithelial resistance (Rt). $^*P < 0.05$ compared with the medium group ($n = 3$ per group).

cultures as described earlier (6). The protocol was approved by the ethics committee of the University of Marburg and informed consent was obtained from the donors. We selected cultures for experiments by measuring the transepithelial resistance (Rt) using an epithelial ohmmeter (EVOM; World Precision Instruments, Sarasota, FL). Cultures were considered confluent and differentiated if the Rt was $>500\ \Omega \cdot cm^2$.

In vitro wound closure assay. NCI-H292 and primary cells were grown to confluence in six-well tissue culture plates. Three to ten circular wounds (from 1 to 2.5 mm in diameter) were scraped in each well with a sterile pipette tip. The wounded monolayers were rinsed with culture medium to remove all cellular debris, and the following experimental conditions were applied: *1*) LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES-COOH) at the indicated concentrations, *2*) transforming growth factor alpha (TGF-α 20 ng/ml, positive control; Sigma-Aldrich Chemie, Munich, Germany), and *3*) culture medium with or without 10% FBS. A scrambled version of LL-37 (sLL-37, sequence RSLEGTDRFPFVRLKNSRKLEFKDIKGIKREQFVKIL-COOH) was used in selected experiments to exclude a nonspecific effect of LL-37. The wound area was determined videomicroscopically immediately after wound creation and at different postwounding time points (24 and 48 h) and expressed as percentage of the area measured immediately after wounding. The wound area was examined with an inverted microscope (Axiovert 25; Carl Zeiss, Oberkochen, Germany), using the Evolution LC Megapixel FireWire Camera Kit bundled with Image-Pro Discovery software (Media Cybernetics, Silver Spring, MD) and a video monitor. After the image was acquired, the measurement data were converted from pixels to millimeters using a calibration image. The wound edges were examined for the presence and intensity of lamellipodia formation by a videomicroscopy technique. We calculated the relative lamellipodia area by dividing the total lamellipodia area by the cell number within the examined wound edge. For inhibitor studies, mechanically wounded NCI-H292 cells were exposed to inhibitors of MEK (PD-98059, 25 μM; Alexis, Nottingham, UK), p38 MAPK (SB-203580, 10 μM; Calbiochem, La Jolla, CA), P2X7 receptor (KN-62, 10 mM; Calbiochem), G protein-coupled receptor (GPCR) signaling [pertussis toxin (PTx) 50 ng/ml; Sigma], or EGF receptor (EGFR) tyrosine kinase (AG-1478, 1 μM; Calbiochem) 1 h before addition of stimuli. To evaluate the role of FPRL1 as a possible receptor for LL-37, some wounded monolayers were incubated with HRYLPM-COOH peptide recently described as a full and selective activator of FPRL1 (5). Because most inhibitors used in our study affect the principal signaling mechanisms essential for basal epithelial repair process, we calculated the inhibition index (the "inhibition" area related to the remaining wound area at the given time point). Such a parameter allows us to compare contribution of a targeted pathway on the wound closure in differently stimulated groups when both of the groups are sensitive to the inhibitor. To study the effect of LL-37 on the repair of differentiated airway epithelial cells, we mechanically damaged the cultures in air-liquid interface with sterile pipette tip, scraping off a ring of cells (from 2 to 3 mm in diameter) without damaging to the filter support. Wound healing was assessed by the measurement of Rt at different time points. The results were expressed as Rt recovery, indicating an increase of Rt (%) compared with the immediate postwounding value.

Cell migration assay. Cell migration of NCI-H292 cells was measured by a modified Boyden's chamber technique using a 96-well

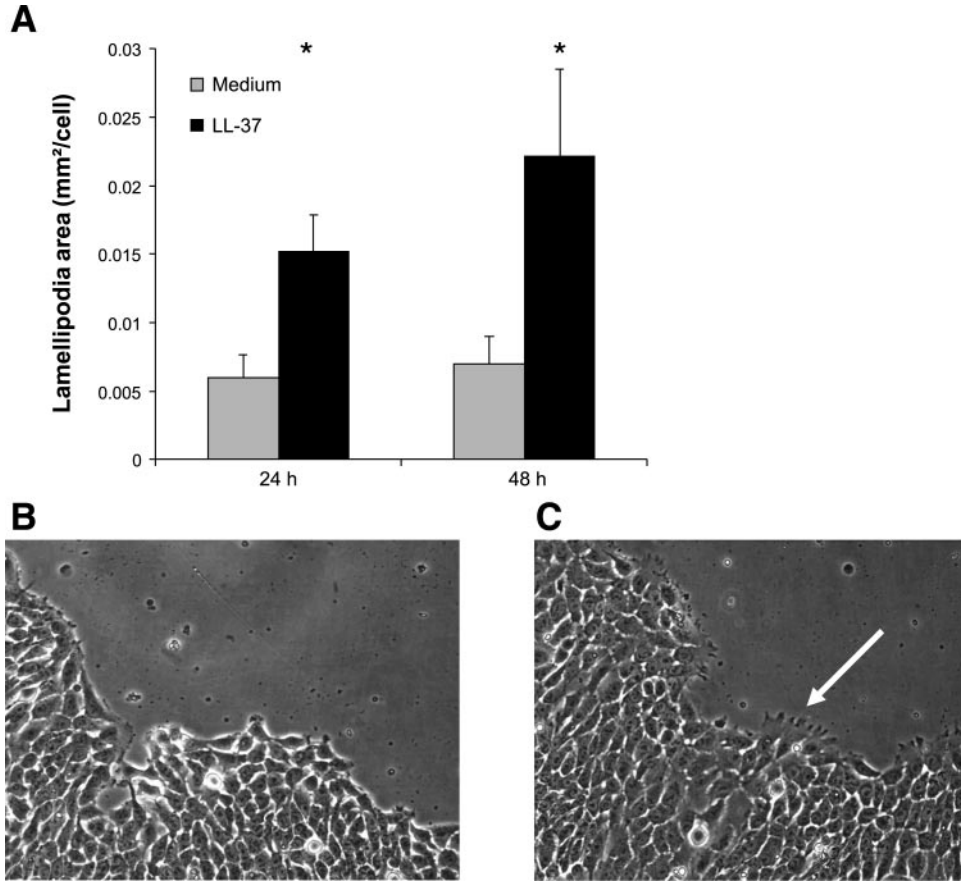

Fig. 2. LL-37 increases the formation of lamellipodia at the wound edge of NCI-H292 cells treated with 5 μg/ml LL-37 (*A*). Formation of lamellipodia (arrow, *C*) at the wound edge treated with 5 μg/ml LL-37 (*A*) or in control cells (*B*). *$P < 0.05$ ($n = 5$ per group).

multiwell chamber (Neuroprobe, Bethesda, MD) (10). Culture medium containing one of the following stimuli was placed into the bottom wells: *1*) LL-37 at concentrations from 1 to 20 μg/ml, *2*) insulin-like growth factor 1 (IGF-1, 100 ng/ml; Sigma Aldrich) as positive control, *3*) TGF-α (20 ng/ml) as additional positive control, *4*) sLL-37, or *5*) culture medium alone. The wells were covered with a polyvinylpyrrolidone-free 8-μm-pore polycarbonate filter (Neuroprobe, Gaithersburg, MD) coated with 0.01% collagen type I solution (Sigma Aldrich). Shortly before the experiment, the cells were detached with 0.05% trypsin and 5 mM EDTA. We placed 10^5 cells in 50 μl into each of the top wells. Then the chamber was incubated for 6 h. After incubation, we removed the filter and removed cells on the top surface of the filter by scraping. We fixed the migrated cells on the lower side of the filter by placing the filter in 100% methanol overnight. The filter was stained with Haemalaun (Waldeck, Division Chroma, Münster, Germany) for 20 min and then washed in water. Cell migration was quantified and expressed as the number of stained cells on the lower surface of the filter in three random fields viewed under a light microscope at ×20 magnification. The experiments were performed under both serum-free and serum-containing (10% FBS) culture conditions.

Cell proliferation assay. NCI-H292 cells were seeded at a density of 10^4 cells/ml on Lab-Tek II eight-chamber glass slides (Nalge Nunc International, Naperville, IL). At subconfluence, the cells were incubated with various stimuli (LL-37, sLL-37, TGF-α) at concentrations as in the migration assay or medium alone for 48 h. Cell proliferation was evaluated by 5-bromo-2-deoxy-uridine (BrdU) incorporation method using the BrdU labeling and detection kit II (Roche Diagnostics) following the manufacturer's protocol. The cells were incubated with 10 μM BrdU for 1 h. After the aspiration of the BrdU-labeling medium and washing, the cells were fixed in 70% ethanol at −20°C for 30 min. The proliferation index was calculated as the number of BrdU-positive cells divided by the total number of cells, multiplied by 100. At least 600 cells from each experimental group were counted. To investigate the possible involvement of selected signaling pathways, we performed inhibitor studies in each individual experiment, using the inhibitors described above. Confluent NCI-H292 cell monolayers grown on Lab-Tek II glass slides were wounded and incubated with various stimuli as described above. At different time points after injury (24 and 48 h), a BrdU assay was performed. Then the wound edges and postwounding areas were analyzed for the presence of BrdU-positive cells.

Measurement of FPRL1, P2Y7, and LL-37/hCAP-18 expression. RT-PCR was performed using total RNA from NCI-H292 or primary airway epithelial cells (RNeasy Mini Kit; Qiagen, Hilden, Germany), the cDNA synthesis kit (MBI Fermentas, St. Leon-Rot, Germany), and the following primers (TIB Molbiol, Berlin, Germany): human β-actin (sense 5′-AGA GCT ACG AGC TGC CTG AC-3′, antisense 5′-AGC ACT GTG TTG GCG TAC AG-3′, annealing temperature 60°C), LL-37 (sense 5′-CCA CCA TGG GCC TGG TGA TGC CTC TGG CCA TC-3′, antisense 5′-TGT ACA CTA GGA CTC TGT CCT GGG TAC AAG-3′, annealing temperature 65°C), FPRL1 (sense 5′-TGG TTG CCC TTC TGG GCA CC-3′, antisense 5′-CTC TCG GAA GTC TTG GCC CAC-3′, annealing temperature 65°C), P2X7 (18) (sense 5′-CCC CGG CCA CAA CTA CAC CAC GAG AAA C-3′, antisense 5′-CCG AG TAG GAG AGG GTT GAG CCG ATG-3′, annealing temperature 67°C). Quantitative PCR results were obtained by the ΔΔCt (cycle threshold) method. For qualitative analysis, PCR products were subjected to electrophoresis on a 1.5% agarose gel, and DNA was visualized by ethidium bromide staining.

Cytotoxicity and apoptosis assays. The cytotoxic effect of different concentrations of LL-37 and sLL-37 on NCI-H292 and primary airway epithelial cells was assessed by colorimetric quantification of the lactate dehydrogenase (LDH) concentration in cell supernatants

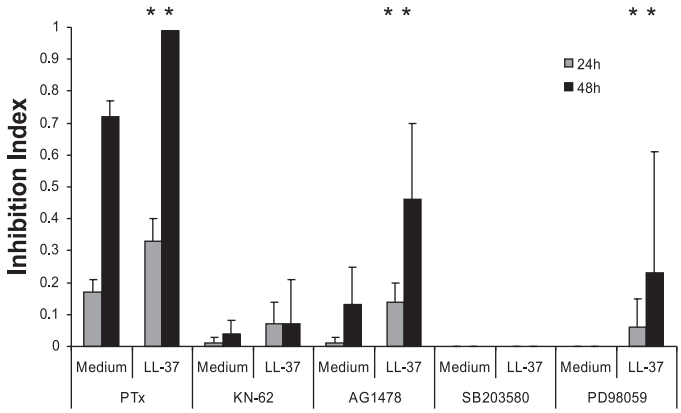

Fig. 3. Effects of inhibitors of selected signaling pathways on LL-37-stimulated bronchial epithelial wound closure. Wounded NCI-H292 monolayers were preincubated with pertussis toxin (PTx), AG-1478 (AG), KN-62, PD-98059 (PD), or SB-203580 (SB) before addition of LL-37 (5 μg/ml). The remaining wound area was determined 24 and 48 h after stimulation, and the inhibition index was calculated as described in MATERIALS AND METHODS. $*P <$ 0.05 compared with the medium group ($n = 5$ per group).

using the Cytotoxicity Detection Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. The effect of LL-37 on apoptosis of NCI-H292 cells was determined by the annexin binding apoptosis assay according to the manufacturer's instruction (Vybrant apoptosis assay; Molecular Probes, Eugene, OR).

Statistical analysis. For all experiments, duplicate or triplicate determinations were made for each experimental condition. The results of replicates were averaged and expressed as one data point. Each experiment was repeated at least two times. All data are expressed as means ± SE. Comparisons between experimental groups were performed by Student's *t*-test or the analysis of variance (ANOVA) as appropriate. For time-course experiments, ANOVA was used to compare the data for multiple time points and different conditions. Post hoc range tests were performed with the *t*-test (two-sided) with Bonferroni adjustment. Results were considered statistically significant for P values <0.05.

RESULTS

LL-37 increases the closure of airway epithelial wounds. To evaluate the effect of LL-37 on bronchial epithelial wound closure in vitro, confluent monolayers of NCI-H292 and primary cells were mechanically damaged and then exposed to LL-37, TGF-α, or medium. LL-37 treatment resulted in a dose-dependent increase of wound closure in NCI-H292 (Fig. 1*A*) cell monolayers. The stimulatory effect of LL-37 on wound closure was dependent on the presence of serum (Fig. 1*A*). sLL-37 did not have any stimulatory effect, suggesting that LL-37-stimulated wound closure is a specific effect of the peptide. LL-37 stimulates airway epithelial wound closure in vitro at 5 μg/ml. The biological properties of cell lines differ significantly from those of primary cells. Therefore, the effect of LL-37 on primary airway epithelial cells was tested (Fig. 1*B*). LL-37 had no effect on primary cells grown as submersed nondifferentiated monolayers (data not shown). When the epithelial cells were allowed to differentiate in air-liquid inter-

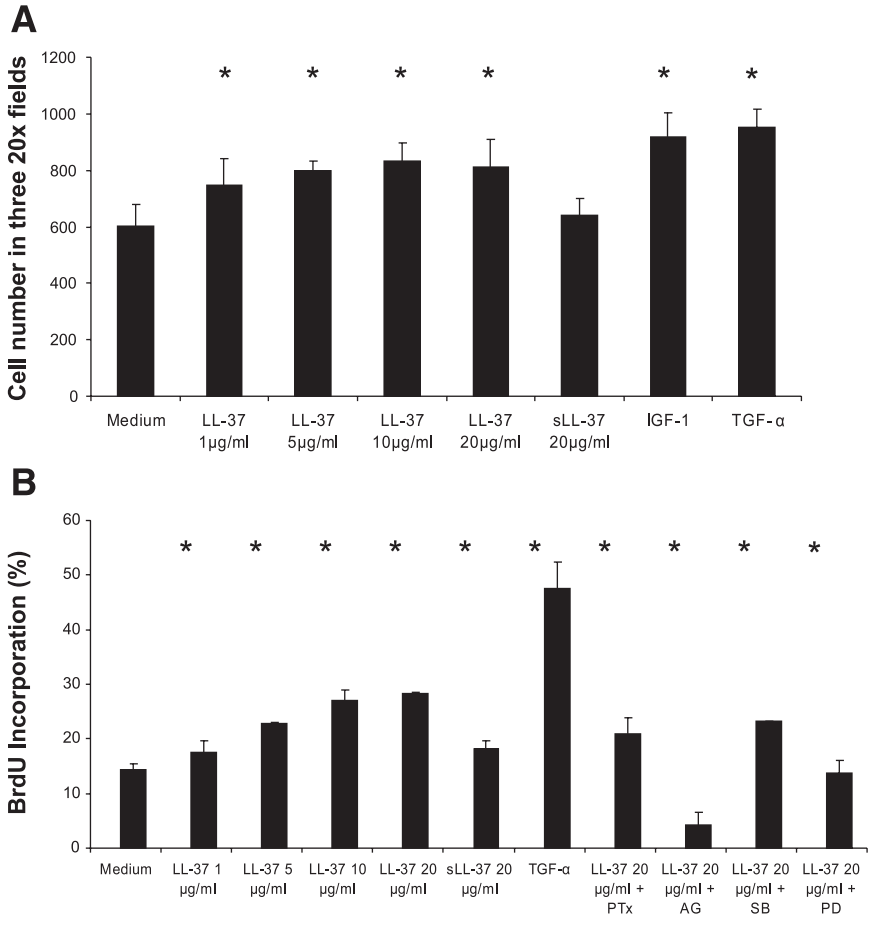

Fig. 4. Dose-dependent effects of LL-37 on NCI-H292 cell migration and proliferation. *A*: Boyden chamber assay. LL-37, IGF-1, TGF-α, or sLL-37 was placed into each of the bottom wells. Cell migration was quantified as the number of stained (migrated) cells on the lower surface of the filter in 3 random fields under a light microscope at ×20 magnification. $*P < 0.05$ compared with the cells in the medium group ($n = 4$ per group). *B*: 5-bromo-2-deoxy-uridine (BrdU) incorporation assay. NCI-H292 cells were incubated with BrdU in the presence of LL-37 or under control conditions (medium, sLL-37, TGF-α) for 48 h. To assess the signaling mechanisms the inhibitors PTx, AG-1478, SB-203580, and PD-98059 were applied before stimulation with LL-37 (20 μg/ml). The number of BrdU-positive cells was determined 48 h after incubation with LL-37. Data are expressed as a percentage of the BrdU-positive cells (BrdU incorporation index). $*P < 0.05$ compared with the medium group (LL-37, TGF-α) or to the 20 μg/ml LL-37 group (sLL-37, inhibitor groups) ($n = 4$ per group).

face culture, a significantly faster increase of Rt was detected in the LL-37-treated group compared with the negative control (Fig. 1*B*). To study whether the effect of LL-37 is due to accelerated migration, the wound edges were examined for the presence of lamellipodia, which were significantly increased in the LL-37-treated group (Fig. 2).

LL-37-induced wound closure involves multiple signaling pathways. To investigate whether the stimulatory effect of LL-37 on airway epithelial wound closure involves an EGFR-dependent mechanism or GPCR-mediated signaling, we exposed mechanically wounded NCI-H292 monolayers to the EGFR tyrosine kinase inhibitor (AG-1478) or PTx. LL-37 was used at a concentration of 5 μg/ml. To gain insight into the possible downstream signaling mechanisms, inhibitors of MEK (PD-98059) and p38 MAPK (SB-203580) were used. PTx pretreatment significantly decreased the stimulatory effect of LL-37 at both time points (24 and 48 h after wounding), as did AG-1478 and to a lesser degree PD-98059 (Fig. 3). LL-37 is known to activate the receptors FPRL1 and P2X7. FPLR1 transcripts were found to be expressed by primary but not NCI-H292 cells; P2X7 transcripts were detected in both cell types (data not shown). A specific ligand of FPRL1, the peptide HRYLPM, did not induce epithelial wound healing. A specific inhibitor of P2X7 (KN-62) did not reduce LL-37-induced wound healing. Levels of FPRL1 and P2Y7 expression were not increased in wounded NCI-H292 cells. As described earlier, LL-37 is expressed in airway epithelial cells (7) and was not found to be increased after wounding in NCI-H292 cells in the present study (data not shown).

LL-37 is chemotactic for bronchial epithelial cells. Because LL-37 enhances airway epithelial wound closure, it is reasonable to believe that LL-37 stimulates epithelial cell migration. We used LL-37 in a modified Boyden's chamber and found that LL-37 stimulated NCI-H292 migration in a concentration-dependent manner (Fig. 4*A*). There was no stimulation of migration in cells treated with sLL-37. In contrast to wound closure, the effect of LL-37 on NCI-H292 cell migration in this chemotaxis assay was not modified induced by the presence of serum (data not shown).

LL-37 stimulates bronchial epithelial cell proliferation. Cell proliferation is one component of wound healing. To determine whether LL-37 stimulates bronchial epithelial cell proliferation, we measured BrdU incorporation in NCI-H292 cells 48 h after stimulation with LL-37 (1–20 μg/ml), sLL-37, TGF-α, and IGF-1 (positive controls), or medium alone. LL-37 increased NCI-H292 labeling with BrdU in a concentration-dependent manner (Fig. 4*B*). Treatment with sLL-37 at the equivalent concentrations had no significant effect on NCI-H292 proliferation. To characterize the signaling pathways involved, we preincubated NCI-H292 cells with one of the following inhibitors: PTx, EGFR inhibitor AG-1478, MEK inhibitor PD-98059, or p38 MAPK inhibitor SB-203580, before stimulation with 20 μg/ml LL-37 or serum-free medium alone. As shown in Fig. 4*B*, pretreatment with AG-1478 completely blocked LL-37-stimulated NCI-H292 cell proliferation, whereas PTx caused only moderate decrease of BrdU incorporation in LL-37-treated cells. LL-37 increased NCI-H292 proliferation was minimally inhibited by p38 MAPK inhibitor SB-203580 but was significantly suppressed by preincubation with MEK inhibitor PD-98059. To determine whether cell proliferation contributes to the LL-37-induced wound healing, the wounded monolayers were incubated with BrdU, and the incorporation was detected. We found no increase of stained cells at the wound edge compared with the control group or a region remote from the wound edge (data not shown).

High concentration of LL-37 is cytotoxic for airway epithelial cells. To determine the cytotoxic effects of LL-37, we performed LDH release assays with NCI-H292 and primary airway epithelial cells. LL-37 induced significant release of LDH at concentrations >20 μg/ml (Fig. 5, *A* and *B*). No significant effect of LL-37 on apoptosis was found in an

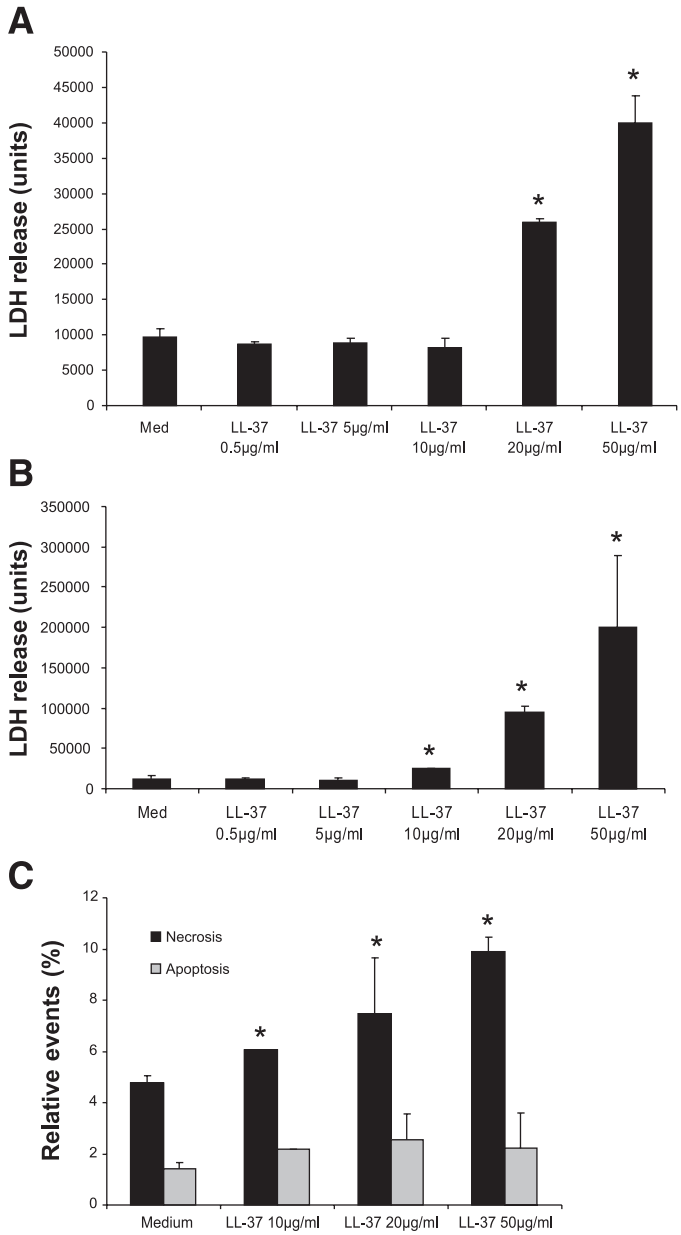

Fig. 5. Cytotoxic effects of LL-37 on airway epithelial cells. LDH release from NCI-H292 (*A*) and primary cells (*B*) was measured in response to the indicated concentrations of LL-37. *C*: LL-37 exposure did not induce apoptosis in an annexin binding assay in NCI-H292 cells but did result in increased necrotic cell death. *$P < 0.05$ compared with the medium group ($n = 4$ per group).

annexin binding assay compared with the induction of necrotic cell death (Fig. 5*C*).

DISCUSSION

The main finding of the present study is that the cathelicidin antimicrobial peptide LL-37 stimulates wound healing of human airway epithelial cells. The peptide induces cell proliferation and chemotaxis of epithelial cells. The intracellular signaling after stimulation with LL-37 involves GPCR, EGFR, and MEK.

Antimicrobial peptides are classically regarded as endogenous antibiotics that provide a first line of host defense until other components of the innate immune system or the adaptive immune system becomes active and functional. Increasing evidence from in vivo studies accumulated during the last few years and proved that host defense is indeed one of the main functions of vertebrate antimicrobial peptides (34). In parallel it became clear that antimicrobial peptides may possess additional functions. It is known that several antimicrobial peptides bind to cellular receptors and induce specific cellular reactions (30). Human neutrophil defensins induce lung epithelial cell proliferation and wound closure in vitro (1, 2). Also LL-37 appears to be involved in the regulation of processes of epithelial cells. The peptide regulates the wound repair of cutaneous wounds (16, 33). Furthermore, it activates epithelial cells of the airways (27). LL-37 interacts at least with two receptors, the FPRL1 and the P2X7 receptor. The data demonstrated here show that the peptide LL-37 is involved in the regulation of airway epithelial wound closure.

The induction of wound closure by LL-37 occurs at relatively low levels that are well in the range of the concentration that can be found during inflammation or infection (4, 11, 25). The exact concentrations of LL-37 at which cells are physiologically exposed are not known. Higher concentrations have detrimental effects on airway epithelial cells as indicated by the LL-37-induced release of IL-8 (27) or the induction of necrosis as demonstrated here. Epithelial injury is normally followed by a complex repair process that comprises epithelial migration, proliferation, and differentiation. These processes are mediated by intracellular signaling cascades including the EGFR pathway (24). The proinflammatory effect of LL-37 on airway epithelial cells seen at high concentrations is mediated by transactivation of the EGFR via metalloprotease-mediated cleavage of membrane-anchored EGFR-ligands and mediated by MAPK (27). The present results also indicate that GPCR signaling and EGF receptor activation are the principal pathways used by LL-37 to stimulate bronchial epithelial restitution after mechanical injury. MEK appears to act as a downstream signaling element. Activation of cell proliferation involves a specific pattern of intracellular signaling where the GPCR and MAPK p38 have a less important role compared with wound healing. It is unclear which cellular receptors mediate the LL-37-induced wound healing. The involvement of the GPCR in the wound healing process is likely since PTx inhibits wound healing significantly. FPRL1 has been shown to be expressed in primary airway epithelial cells (17), but its involvement in LL-37 induced wound healing is uncertain. In the present study the LL-37-dependent effect could not be duplicated by an agonist of FPRL1. The relative independence of the cell proliferation from GPCR indicates that the effect of LL-37 could be mediated by several distinct receptors. It is also not clear whether P2X7 is involved in this process. An inhibitor of this receptors did not influence LL-37-induced wound healing. The effect of LL-37 on wound closure was observed only in the presence of serum, suggesting a possible interaction between the peptide and one or several components of serum.

The effect of LL-37 was detected in a cell line and in primary airway epithelial cells. This is important because it is known that cells lines often behave quite differently from primary cells. Intriguingly, we could observe the peptide's effect only on well-differentiated epithelial cells. Air-liquid interface cultures are often used to study effects of the airway epithelium that are dependent on differentiation, polarization, and the formation of tight junctions. Wound healing involving the EGF system is known to depend on the polarization of the epithelium (28). Several reasons could account for the dependence of LL-37 on the differentiation status: *1*) the receptor necessary for LL-37-induced wound healing is present only in differentiated primary cells, *2*) the assay used to study wound closure in nondifferentiated primary cells is inadequate to detect LL-37-induced wound healing, *3*) the effect of LL-37 depends on a polarized state of the epithelium. Primary cells and also the cell line used in the study are capable of producing LL-37, making an autocrine mechanism for regulation of wound healing likely. Other epithelial cells are also capable to regulate repair processes in an autocrine manner (26).

In conclusion, we show that LL-37 induces airway epithelial wound healing. LL-37 induces proliferation and migration of airway epithelial cells. This effect is mediated by signaling pathways involving the EGFR, GPCR, and MAPK. In addition to its host defense function and modulatory effect on the innate immune system, LL-37 might play an important role in the homeostasis of the airway epithelium and the maintenance of the integrity of the respiratory tract.

GRANTS

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