Ramotar *et al. Respiratory Research* (2026) 27:21
https://doi.org/10.1186/s12931-026-03493-w

Respiratory Research

RESEARCH **Open Access**

LL-37 and citrullinated-LL-37 enhances oxylipins: citrullination attenuates LL-37-mediated COX-2-dependent chemokine response in human bronchial epithelial cells

Padmanie Ramotar[1], Mahadevappa Hemshekhar[2,3], Anthony Altieri[1,4], Anne M van der Does[5], Christopher D Pascoe[6] and Neeloffer Mookherjee[1,2*]

Abstract

Background During airway inflammation, chemokines, oxylipins (bioactive lipids) and cationic host defence peptides (CHDP) are enhanced in the lungs. However, the interplay of these molecules in the process of airway inflammation is not fully resolved. The human cathelicidin CHDP, LL-37, can enhance the expression of chemokines which is turn facilitates influx of leukocytes into the lungs. Moreover, LL-37 can be citrullinated during inflammation and the effect of this post-translational modification on LL-37-mediated immunomodulatory functions remains unclear. Therefore, in this study we aimed to define the impact of LL-37 and citrullinated-LL-37 (citLL-37) on oxylipins and its association with downstream chemokine production in human bronchial epithelial cells (HBEC), and its functional impact on leukocyte influx.

Methods We used a lipidomics approach to identify oxylipins that are enhanced in response to LL-37 and citLL-37 in HBEC. We further examined the role of selected oxylipins in LL-37- and citLL-37-mediated chemokine production by ELISA, and related leukocyte migration using a transwell migration assay.

Results We showed that LL-37, but not citLL-37, enhances oxylipins that are known to promote inflammation such as prostaglandins regulated by the cyclooxygenase (COX pathway). LL-37-mediated increase in COX-2 expression was significantly higher than that mediated by citLL-37. We showed that upregulation of COX-2 expression was dependent on the $P2X_7$ purinergic receptor. Our mechanistic studies revealed that LL-37-mediated increase in chemokines, GROα, IL-8 and MIP-3α, was dependent on the COX-2 pathway, and that this was significantly attenuated by citrullination of the peptide. Our results also indicated that COX-2-induced PGE_2 may act in an autocrine manner signaling through its EP receptors to facilitate LL-37-induced chemokine production. We functionally confirmed that factors secreted from HBEC in response to LL-37, but not citLL-37, promotes neutrophil migration which is COX-2 dependent.

---

*Correspondence:
Neeloffer Mookherjee
neeloffer.mookherjee@umanitoba.ca

Full list of author information is available at the end of the article

Conclusion The results of this study indicate that pro-inflammatory responses mediated by LL-37 is attenuated by citrullination of the peptide. The findings of this study underscore the role of LL-37 in influencing the enhancement of bioactive lipids and metabolic pathways such as COX-2 and its link to the peptide-mediated immunomodulatory functions in the lungs.

Keywords Cathelicidin, LL-37, Lung, Oxylipin, COX-2, Airway inflammation

Background

Inflammation is a critical component of immune response that involves an intricate network of cellular and molecular events. During airway inflammation, human bronchial epithelial cells (HBEC) releases chemokines that facilitate recruitment of leukocytes such as neutrophils to the lungs. In addition, during airway inflammation immunomodulators such as oxylipins and cationic host defense peptides (CHDP) are also released in the lungs, which play a role in the modulation of inflammation [1–3]. Although chemokines, oxylipins and CHDPs are all released in the lungs, the interplay of these immunomodulators remain largely unexplored in the process of airway inflammation. Thus, the focus of this study was to characterize oxylipins enhanced in response to the human CHDP LL-37 in HBEC, and its impact on LL-37-mediated chemokine production and neutrophil migration.

Oxylipins are bioactive lipids that can facilitate inflammation [1], and are generated from the oxidation of polyunsaturated acids such as arachidonic acid which is released from the lipid membrane by phospholipase A_2 [4]. Arachidonic acid serves as a substrate for three different metabolic pathways to produce oxylipins; the cyclooxygenase (COX) pathway which generates prostaglandins, the lipoxygenase (LOX) pathway which produces leukotrienes, and the cytochrome P450 monooxygenases pathway which produces epoxides [5]. Oxylipins bind to specific G protein-coupled receptors (GPCRs) to mediate downstream signaling pathways related to immune response and inflammation [1]. Prostaglandin E_2 (PGE_2) and the COX-2 pathway are the most well characterized mediators implicated in the process of inflammation, including in airway inflammation [6]. Previous studies have demonstrated that influence of LL-37 on oxylipins in different cell types. For example, LL-37 was shown to enhance PGE_2 in endothelial cells, fibroblasts and keratinocytes [7–9], and induce leukotriene production in eosinophils [10] and macrophages [11]. However, LL-37-mediated modulation of oxylipin profile and its relationship with chemokine responses is not understood in the context of airway inflammation.

A consideration in the biological function of LL-37 in airway inflammation is the aspect of post-translational modification of the peptide. Citrullination is a post-translational modification wherein the positively charged arginine residues of LL-37 are converted to neutral citrulline by peptidyl-arginine deiminases [12, 13]. Consequently both LL-37 and citrullinated forms of LL-37 are found in the lungs [12, 13]. While citrullination of LL-37 has been shown to impair its antimicrobial and antiviral properties [14, 15], the effect of citrullination on the immunomodulatory functions of LL-37 remains largely elusive. We have recently shown that citrullination does not impair all immunomodulatory functions of LL-37, instead it selectively suppress the pro-inflammatory functions of LL-37 [16, 17]. The impact of citrullination on LL-37-mediated oxylipin response is unknown.

In this study, we aimed to define oxylipins that are enhanced in response to LL-37 and citLL-37 in HBEC, using a lipidomics approach. We assessed the effect of LL-37 and citLL-37 on oxylipins and chemokine production. Using pharmacological inhibitors, we investigated the relationship of selected oxylipins with peptide-induced chemokine production, and its functional impact on neutrophil migration. The findings of this study indicate that citrullination may be a molecular switch to attenuate LL-37-mediated inflammatory responses in the lungs. Notably, this study highlights the role of LL-37 in influencing metabolic pathways to facilitate the peptide's immunomodulatory functions, thus defining a role for LL-37 in immunometabolism.

Methods

Reagents

The cationic peptides LL-37, citLL-37, and scrambled LL-37 (sLL-37) were synthesized and purchased from Innovagen AB (Lund, Sweden) and stored in -20 °C until use (Table 1). The quality control for the purity of LL-37 and citLL-37 including the mass spectrometry profile is shown in Supplementary Figure 1. Peptides were reconstituted in endotoxin-free water (Fisher Scientific, Cat# SH3052901), aliquoted and stored in glass vials at -20°C for a maximum of 3 months. Each reconstituted peptide aliquot was thawed at room temperature (RT), sonicated for 30 s in a water bath and vortexed for 15 s before use. Human recombinant IL-8 (carrier-free) was purchased from R&D Systems (Oakville, ON, CA; Cat# 208-IL), aliquoted and stored at -80 °C until use. The COX-2 inhibitor Rofecoxib was obtained from Selleckchem (Burlington, ON, CA; Cat# S3043), reconstituted in dimethyl sulfoxide (DMSO; Fisher Scientific) according to the manufacturer's instructions. $P2X_7$ inhibitor, KN62 (Cat# 13318) and PGE_2 receptors (EP_{1-4}) inhibitors, SC-19,220

Table 1 Peptides and sequences

Peptides	Sequence
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES
citLL-37	LLGDFF(cit)KSKEKIGKEFK(cit)IVQ(cit)KDFL(cit)NLVP(cit)TES
sLL-37	RSLEGTDRFPFVRLKNSRKLEFKDIKGIKREQFVKIL

(EP_1; Cat# 14060), PF-04418948 (EP_2; Cat# 15016), L-798,106 (EP_3; Cat# 11129) and MF498 (EP_4; Cat# 15973), were purchased from Cayman Chemical (Ann Arbor, MI, USA) and reconstituted in DMSO (Fisher Scientific) according to the manufacturer's instructions.

Human bronchial epithelial cell cultures; cell line and human primary cells

The human bronchial epithelial cell line HBEC-3KT (CRL-4051™) was purchased from American Type Culture Collection, cultured in airway epithelial cell basal medium (ATCC® PCS-300-030™) supplemented with bronchial epithelial cell growth kit (ATCC® PCS-300-040™), according to the manufacturer's instructions and as previously described by us [16, 18, 19]. The cell culture medium was replaced with airway epithelial cell basal medium containing 6 mM L-glutamine, without the growth supplements, 24 hours (h) prior to stimulation with peptides in the presence or absence of various inhibitors. HBEC-3KT cells were stimulated with either LL-37, citLL-37, and sLL-37 (concentrations as indicated), mRNA was isolated after 4 h and tissue culture (TC) supernatants were collected after 24 h.

Human primary bronchial epithelial cells (PBEC) were isolated from macroscopically normal lung tissue obtained from patients undergoing resection surgery for lung cancer at the Leiden University Medical Center (LUMC), the Netherlands, using a protocol previously described by us [20]. These cells can be differentiated to a multiciliated mucus-producing airway epithelial cell culture, supporting their airway phenotype [21]. Lung tissue donors were enrolled in the biobank via a no-objection system for coded anonymous further use of such tissue (www.coreon.org). Samples from this Biobank were approved for research use by the institutional medical ethical committee (BB22.006/AB/ab). Since 01-09-2022, patients are enrolled in the biobank using written informed consent in accordance with local regulations from the LUMC biobank with approval by the institutional medical ethical committee (B20.042/KB/kb). Briefly, human PBECs were expanded in T75 TC flasks precoated with coating media (10 µg/mL fibronectin (Sigma), 30 µg/mL PureCol (Advanced Biomatrix, California, USA) and 10 µg/mL bovine serum albumin [(BSA; Sigma) in phosphate buffered saline (PBS)] in keratinocyte serum-free medium (KSFM; Gibco) supplemented with 25 µg/mL of bovine pituitary extract (BPE; Gibco), 0.2 ng/mL of epidermal growth factor (EGF; Life Technologies), 1:100 dilution of antibiotics Penicillin and Streptomycin (Lonza, Kingston, ON, CA) and 1 µM isoproterenol (Sigma-Aldrich, Oakville, ON, CA). Cells were expanded to approximately 80% confluency, followed by trypsinization with 0.3 mg/mL trypsin in PBS containing 1 mg/mL glucose (Gibco), 0.1 mg/mL EDTA (Gibco) and 1:100 dilution of Penicillin and Streptomycin. PBEC were seeded at a density of 1×10^4 cells/mL in 12-well TC plates (Costar®) precoated with coating media, and cultured in a 1:1 mixture of basal bronchial epithelial cell medium (ScienCell, CA, USA) containing bronchial epithelial cell growth supplement (ScienCell), 1 nM of EC-23 (Tocris, UK), along with Dulbecco's modified Eagle's medium (Gibco) containing a 1:40 dilution of HEPES buffer (Invitrogen), and 1:100 dilution of Penicillin and Streptomycin. The cell culture medium was replaced every 48 h until, the medium was replaced without EGF, BPE and BSA 24 h prior to stimulation with either peptides or inhibitors as indicated.

Cytotoxicity assay

Cytotoxicity was determined by examining TC supernatants for the release of the lactate dehydrogenase (LDH) enzyme using a colorimetric assay (Roche Diagnostic, Laval, QC, CA), according to the manufacturer's instructions. Briefly, TC supernatants were centrifuged (250 x g for 5 min (min)) to obtain cell-free samples. TC supernatants (50 µL) were incubated with the LDH substrate mix (50 µL) for ~ 30 min in dark at RT, followed by colorimetric detection at 490 nm. Cytotoxicity was calculated relative to TC supernatants obtained from cells treated with 2% Triton X-100 (Sigma) as a measure for 100% cytotoxicity.

Oxylipin profiling with mass spectrometry

HBEC-3KT cells were stimulated with either LL-37, citLL-37 or sLL-37 (0.25 µM each) as indicated for 24 h. TC supernatants were centrifuged at 250 x g for 5 min to obtain cell-free samples. Oxylipin abundance was measured in TC supernatants using LC-MS/MS as previously described by us [22]. Briefly, oxylipins were extracted from the TC supernatants with solid phase extraction using Strata-X SPE columns (Phenomenex, CA, USA) preconditioned with methanol and pH 3 water and eluted with methanol. The extracted oxylipins were dried down under a gentle stream of nitrogen and resuspended in solvent A (Water – Acetonitrile – Acetic Acid [70:30:0.02; v/v/v]) & Solvent B (Acetonitrile – Isopropyl Alcohol [50:50; v/v]). Samples were separated using reverse phase HPLC using a Luna column (Luna 5u C18 [2] 100 A; 250 × 2.00 mm, Phenomenex) with gradient elution using Solvent A (Water – Acetonitrile – Acetic Acid [70:30:0.02; v/v/v]) and Solvent B (Acetonitrile – Isopropyl Alcohol [50:50; v/v]), on a Nexera-XR LC-20AD XR (Shimadzu)

HPLC. The HPLC was coupled to a Qtrap 6500 (SCIEX) mass spectrometer equipped with a IonDrive Turbo V Electrospray Ion source. Polarity was set to negative mode, and responses were measured using selective reaction monitoring (SRM). Pairwise differential analysis was conducted on normalized $\log_2$ oxylipins values. Oxylipins enhanced by sLL-37 were removed from the data analysis. Welch's t-test with a cut-off of $p < 0.05$ was used to select oxylipins that were significantly enhanced by LL-37 and citLL-37. Fold changes were computed for LL-37 and citLL-37-mediated oxylipin abundance relative to unstimulated cells.

ELISA

Cells were stimulated with either LL-37, citLL-37 or sLL-37 as indicated, and TC supernatants were collected after 24 h. TC supernatants were centrifuged (250 x g for 5 min) to obtain cell-free samples. The abundance of IL-8 (Cat# DY208), GROα (Cat# DY275) and MIP-3α (Cat# DY360) were examined in TC supernatants using ELISA kits obtained from R&D Systems, according to the manufacturer's instructions.

Quantitative real-time PCR

Cells were stimulated with either LL-37, citLL-37 or sLL-37 as indicated for 2, 4, 6, or 24 h. The cells were washed with cold PBS and cell lysate collected using lysis buffer (ThermoFisher Scientific; Cat# AC149320050) containing MagMAX™ Lysis/Binding Solution Concentrate (ThermoFisher Scientific; Cat# AM8500). Total RNA was isolated from the cell lysates using the MagMAX™ RNA isolation kit (ThermoFisher Scientific; Cat# AM1830) and the KingFisher Flex711 Automated Extraction & Purification System-GL (ThermoFisher Scientific), which included treatment with DNase to remove any residual genomic DNA, as previously described by us [17, 18]. Total RNA was quantified using a NanoDrop 2000 Spectrophotometer (ThermoFisher Scientific). Superscript III First-Strand Synthesis SuperMix for qRT-PCR was used for first strand cDNA synthesis from total RNA input of 200 ng per sample (ThermoFisher Scientific; Cat# 11752050), as previously described by us [17, 18]. QuantiTect Primer Assays (Qiagen) for CXCL8 (GeneGlobe ID QT00000322), CXCL1 (GeneGlobe ID QT00199752), CCL20 (GeneGlobe ID QT00012971), PTGS1 (GeneGlobe ID QT00210280), PTGS2 (GeneGlobe ID QT00040586) and 18s (GeneGlobe ID QT00199367) were used for qRT-PCR. These primers are designed to span exon-exon boundaries. A melt curve analysis was added to all qRT-PCR reactions to ensure a single, specific amplicon for each target. Fold change for each mRNA target was calculated using the comparative ΔΔCt method after normalization with 18s RNA as the reference gene, followed by Log_2 transformation, as previously described by us [17, 18].

Neutrophil isolation and migration assay

Human blood was obtained from healthy volunteers with informed written consent, approved by the University of Manitoba's Human Research Ethics Board (Protocol #: HS11105, H2010:259). Venous blood was collected in EDTA vacutainer tubes and neutrophils isolated using the EasySep™ Direct Human Neutrophil Isolation Kit (STEMCELL technologies, Vancouver, BC, Canada; Cat# 19666), according to the manufacturer's instructions and described by us [18]. Briefly, 25 mL of blood was gently mixed with the isolation cocktail and 50 μL of RapidSpheres™ provided in the kit, followed by incubation at RT for 5 min. The beads were washed, and neutrophils isolated using negative selection.

HBEC-3KT cells were stimulated with either LL-37, citLL-37 or sLL-37 (0.50 μM each), in the presence and absence of Rofecoxib (20 nM), and TC supernatants were collected after 24 h. TC supernatants (600 μL) were added to the bottom chamber of a 12-well, 12 mm Transwell plate with 12 (5.0 μM Pore Polyester membrane) inserts (Costar®). The plates were incubated at 37 °C in a 5% of CO_2 incubator for 30 min. Subsequently, human blood-derived neutrophils (6×10^5 cells/ insert, 200 μL) were added to the upper chamber of the Transwell insert. As a positive control, human recombinant chemokine IL-8 (30 ng/mL) was added to the bottom chamber of one of the wells in the Transwell plates containing airway epithelial cells basal medium (containing 6 mM L-glutamine), as previously described by us [18]. The Transwell plates were incubated for 2 h at 37 °C, followed by counting the number of migrated neutrophils in the bottom chamber using a Scepter 3.0 Handheld Automated Cell Counter (Millipore Ltd, ON, Canada).

Statistical analysis

All statistical analyses were performed using GraphPad Prism (version 10.1.1; GraphPad Software). Briefly, paired baseline correction was done for all experiments by subtracting values obtained from unstimulated cells. An unpaired t-test was used for qRT-PCR statistical analysis. Two-Way ANOVA was used to compare protein levels of IL-8, GROα, and MIP-3α between different experimental conditions in experiments with pharmacological inhibitors, both in the presence and absence of peptide treatments (LL-37, citLL-37, and sLL-37). A p-value of $p < 0.05$ was considered statistically significant. All statistical analyses are detailed in each of the figure legends.

Results

LL-37 and citLL-37 disparately enhances Oxylipins in human bronchial epithelial cells

As LL-37 enhances PGE_2 in fibroblasts, keratinocytes and endothelial cells [7–9], we examined oxylipin profile in HBECs in response to LL-37 and citLL-37 using a lipidomics approach as previously described by us [22]. HBEC-3KT cells were stimulated with the peptides LL-37, citLL-37 or sLL-37 (0.25 µM each) and TC supernatant was collected after 24 h ($n = 3$). Concentration of the peptides used was based on our previous study demonstrating that 0.25 µM of LL-37 and citLL-37 enhances specific chemokines and cytokines in HBEC [17]. The scrambled peptide, sLL-37, was used as a paired negative peptide control, as we have previously shown that sLL-37 does not elicit the immunomodulatory functions of LL-37 [17, 23]. Thus, any oxylipin enhanced in response to sLL-37 was removed from the data analysis. Our results demonstrated that prostaglandins were enhanced in response to LL-37 (> 2-fold compared to unstimulated cells). LL-37 specifically enhanced the abundance of PGE_2, 11b PGE_2, Dihomo PGE_2, Dihomo $PGF_2\alpha$, PGA_2 and PGE_1 (Table 2), all products of the COX pathway [6, 24, 25]. Whereas none of these prostaglandins were enhanced in response to citLL-37 (Table 2). LL-37 also significantly enhanced the abundance of 11-HETE (> 2-fold), 15-HETE (> 26-fold) and 15-HETrE (> 2-fold compared to unstimulated cells), oxylipins produced by both the COX and LOX pathways (Table 2). Whereas citLL-37 only enhanced the abundance of 15-HETrE significantly (Table 2). In contrast, both LL-37 and citLL-37 significantly enhanced the abundance of 9,10 EpOME (> 5-fold compared to unstimulated cells) and 12, 13 EpOME (> 23-fold compared to unstimulated cells), oxylipins that are products of the cytochrome P450 pathway [26, 27]. However, abundance of both 9,10 EpOME and 12, 13 EpOME enhanced in response to citLL-37 were > 50% lower than that elicited in response to LL-37 (Table 2).

Table 2 Disparate enhancement of Oxylipins by LL-37 and citLL-37 in human bronchial epithelial cells

Pathways	Oxylipins	LL-37		citLL-37	
		Avg Log_2 Fold Change	*p*-value	Avg Log_2 Fold Change	*p*-value
COX Pathway	PGE_2	2.63 ± 1.19	0.03	1.28 ± 0.144	ns
	11b PGE_2	2.44 ± 1.04	0.03	1.27 ± 0.162	ns
	Dihomo PGE_2	2.34 ± 0.813	0.02	1.21 ± 0.291	ns
	Dihomo $PGF_2\alpha$	1.90 ± 0.415	0.03	1.15 ± 0.178	ns
	PGE_1	2.19 ± 0.9	0.04	1.16 ± 0.102	ns
COX/LOX Pathway	11-HETE	3.47 ± 1.04	0.02	5.50 ± 3.00	ns
	15-HETE	26.47 ± 24.69	0.02	2.90 ± 0.92	ns
	15-HETrE	2.49 ± 0.73	0.02	2.39 ± 0.490	0.03
Cytochrome P450 Pathway	9,10 EpOMe*	5.83 ± 2.27	0.005	2.49 ± 0.522	0.02
	12,13 EpOMe*	23.60 ± 13.74	0.01	9.25 ± 3.15	0.004

*Can also be produced non-enzymatically

LL-37 and citLL-37 differentially upregulates COX-2 mRNA via the $P2X_7$ receptor

As LL-37 significantly enhanced prostaglandins (Table 2), and as COX enzymes are critical in the production of prostaglandins, we next examined the COX-1 and COX-2 mRNA abundance in response to LL-37 and citLL-37. However, before examining COX expressions we needed to optimize the concentration of LL-37 within physiologically representative range for further mechanistic studies. Concentration of LL-37 in the lungs has been shown to be between 0.25 and 1 µM in airway inflammation [28]. We have previously shown that LL-37 (0.25 and 0.5 µM) enhances the levels of chemokines IL-8, GROα and MIP-3α in HBEC-3KT cells [17]. Therefore, we examined chemokine production in response to LL-37 (0.25 and 0.5 µM) in HBEC-3KT cells. Both 0.25 and 0.5 µM of LL-37 significantly enhanced the levels of IL-8, GROα and MIP-3α in TC supernatants after 24 h, however the response was more than 50% higher following stimulation with 0.5 µM compared to 0.25 µM of LL-37 (Supplementary Figure 2). Based on these results, we selected the concentration of 0.5 µM for peptide stimulation for further studies.

HBEC-3KT cells were stimulated with LL-37, citLL-37 or sLL-37 (0.5 µM each) for 4 h, and the mRNA abundance of COX-1 and COX-2 were determined by qRT-PCR ($n = 5$). COX-1 mRNA abundance was not significantly enhanced by either LL-37 or citLL-37 (Fig. 1a). Although both LL-37 and citLL-37 significantly enhanced the mRNA abundance of COX-2 compared to unstimulated cells, LL-37-mediated enhancement of COX-2 was ~ 50% higher than that mediated by citLL-37 (Fig. 1b). These results were consistent with the lipidomics data showing an increase in prostaglandins (between 2 and 3-fold increase compared to unstimulated cells) in response to LL-37, but not significantly with citLL-37 (Table 2). These results suggest that citrullination of LL-37 suppresses the ability of the peptide to enhance elements in the COX-2 pathway.

A previous study demonstrated that LL-37 engages the $P2X_7$ purinergic receptor to induce COX-2 expression

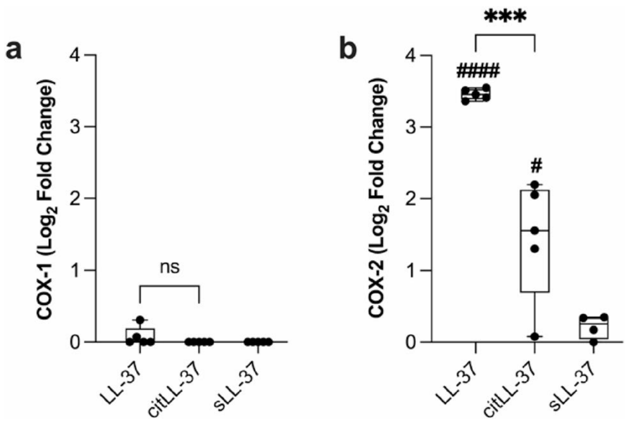

Fig. 1 COXmRNA abundance in response to LL-37 and citLL-37. HBEC-3KT cells were stimulated with either LL-37, citLL-37 or sLL-37 (0.50 μM each). **a** COX-1 and (**b**) COX-2 mRNA abundance were examined in cell lysates using qRT-PCR after 4 h. Relative fold changes were calculated compared to unstimulated cells normalized to 1, using the ΔΔCt method after normalization with 18s RNA expression. Fold changes compared to unstimulated cells are shown after Log$_2$ transformation. Each dot represents an independent experiment ($n = 5$), the bars show IQR with the median line and the whiskers represent the min-max range. Statistical significance was determined by paired t-test, and # represents statistical significance compared to unstimulated cells ($^{\#}p < 0.05$, $^{***}p < 0.0005$, and $^{\#\#\#\#}p < 0.0001$)

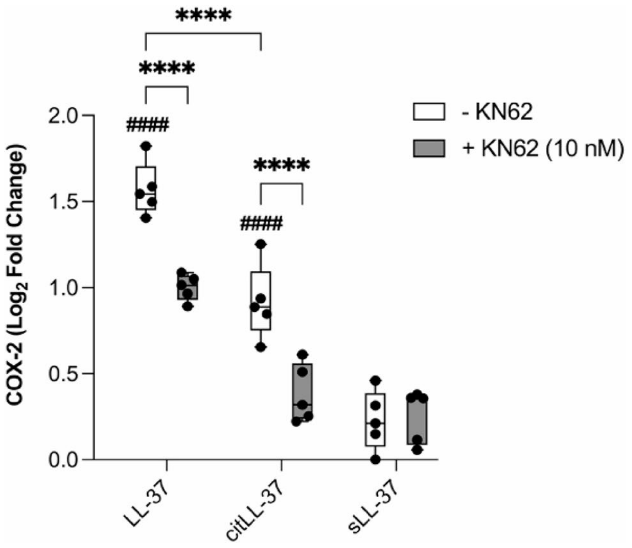

Fig. 2 Inhibition of P2X$_7$ receptor suppresses COX-2 mRNA. HBEC-3KT cells were pre-treated with KN62 (10 nM) for 1 h, followed by stimulation with either LL-37, citLL-37 or sLL-37 (0.50 μM each) for 4 h (n = 5). Abundance of COX-2 mRNA was examined in cell lysates by qRT-PCR. Relative fold changes were calculated compared to unstimulated cells normalized to 1, using the ΔΔCt method after normalization with 18s RNA expression. Cells stimulated with KN62 were normalized to cells stimulated with inhibitor alone. Fold changes compared to unstimulated cells are shown after Log2 transformation. Each dot represents an independent experiment (n = 5), the bars show IQR with the median line, and the whiskers represent the min-max range. Statistical significance was determined by Two-Way ANOVA (#### or ****$p < 0.0001$), where $^{\#}$ represents statistical significance compared to unstimulated cells

in human gingival fibroblasts [8]. Therefore, we next examined the involvement of the P2X$_7$ receptor in the enhancement of COX-2 mRNA, in response to LL-37 and citLL-37 in HBEC. HBEC-3KT cells were pre-treated with the P2X$_7$ inhibitor, KN62 (10 nM) for 1 h, subsequently the cells were stimulated with either LL-37, citLL-37 or sLL-37 (0.5 μM each) for 4 h, and the mRNA abundance of COX-2 was determined by qRT-PCR. The inhibitor concentration was selected based on dose titration performed based on IC50 values provided by the manufacturer and existing literature [29] (Supplementary Figure 3). Cells were monitored for cytotoxicity by examining the release of LDH in TC supernatants. KN62 (10 nM) did not significantly increase cytotoxicity, in the presence or absence of the peptides, compared to unstimulated cells (Supplementary Figure 4). Inhibition of the P2X$_7$ receptor significantly suppressed both LL-37 and citLL-37-mediated enhancement of COX-2 mRNA (Fig. 2). These findings indicate that both LL-37 and citLL-3 engages the P2X$_7$ receptor to enhance COX-2 mRNA in HBECs.

LL-37 and citLL-37-mediated chemokine response positively correlates with COX-2 expression

We showed that LL-37 and citLL-37 can upregulate the expression of COX-2, albeit with quantitative differences (Fig. 1). The COX-2 pathway is associated with the production of chemokines [24, 25], and we have previously shown that LL-37 enhances the levels of chemokines IL-8, GROα and MIP-3α in HBECs [17]. Therefore, we examined the relationship of peptide-mediated upregulation of COX-2 and chemokines. HBEC-3KT cells were stimulated with LL-37, citLL-37 or sLL-37 (0.5 μM each) for 4 h, and the mRNA abundance of chemokines IL-8, GROα and MIP-3α, as well as COX-2, was determined by qRT-PCR. Similar to COX-2 (Fig. 1b), LL-37 significantly enhanced mRNA abundance of IL-8, GROα and MIP-3α, and these chemokine responses were significantly less following stimulation with citLL-37 (Supplementary Figure 5). Additionally, LL-37 and citLL-37-mediated increase in mRNA abundance of COX-2 showed a significant positive correlation with chemokine response, however, the magnitude of response by LL-37 was greatly reduced when the peptide was citrullinated (Fig. 3).

LL-37-mediated chemokine production is dependent on COX-2

As there was a positive correlation between LL-37 and citLL-37-mediated upregulation of COX-2 and chemokines (Fig. 3), we next examined the effect of COX-2 inhibition on peptide-mediated increase in IL-8, GROα and MIP-3α. HBEC-3KT cells were pre-treated with the COX-2 inhibitor Rofecoxib (20 nM) for 1 h. Concentration of the inhibitor was based on dose titration

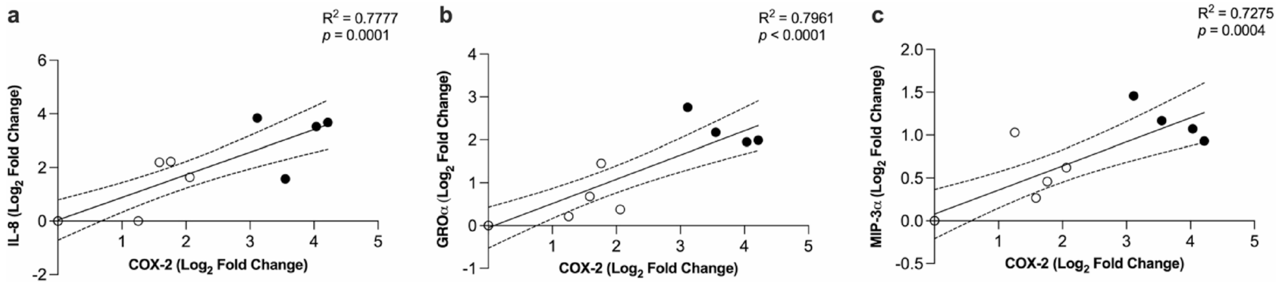

Fig. 3 Correlations between LL-37 and citLL-37-mediated increase in the mRNA abundance of COX-2 and chemokines. HBEC-3KT cells were stimulated with either (●) LL-37 or (○) citLL-37 (0.50 µM each). mRNA abundance of COX-2, IL-8, GROα and MIP-3α were examined in cell lysates using qRT-PCR after 4 h. Relative fold changes were calculated compared to unstimulated cells normalized to 1, using the ΔΔCt method after normalization with 18s RNA expression. Pearson's correlation analysis was performed to determine the correlation between COX-2 and (**a**) IL-8, (**b**) GROα and (**c**) MIP-3α mRNA abundance (fold changes compared to unstimulated cells)

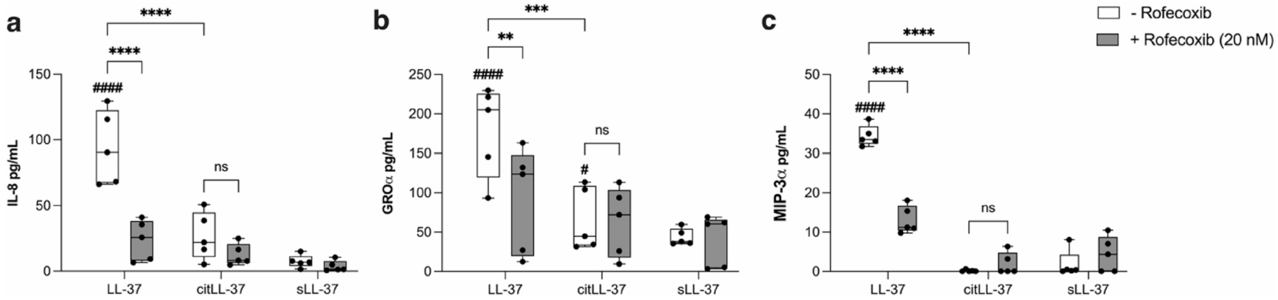

Fig. 4 Inhibition of COX-2 suppresses LL-37-mediated chemokine production. HBEC-3KT cells were pre-treated with COX-2 inhibitor Rofecoxib (20 nM) for 1 h, followed by stimulation with either LL-37, citLL-37 or sLL-37 (0.50 µM each) for 24 h (n = 5). TC supernatants were examined for the abundance of (**a**) IL-8, (**b**) GROα and (**c**) MIP-3α by ELISA. Chemokine levels shown in pg/mL after background subtraction of levels in unstimulated cells. Each dot represents an independent experiment (n = 5), the bars show IQR with the median line and the whiskers represent the min-max range. Statistical significance was measured using Two-Way ANOVA, and # represents statistical significance compared to unstimulated cells (**$p < 0.005$, ***$p < 0.0005$, #### or ****$p < 0.0001$)

performed based on IC50 value provided by the manufacturer (Supplementary Figure 6). Subsequently, the cells were stimulated with LL-37, citLL-37 or sLL-37 (0.5 µM each) for 24 h and the protein abundance of IL-8, GROα and MIP-3α was examined in TC supernatants by ELISA. Cells were monitored for cytotoxicity by examining the release of LDH in TC supernatants under all conditions. The inhibitor did not increase cytotoxicity, in the presence or absence of the peptides, compared to unstimulated cells (Supplementary Fig. 7). Presence of the COX-2 inhibitor significantly suppressed LL-37-mediated increase in the abundance of IL-8, GROα and MIP-3α proteins (Fig. 4). In contrast, increase of GROα in response to citLL-37 was not significantly altered by inhibition of COX-2 (Fig. 4). These results were also confirmed in human primary bronchial epithelial cells (PBEC) isolated from resected lung tissues. Like the results in HBEC-3KT cells (Fig. 4), inhibition of COX-2 suppressed LL-37-mediated enhancement of chemokine production (Supplementary Figure 8). These results indicated that LL-37-mediated chemokine response is dependent on the COX-2 pathway. These results also aligned with the lipidomics data demonstrating enhancement of PGE_2, an oxylipin downstream of COX-2, in response to LL-37 but not citLL-37 (Table 2).

LL-37-mediated neutrophil migration is dependent on the COX-2 pathway

LL-37-induced chemokines IL-8 and GROα are both known to facilitate neutrophil recruitment, and LL-37 was previously shown to promote neutrophil migration [30]. Similarly, COX-2-derived PGE_2 can promote neutrophil migration [31]. Our results demonstrated that LL-37-mediated IL-8 and GROα production were dependent on COX-2 (Fig. 4). In addition, citLL-37 also modestly increased the abundance of GROα, albeit more than 50% less than that elicited in response to LL-37 (Fig. 4). Therefore, we performed a functional assay to examine the impact LL-37 and citLL-37 on neutrophil migration, and its dependency on the COX-2 pathway. We examined the effect of a COX-2 inhibitor on neutrophil migration mediated by the secreted milieu of HBECs treated with the peptides. HBEC-3KT cells were pre-treated with the COX-2 inhibitor Rofecoxib (20 nM) for 1 h. Subsequently, the cells were stimulated with either LL-37, citLL-37 or sLL-37 (0.5 µM each) for 24 h. TC supernatants collected

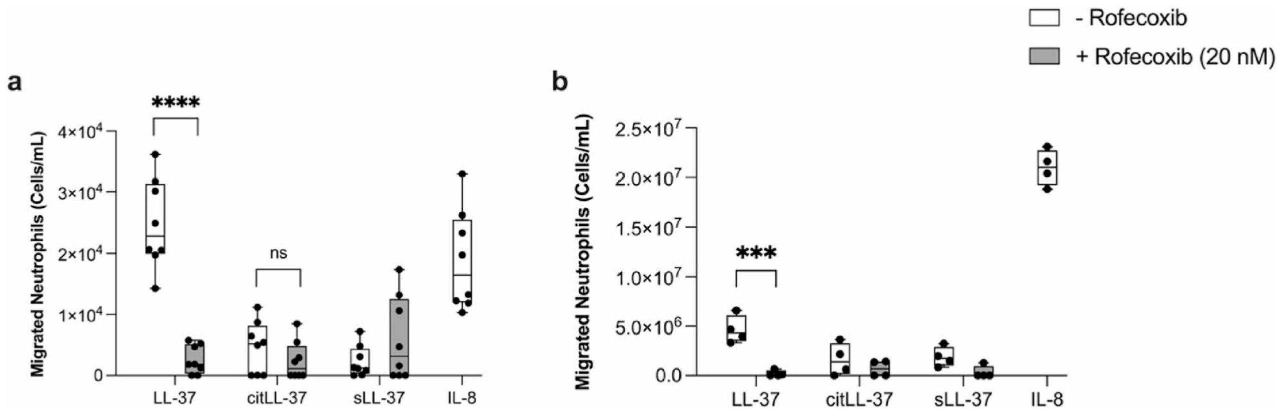

Fig. 5 LL-37-mediated neutrophil migration is dependent on the COX-2 pathway. **a** HBEC-3KT cells and (**b**) human primary bronchial epithelial cells (PBEC) were pre-treated with the COX-2 inhibitor Rofecoxib (20 nM) for 1 h, followed by stimulation with either LL-37, citLL-37 or sLL-37 (0.50 µM each). TC supernatants collected after 24 h were used in the bottom chamber of a Transwell plates. IL-8 (30 ng/mL) was used in the bottom chamber of an independent well as a positive control for neutrophil migration. Neutrophils isolated from human blood, from two independent donors with two technical replicates each, were placed in the upper chamber of the Transwell plate. Neutrophils were counted in the bottom chamber of the Transwell plate after 3 h. Results shown are after subtracting background counts of neutrophils in the bottom chamber in wells with TC supernatants from unstimulated cells. Each dot represents an independent replicate), the bars show IQR with the median line and the whiskers represent the min-max range. Statistical significance was measured using Two-Way ANOVA (***$p<0.001$ and ****$p<0.0001$)

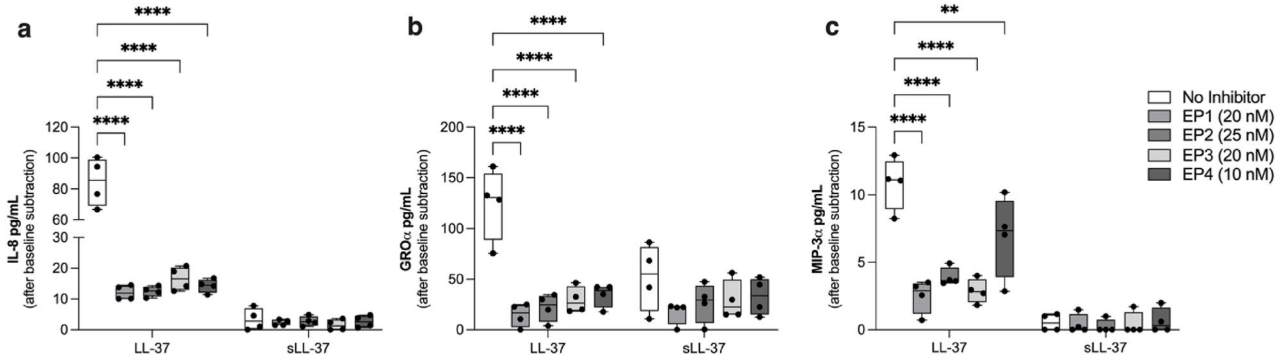

Fig. 6 Inhibition of PGE2 receptors (EP1-4) suppresses LL-37-mediated enhancement of chemokines. HBEC-3KT cells were pre-treated with specific inhibitors for PGE2 receptors, EP1 (SC-19220; 20 nM), EP2 (PF-044EP2; 25 nM), EP3 (L-798,106; 20 nM), or EP4 (MF498; 10 nM), 1 h prior to stimulation with either LL-37or sLL-37 (0.50 µM). Tissue culture (TC) supernatants were collected after 24 h and the abundance of (**a**) IL-8, (**b**) GROα and (**c**) MIP-3α were measured by ELISA. Each dot represents an independent experiment (n=4), the bars show IQR with the median line and the whiskers represent the min-max range. Results shown are after subtracting baseline values obtained from unstimulated cells in each independent experiment. Statistical significance was determined using Two-Way ANOVA (**$p< 0.001$ and ****$p< 0.0001$)

from these cells were used in the lower chamber of a Transwell plate for a neutrophil migration assay, as previously described by us [18]. Neutrophils isolated from human blood were placed on the upper chamber of the Transwell plates, and the number of neutrophils in the lower chamber was counted after 3 h. TC supernatants obtained from HBEC-3KT cells treated with LL-37 significantly enhanced neutrophil migration, and this was significantly suppressed to baseline levels in the presence of the COX-2 inhibitor (Fig. 5a).

We further confirmed these results in primary cells using human PBEC isolated from lungs. PBECs were pre-treated with COX-2 inhibitor for 1 h, followed by stimulation of the cells with the peptides, as indicated above, for 24 h. TC supernatants obtained from PBECs under different conditions was used in the lower chamber of a Transwell plate for the neutrophil migration assay detailed above. Similar to the results in HBEC-3KT cells, TC supernatants obtained from human PBEC stimulated with LL-37 significantly enhanced neutrophil migration which was significantly inhibited in the presence of the COX-2 inhibitor (Fig. 5b).

Overall, TC supernatants obtained from both HBEC-3KT cell and human PBEC treated with LL-37, but not citLL-37, promoted neutrophil migration which was dependent on the COX-2 pathway (Fig. 6).

Inhibition of PGE_2 receptors suppresses LL-37-mediated enhancement of chemokines

Previous studies have shown that COX-2-mediated PGE_2 can act in an autocrine manner via EP receptors to induce the production of chemokines, including IL-8 [32]. We demonstrated that LL-37-mediated chemokine production, but not citLL-37, was dependent on the COX-2 pathway (Fig. 4), and that LL-37 facilitates neutrophil migration (Fig. 5). Therefore, we further examined whether LL-37-mediated chemokine production was dependent on the PGE_2 receptors EP_{1-4}. HBEC-3KT cells were treated with specific inhibitors for PGE_2 receptors, EP_1 (SC-19220; 20 nM), EP_2 (PF-044EP2; 25 nM), EP_3 (L-798,106; 20 nM), and EP_4 (MF498; 10 nM), for 1 h. The inhibitor concentration was selected based on IC50 values provided by the manufacturer. In the presence or absence of the peptides, the inhibitors did not increase cytotoxicity compared to unstimulated cells (Supplementary Figure 9). Subsequently, the cells were stimulated with either LL-37 or sLL-37 (0.5 µM each) for 24 h, and the protein abundance of IL-8, GROα, and MIP-3α in TC supernatants was measured by ELISA. Inhibition of PGE_2 receptors EP_{1-4} significantly suppressed LL-37-mediated increase of chemokines IL-8, GROα and MIP-3α. These results indicated that LL-37-mediated release of PGE_2 (Table 2) may signal in an autocrine manner via the EP_{1-4} receptors to enhance chemokines production in HBECs.

Discussion

The findings of this study provide an insight into differential production of oxylipins by LL-37 and the modified citrullinated peptide. Our findings show that LL-37 but not citLL-37 robustly enhances the production of prostaglandins in HBECs. We further demonstrate that LL-37 upregulates the expression of COX-2, which is significantly higher than that mediated by citLL-37. We also show that LL-37-mediated chemokine production and neutrophil migration is dependent on COX-2, a key enzyme within the metabolic pathway that produces prostaglandins. Overall, the findings of this study indicate a role for LL-37 in immunometabolism, linking bioactive lipids oxylipins produced by bronchial epithelial cells to the peptide's functions in modulating chemokine responses and facilitating neutrophil migration, relevant to airway inflammation. Our findings also suggest that citrullination of LL-37 attenuates the peptide's ability to enhance COX-2, pro-inflammatory oxylipins and chemokines in HBECs, and likely mitigates LL-37-mediated neutrophil migration. These findings suggest that citrullination during airway inflammation may be a post-translational regulatory mechanism to dampen the pro-inflammatory functions of LL-37.

Both LL-37 and oxylipins function as immunomodulators that can either promote or suppress inflammation depending on the cellular milieu [33, 34]. Some previous studies have demonstrated an impact of LL-37 on oxylipins in different cell types. For example, LL-37 was shown to facilitate the release of PGE_2 in endothelial cells [7], and upregulate the expression of COX-2 to promote PGE_2 release in keratinocytes and gingival fibroblasts [8, 9]. These studies are corroborated by our findings demonstrating that LL-37 enhances COX-2 expression and PGE_2 abundance in HBECs. A possible mechanism may be that LL-37 enhances oxylipin production through its involvement in lipid peroxidation. Oxylipins are products of polyunsaturated fatty acids (PUFA) oxidation, a complex process where PUFAs undergo reactions with oxygen to form new compounds [33]. Reactive oxygen species (ROS) are critical in initiating lipid peroxidation [35]. LL-37 has been shown to facilitate increase in ROS production in neutrophils and macrophages [36]. Thus, it is possible that LL-37 enhances ROS levels in the lungs and promotes oxidation of PUFAs to generate oxylipins, which warrants further investigation. Additionally, our lipidomics data demonstrate differential enhancement of other oxylipins such as those in the LOX and Cytochrome P450 pathways by LL-37 and citLL-37. While previous studies have shown that LL-37 can induce the release of leukotrienes [37], to our knowledge, this is the first study to show that LL-37 can enhance products from the LOX pathway, such as 11-HETE, 15-HETE, and 15-HETrE. To our knowledge, we also demonstrate for the first time that LL-37 enhances 9,10- and 12,13 EpOME, products of the CYP pathway. EpOMEs can also be generated non-enzymatically primarily by processes related to oxidative stress [38]. As mentioned above, as LL-37 can facilitate increase in ROS, it is possible that LL-37-mediated enhancement of EpOMEs may be generated non-enzymatically. Although here we focus on the COX pathway, which regulates prostaglandins, involvement of the LOX and Cytochrome P450 pathways in mechanisms related to LL-37- and citLL-37-mediated immunomodulatory functions warrants further investigation, which is beyond the scope of this study.

In this study, we showed a direct association of COX-2 with LL-37-mediated enhancement of chemokines such as GROα and IL-8 that can facilitate neutrophilia. We confirm this functionally by demonstrating that LL-37-mediated neutrophil migration is dependent on the COX-2 pathway. Thus, our findings clearly indicate that the pro-inflammatory functions of LL-37 to facilitate airway inflammation, in particular neutrophilic airway inflammation, may be dependent on the COX-2 pathway. Here, we also showed that the ability of LL-37 to enhance COX-2 is dependent on the $P2X_7$ purinergic receptor in HBEC. This is consistent with a previous

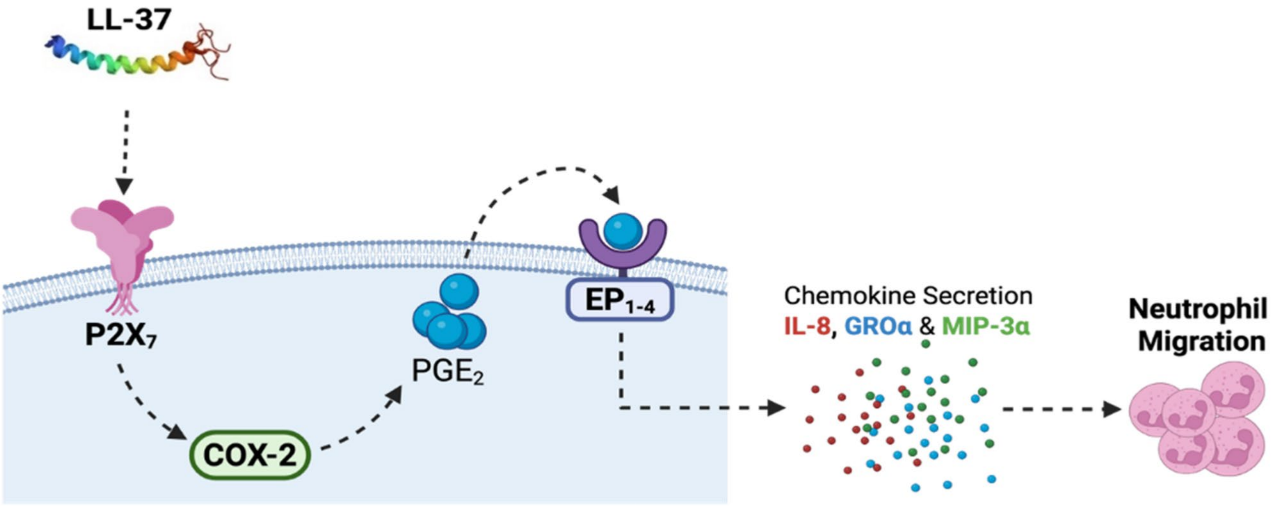

Fig. 7 Proposed mechanism of LL-37-COX-2 axis for chemokine production and neutrophil migration in the lungs. LL-37 engages the P2X$_7$ receptor in upregulating COX-2 expression which facilitates increase in the abundance of prostaglandin PGE2. Release of PGE2 may act in an autocrine manner through PGE2 receptors (EP1 – 4), resulting in enhanced production of chemokines including IL-8 and GROα, which facilitates neutrophil migration contributing to airway inflammation. However, citrullination of LL-37 dampens this pathway, potentially acting as a regulatory switch that limits the pro-inflammatory functions of LL-37 in the lungs (Figure created in BioRender.com)

study demonstrating that LL-37 enhances COX-2 and PGE$_2$ via the P2X$_7$ receptor in gingival fibroblasts [8]. A nuance to consider is that COX-2 expression is regulated by calmodulin-dependent protein kinase II (CaMKII) which can also be suppressed by the KN62 inhibitor [39, 40]. As calmodulin-dependent processes are not dependent on the P2X$_7$ receptor in human cells [41], the distinct influence of the P2X$_7$ receptor and CAMKII in LL-37-mediated COX-2 response needs further investigation. Additionally, we have previously shown that LL-37-mediated increase in chemokine production including GROα is dependent on GPCRs in macrophages [23]. Interestingly, the biological functions of PGE$_2$ is mediated by four different GPCRs subtypes EP$_{1-4}$ [42]. Previous studies have shown that COX-2-mediated PGE$_2$ can act in an autocrine manner via EP receptors to induce the production of chemokines, including IL-8 [32]. This is corroborated by our findings that inhibition of GPCRs EP$_{1-4}$ receptors significantly suppresses the enhancement of chemokines in response to LL-37 in HBECs. Overall, based on the findings of this study we propose that LL-37 engages the P2X$_7$ receptor on HBEC to enhance COX-2 expression facilitating the induction and release of PGE$_2$, which subsequently acts in an autocrine manner via the PGE$_2$ receptors (EP$_{1-4}$), resulting in enhancement of chemokines such as IL-8 and GROα to facilitate neutrophil migration during airway inflammation (Fig. 7).

As LL-37 can be citrullinated during lung inflammation [12, 14], it is important to compare the biological functions of LL-37 with that of citLL-37 to provide an insight into how the peptide activity may be altered in the lungs during airway inflammation. Our findings demonstrate that the ability of LL-37 to enhance pro-inflammatory oxylipins such as PGE$_2$, chemokine production and facilitate neutrophil migration in the lungs is attenuated by citrullination. Previous studies have shown that citrullination of LL-37 results in impairment of LL-37's antimicrobial functions [12, 14, 15]. However, we have previously shown that citrullination of LL-37 does not abrogate all immunomodulatory functions of the peptide, instead selectively suppresses certain pro-inflammatory responses mediated by LL-37 [16, 17]. This is corroborated by the findings in this study demonstrating that although citLL-37 can modestly enhance COX-2 expression and GROα production, this is significantly less than that elicited by unmodified LL-37. We also demonstrate with a functional assay that citLL-37 is not able to promote neutrophil migration, which contrasts with LL-37. These findings suggest that citrullination of LL-37 during inflammation may be a post-translational mechanism to dampen the peptide's pro-inflammatory functions, potentially contributing to the resolution of inflammation and restoring immune homeostasis.

Here, we show that the modest upregulation of COX-2 in response citLL-37 is also dependent on the P2X$_7$ receptor, suggesting engagement of a common receptor by both LL-37 and citLL-37. It is possible that the affinity of LL-37 to interact with P2X$_7$ is reduced by citrullination, thereby impairing its ability to mediate downstream pro-inflammatory responses. It may also be that citLL-37 interacts with other receptors or protein partners which leads to the selective loss of pro-inflammatory responses of LL-37 [16, 17]. In this study, we have used fully citrullinated LL-37 where all five arginine residues are modified. However, other citrullinated variants of LL-37 are found in the bronchoalveolar lavage fluid, for example with three arginine residues of LL-37 citrullinated [12]. Thus, future studies to investigate how LL-37-mediated functions are altered depending on the degree of citrullination of the peptide are warranted. Studies defining the LL-37 interactome indicate that the peptide directly interacts with 16 different protein partners and indirectly with more than 1000 secondary effector proteins [43]. How these interactions change following citrullination of LL-37 and consequent effects on the peptide's immunomodulatory functions merits further systematic examination in the future.

A limitation of this study is that the findings are from in vitro experiments using undifferentiated HBECs, albeit using both cell line and human primary cells. A previous study showed that bronchial epithelial cells following mucociliary differentiation using air-liquid interface (ALI) cultures are more efficient in metabolic conversion of substrates in the generation of oxylipins [44]. Nevertheless, the results of this study provide the foundation for future studies to systematically confirm the proposed mechanism (Fig. 7) using ALI cultures and in vivo models of airway inflammation. Also, in this study we have used citLL-37 where all five arginine residues were modified to citrulline. However, under physiological conditions different forms of citrullinated LL-37 are detected in the lungs, and altered biological activity of LL-37 seem to be associated with the number of arginine residues of the peptide that are citrullinated [14]. Therefore, the impact of citLL-37 on the peptide's immunomodulatory functions and oxylipin production in the lungs may be quantitatively different depending on the number of arginine residues that are citrullinated, which also needs to be further examined.

Conclusion

In this study, we have defined oxylipins that are enhanced in response to LL-37 and citLL-37 in human bronchial epithelial cells. We demonstrated that LL-37 enhances specific chemokines in HBECs, and that the resulting secreted milieu promotes neutrophil migration, via a COX-2-dependent mechanism. To our knowledge, this is the first study to link bioactive lipids and metabolic enzymes such as COX-2 to immunomodulatory functions of LL-37 in bronchial epithelial cells. This study underscores a role for LL-37 in immunometabolism during lung inflammation.

Abbreviations

CHDP	Cationic host defence peptides
citLL-37	Citrullinated-LL-3
COX	Cyclooxygenase
GPCRs	G protein-coupled receptors
HBEC	Human bronchial epithelial cells
LDH	Lactate dehydrogenase
LOX	Lipoxygenase
PGE_2	Prostaglandin E_2
TC	Tissue culture

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-026-03493-w.

Supplementary Material 1.

Acknowledgements
We gratefully acknowledge Dr. Harold Aukema at the Canadian Centre for Agri-Food Research in Health and Medicine, for oxylipin profiling with mass spectrometry.

Authors' contributions

PR and NM conceived and designed the study. PR performed majority of the experiments, analyzed the data, and wrote the manuscript. MH contributed to the optimization of some of the methods, provided intellectual input in study design and performed some of the experiments with primary cells. CP provided significant intellectual input for the study, performed the lipidomics sample processing and data analysis. AA performed initial optimization of peptides and chemokine endpoints in human bronchial epithelial cells. AMD provided the human primary bronchial epithelial cells and provided methodological guidance for primary cell cultures. NM obtained funding and resources for this study, provided overall supervision, and extensively edited the manuscript. All the authors reviewed the manuscript.

Funding
This study was supported by The Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (RGPIN-2020-06599). PR was supported by the Canada Graduate Scholarship – Master's (CGSM).

Data availability

All data generated and analyzed during this study are included in the published article and supplementary information.

Declarations

Ethics approval and consent to participate

Venous blood was obtained from healthy volunteers with informed written consent, approved by the University of Manitoba's Human Research Ethics Board (Protocol #: HS11105, H2010:259). Human primary bronchial epithelial cells (PBEC) were isolated from macroscopically normal lung tissue obtained from patients undergoing resection surgery for lung cancer at the Leiden University Medical Center (LUMC), the Netherlands. Lung tissue donors were enrolled in the biobank via a no-objection system for coded anonymous further use of such tissue (www.coreon.org) and samples were approved for research use by the institutional medical ethical committee (BB22.006/AB/ab). Since 01-09-2022, patients are enrolled in the biobank using written informed consent in accordance with local regulations from the LUMC biobank with approval by the institutional medical ethical committee (B20.042/KB/kb).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details
[1]Department of Immunology, University of Manitoba, Winnipeg, MB, Canada
[2]Department of Internal Medicine, Manitoba Centre for Proteomics & Systems Biology, University of Manitoba, Winnipeg, MB, Canada
[3]Nursing Department, School of Health and Community Services, Red River College Polytechnic, Winnipeg, MB, Canada
[4]Department of Immunology, University of Toronto, Toronto, ON, Canada

[5]Department of Pulmonology, PulmoScience Lab, Leiden University Medical Centre, Leiden, The Netherlands
[6]Department of Physiology & Pathophysiology, University of Manitoba, Winnipeg, MB, Canada

Received: 14 August 2025 / Accepted: 31 December 2025

References

1. Chiurchiù V, Leuti A, Maccarrone M. Bioactive lipids and chronic inflammation: managing the fire within. Front Immunol. 2018;9:38.
2. Rohmann K, Tschernig T, Pabst R, Goldmann T, Drömann D. Innate immunity in the human lung: pathogen recognition and lung disease. Cell Tissue Res. 2011;343(1):167–74.
3. Mookherjee N, Anderson MA, Haagsman HP, Davidson DJ. Antimicrobial host defence peptides: functions and clinical potential. Nat Rev Drug Discov. 2020;19(5):311–32.
4. Dennis EA, Norris PC. Eicosanoid storm in infection and inflammation. Nat Rev Immunol. 2015;15(8):511–23.
5. Shinto LH, Raber J, Mishra A, Roese N, Silbert LC. A review of Oxylipins in alzheimer's disease and related dementias (ADRD): potential therapeutic targets for the modulation of vascular tone and inflammation. Metabolites. 2022;12(9):826.
6. Claar D, Hartert TV, Peebles RS. The role of prostaglandins in allergic lung inflammation and asthma. Expert Rev Respir Med. 2015;9(1):55–72.
7. Salvado MD, Di Gennaro A, Lindbom L, Agerberth B, Haeggström JZ. Cathelicidin LL-37 induces angiogenesis via PGE2-EP3 signaling in endothelial cells, in vivo Inhibition by aspirin. Arterioscler Thromb Vasc Biol. 2013;33(8):1965–72.
8. Chotjumlong P, Bolscher JG, Nazmi K, Reutrakul V, Supanchart C, Buranaphatthana W, et al. Involvement of the P2X7 purinergic receptor and c-Jun N-terminal and extracellular signal-regulated kinases in cyclooxygenase-2 and prostaglandin E2 induction by LL-37. J Innate Immun. 2013;5(1):72–83.
9. Chamorro CI, Weber G, Grönberg A, Pivarcsi A, Ståhle M. The human antimicrobial peptide LL-37 suppresses apoptosis in keratinocytes. J Invest Dermatology. 2009;129(4):937–44.
10. Sun J, Dahlén B, Agerberth B, Haeggström JZ. The antimicrobial peptide LL-37 induces synthesis and release of Cysteinyl leukotrienes from human eosinophils – implications for asthma. Allergy. 2013;68(3):304–11.
11. Wan M, Soehnlein O, Tang X, van der Does AM, Smedler E, Uhlén P, et al. Cathelicidin LL-37 induces time-resolved release of LTB4 and TXA2 by human macrophages and triggers eicosanoid generation in vivo. FASEB J. 2014;28(8):3456–67.
12. Al-Adwani S, Wallin C, Balhuizen MD, Veldhuizen EJA, Coorens M, Landreh M, et al. Studies on citrullinated LL-37: detection in human airways, antibacterial effects and biophysical properties. Sci Rep. 2020;10(1):2376.
13. Ciesielski O, Biesiekierska M, Panthu B, Soszyński M, Pirola L, Balcerczyk A. Citrullination in the pathology of inflammatory and autoimmune disorders: recent advances and future perspectives. Cell Mol Life Sci. 2022;79(2):94.
14. Casanova V, Sousa FH, Shakamuri P, Svoboda P, Buch C, D'Acremont M, et al. Citrullination alters the antiviral and Immunomodulatory activities of the human Cathelicidin LL-37 during rhinovirus infection. Front Immunol. 2020;11:85.
15. Koziel J, Bryzek D, Sroka A, Maresz K, Glowczyk I, Bielecka E, et al. Citrullination alters Immunomodulatory function of LL-37 essential for prevention of endotoxin-induced sepsis. J Immunol. 2014;192(11):5363–72.
16. Altieri A, Marshall CL, Ramotar P, Lloyd D, Hemshekhar M, Spicer V, et al. Human host defense peptide LL-37 suppresses TNFα-Mediated matrix metalloproteinases MMP9 and MMP13 in human bronchial epithelial cells. J Innate Immun. 2024;16(1):203–15.
17. Altieri A, Lloyd D, Ramotar P, van der Does AM, Hemshekhar M, Mookherjee N. LL-37 and citrullinated-LL-37 modulate IL-17A/F-mediated responses and selectively suppress Lipocalin-2 in bronchial epithelial cells. J Inflamm. 2025;22(1):20.
18. Altieri A, Piyadasa H, Hemshekhar M, Osawa N, Recksiedler B, Spicer V, et al. Combination of IL-17A/F and TNF-α uniquely alters the bronchial epithelial cell proteome to enhance proteins that augment neutrophil migration. J Inflamm (Lond). 2022;19(1):26.
19. Altieri A, Piyadasa H, Recksiedler B, Spicer V, Mookherjee N, Cytokines. IL-17, TNF and IFN-γ alter the expression of antimicrobial peptides and proteins disparately: A targeted proteomics analysis using SOMAscan technology. Vaccines (Basel). 2018;6(3):51.
20. Ninaber DK, van der Does AM, Hiemstra PS. Isolating bronchial epithelial cells from resected lung tissue for biobanking and Establishing Well-Differentiated Air-Liquid interface cultures. J Vis Exp. 2023;195: e65102.
21. Amatngalim GD, Schrumpf JA, Dishchekenian F, Mertens TCJ, Ninaber DK, van der Linden AC, et al. Aberrant epithelial differentiation by cigarette smoke dysregulates respiratory host defence. Eur Respir J. 2018;51(4):1701009.
22. Kahnamoui S, Winter T, Lloyd D, Halayko AJ, Mookherjee N, Aukema HM, et al. Oxylipin profiling of airway structural cells is unique and modified by relevant stimuli. J Proteome Res. 2025;24(2):672–84.
23. Hemshekhar M, Choi KYG, Mookherjee N. Host defense peptide LL-37-Mediated chemoattractant Properties, but not Anti-Inflammatory cytokine IL-1RA Production, is selectively controlled by Cdc42 Rho GTPase via G protein-Coupled receptors and JNK Mitogen-Activated protein kinase. Front Immunol. 2018;9:1871.
24. Harris SG, Padilla J, Koumas L, Ray D, Phipps RP. Prostaglandins as modulators of immunity. Trends Immunol. 2002;23(3):144–50.
25. Burkett JB, Doran AC, Gannon M. Harnessing prostaglandin E2 signaling to ameliorate autoimmunity. Trends Immunol. 2023;44(3):162–71.
26. Gilroy DW, Edin ML, De Maeyer RPH, Bystrom J, Newson J, Lih FB, et al. CYP450-derived Oxylipins mediate inflammatory resolution. Proc Natl Acad Sci U S A. 2016;113(23):E3240–3249.
27. Hildreth K, Kodani SD, Hammock BD, Zhao L. Cytochrome P450-derived Linoleic acid metabolites EpOMEs and dihomes: a review of recent studies. J Nutr Biochem. 2020;86:108484.
28. Wright TK, Gibson PG, Simpson JL, McDonald VM, Wood LG, Baines KJ. Neutrophil extracellular traps are associated with inflammation in chronic airway disease. Respirology. 2016;21(3):467–75.
29. Tomasinsig L, Pizzirani C, Skerlavaj B, Pellegatti P, Gulinelli S, Tossi A, et al. The Human Cathelicidin LL-37 Modulates the Activities of the P2X7 Receptor in a Structure-dependent Manner. J Biol Chem. 2008;283(45):30471–81.
30. Kahlenberg JM, Kaplan MJ. Little peptide, big effects: the role of LL-37 in inflammation and autoimmune disease. J Immunol. 2013;191(10):4895–901.
31. Sheppe AEF, Edelmann MJ. Roles of eicosanoids in regulating inflammation and neutrophil migration as an innate host response to bacterial infections. Infect Immun. 2021;89(8):e00095–21.
32. Aso H, Ito S, Mori A, Morioka M, Suganuma N, Kondo M, et al. Prostaglandin E2 enhances interleukin-8 production via EP4 receptor in human pulmonary microvascular endothelial cells. Am J Physiol Lung Cell Mol Physiol. 2012;302(2):L266–273.
33. Misheva M, Johnson J, McCullagh J. Role of Oxylipins in the Inflammatory-Related diseases NAFLD, Obesity, and type 2 diabetes. Metabolites. 2022;12(12):1238.
34. Yang B, Good D, Mosaiab T, Liu W, Ni G, Kaur J, et al. Significance of LL-37 on Immunomodulation and disease outcome. Biomed Res Int. 2020;2020:8349712.
35. Endale HT, Tesfaye W, Mengstie TA. ROS induced lipid peroxidation and their role in ferroptosis. Front Cell Dev Biol. 2023;11:1226044.
36. Zheng Y, Niyonsaba F, Ushio H, Nagaoka I, Ikeda S, Okumura K, et al. Cathelicidin LL-37 induces the generation of reactive oxygen species and release of human α-defensins from neutrophils. Br J Dermatol. 2007;157(6):1124–31.
37. Jiao D, Wong CK, Tsang MSM, Chu IMT, Liu D, Zhu J, et al. Activation of eosinophils interacting with bronchial epithelial cells by antimicrobial peptide LL-37: implications in allergic asthma. Sci Rep. 2017;7(1):1848.
38. Rund KM, Heylmann D, Seiwert N, Wecklein S, Oger C, Galano JM, et al. Formation of trans-epoxy fatty acids correlates with formation of isoprostanes and could serve as biomarker of oxidative stress. Prostaglandins Other Lipid Mediat. 2019;144:106334.
39. Zhou X, Li J, Yang W. Calcium/calmodulin-dependent protein kinase II regulates cyclooxygenase-2 expression and prostaglandin E2 production by activating cAMP-response element-binding protein in rat peritoneal macrophages. Immunology. 2014;143(2):287–99.
40. Noronha-Matos JB, Sousa-Soares C, Correia-de-Sá P. Differential participation of CaMKII/ROCK and NOS pathways in the cholinergic inhibitory drive operated by nicotinic α7 receptors in perisynaptic Schwann cells. Biochem Pharmacol. 2025;231:116649.
41. Roger S, Gillet L, Baroja-Mazo A, Surprenant A, Pelegrin P. C-terminal Calmodulin-binding Motif Differentially Controls Human and Rat P2X7 Receptor Current Facilitation. J Biol Chem. 2010;285(23):17514–24.

42. Mendez M, LaPointe MC. PGE2-induced hypertrophy of cardiac myocytes involves EP4 receptor-dependent activation of p42/44 MAPK and EGFR transactivation. Am J Physiol Heart Circ Physiol. 2005;288(5):H2111–7.
43. Hancock REW, Haney EF, Gill EE. The immunology of host defence peptides: beyond antimicrobial activity. Nat Rev Immunol. 2016;16(5):321–34.
44. Boei JJWA, Vermeulen S, Klein B, Hiemstra PS, Verhoosel RM, Jennen DGJ, et al. Xenobiotic metabolism in differentiated human bronchial epithelial cells. Arch Toxicol. 2017;91(5):2093–105.

Publisher's Note