SUPPLEMENT FILE

Bronchoscopy

After informed consent was signed, women of childbearing age underwent urine pregnancy screen. Subjects were pre-medicated with atropine (0.6 mg IM) and either morphine (10 mg IM) or meperidine (12.5-25 mg IM). The airways were numbed using topical lidocaine (2-4%). A flexible bronchoscope (model P160 or P180; Olympus) was introduced trans-nasally or trans-orally into the right main bronchium under standard clinical monitoring.

Vitamin D_3 serum measurement

Samples were sent to the Laboratory at the University of Iowa Department of Pathology. $25(OH)D_3$ was measured by Electrochemiluminescence, Multiplex flow immunoassay, and $1,25(OH)_2D_3$ was measured by quantitative chemiluminescent immunoassay.

Bacterial strains

Staphylococcus aureus strain Xen 29® (Caliper LifeSciences Bioware™, MA, USA) was chosen because it is a human isolate strain that possesses a copy of the modified Photorhabdus luminescens luxABCDE operon at a single integration site on the bacterial chromosome. This feature allowed us to measure a thermostable real time luminescent signal in Relative Light Units (RLU). Xen 29® was grown from a bacterial glycerol stock on a tryptic soy agar plate containing kanamycin (0.2 mg/mL) overnight. On the next day, four to five colonies were grown in Tryptic Soy Broth medium (TSB) (BD Difco, New Jersey, USA) overnight. Thereafter, a subculture of log phase bacteria

was centrifuged (1 min, 25 °C, 16 RCF) and washed twice with Phosphate Buffered Saline with no calcium or magnesium (PBS -/-) (Gibco® Life Technologies, Massachusetts, USA). The final bacterial pellet was suspended in minimal media consisting of NaPO$_4$buffer supplemented with 100 mM NaCl and 1% TSB to sustain bacterial luminescence as previously described E[1].

ASL antimicrobial activity

We used a bioluminescent bacterial assay using *Staphylococcus aureus* Xen 29 (Caliper Biosciences). This assay allowed us to determine bacterial viability in a high throughput fashion and previous studies have determined a good correlation between RLU and Colony Forming Units (CFU) E[1]. Corrected samples (10 µl) were combined with equal volume of bacteria Xen 29 (~5x10^6 CFU) suspended in 10 mM sodium phosphate buffer pH 7.4, 100 mM NaCl, 1 % TSB using the precision pump of the luminometer. We measured RLU after 4 minutes of bacterial challenge.

LL-37 antimicrobial assay

Assays were performed as previously described E[1]. LL-37 (Anaspec, Fremont, CA, USA) was diluted with the peptide diluent recommended by the manufacturer. We coincubated 10 µL of LL-37 (50 µg/mL final concentration) with 10 µL of goat anti LL-37 Ab (Santa Cruz) or goat IgG anti mouse IgG (Santa Cruz) at two concentrations (5.5 and 2.75 nM). We sealed the plate with a rubber lid to avoid evaporation, and the plate was kept at 37 ºC for one hour with constant shaking (200 rpm). We then challenged the

samples with 20 µl of bacteria (~5x10^6 CFU) in an opaque white 96 well plate. RLU

were registered at 4 min and percentage of live bacteria was calculated.

LL-37 neutralization in the ASL

To neutralize LL-37 in the ASL, we coincubated ASL (10 µL) with goat LL-37 Ab (10 µL)

at two concentrations (7, 70 nM) or goat IgG anti-mouse IgG control (70 nM). We sealed

the plate with a rubber lid to avoid evaporation, and the plate was kept at 37 °C for one

hour with constant shaking (200 rpm). Thereafter, we challenged the samples with

bacteria (5x10^6 CFU) in an opaque white 96-well plate. RLU were registered at 4 min

and percentage of live bacteria was calculated

Macrophage RNA microarray analysis

RNA preparation, quality analysis, and microarray analysis were done as previously

described E[2]. Genome-wide macrophage mRNA expression measurements were

conducted using the GeneChip Human Exon 1.0 ST Arrays (Affymetrix). We analyzed

microarray data with Partek Genomics Suite, version 6.5, software (Partek, St. Louis,

MO, USA). We analyzed the quality of the data and subjected to robust multi-array

averaging normalization. We used paired *t*-test to compare the effect of the intervention.

A *p* of 10^{-5} was considered significant in this analysis.

Cigarette Smoke Extract (CSE) production

CSE was made as previously described with modifications E[3]. We used a 500 µl pipette

tip connected to a lit University of Kentucky Research Cigarette (Code 3R4F) in one end

and to a 50 mL syringe filled with 12.5 mL of MEM on the other end. The cigarette

smoke was inhaled using the syringe and the MEM was mixed with the smoke by

agitation. The resulting liquid was filtered through a 0.2 µm filter (Millipore). To ensure

standardization between experiments, the absorbance was measured at 320 nm on a

spectrophotometer and an optical density of 1 was defined as 100%.

PCR gene expression analysis

Airway samples were stored at -80°C in RNAlater® (Invitrogen) until we isolated RNA

using the mirVana™ miRNA Isolation Kit (Invitrogen) following manufacturer's

instructions. cDNA was prepared by using High Capacity cDNA Reverse

Transcriptase™ kit (Applied Biosystems). A concentration of 0.0175 µg/µL of RNA in a

20 µL reaction was used to create the cDNA template. qPCR reactions were carried

using Fast SYBR® Green Master Mix (Applied Biosystems) with primers (Integrated

DNA Technologies) already tested in a previous publication (See supplemental Table 1)

E[4]. The endogenous control was HPRT and the relative expression levels were

determined using the $Fold_2$ Change based on the $-\Delta\Delta Ct$ method (Applied Biosystems).

The PCR reactions were carried out in the QuantaStudio 6 Flex following the

manufacturer's instructions (Applied Biosystems).

SUPPLEMENTAL TABLE

Gene	Name	Forward	Reverse
CAMP	Cathelicidin Antimicrobial Peptide	ACACAGCAGTCACCAGAGCATTGT	ATTTCTCAGAGCCCAGAAGCCTGA
CYP27B1	1alpha-hydroxylase	AACCCTGAACAACGTAGTCTGCGA	ATGGTCAACAGCGTGGACACAAA
HPRT	housekeeping gene	GCAGACTTTGCTTTCCTTGG	AAGCAGATGGCCACAGAACT

Table 1. List of primers. From Hansdottir S et al. J Immunol 2008; 181: 7090-7099.

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