The Pseudomonas aeruginosa protease LasB directly activates IL-1β.
The *Pseudomonas aeruginosa* protease LasB directly activates IL-1β

Josh Sun, Doris L. LaRock, Elaine A. Skowronski, Jacqueline M. Kimney, Joshua Olson, Zhenze Jiang, Anthony J. O’Donoghue, Victor Nizer, Christopher N. LaRock

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**A B S T R A C T**

Background: Pulmonary damage by *Pseudomonas aeruginosa* during cystic fibrosis lung infection and ventilator-associated pneumonia is mediated both by pathogen virulence factors and host inflammation. Impaired immune function due to tissue damage and inflammation, coupled with pathogen multidrug resistance, complicates the management of these deep-seated infections. Pathological inflammation during infection is driven by interleukin-1β (IL-1β), but the molecular processes involved are not fully understood.

Methods: We examined IL-1β activation in a pulmonary model infection of *Pseudomonas aeruginosa* and in vitro using genetics, specific inhibitors, recombinant proteins, and targeted reporters of protease activity and IL-1β bioactivity.

Findings: Caspase-family inflammasome proteases can directly regulate maturation of this proinflammatory cytokine, but we report that plasticity in IL-1β proteolytic activation allows for its direct maturation by the pseudomonal protease LasB. LasB promotes IL-1β activation, neutrophilic inflammation, and destruction of lung architecture characteristic of severe *P. aeruginosa* pulmonary infection.

Interpretation: Preservation of lung function and effective immune clearance may be enhanced by selectively controlling inflammation. Discovery of this IL-1β regulatory mechanism provides a distinct target for anti-inflammatory therapeutics, such as matrix metalloprotease inhibitors that inhibit LasB and limit inflammation and pathology during *P. aeruginosa* pulmonary infections.

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1. Introduction

*Pseudomonas aeruginosa* is a prominent cause of severe opportunistic pulmonary infections associated with mechanical ventilation and the genetic disease cystic fibrosis (CF). *P. aeruginosa* infection is often refractory to antibiotic therapy due to multidrug resistance, making it a World Health Organization and U.S. Centers for Disease Control priority pathogen for therapeutic development. *P. aeruginosa* infection destroys lung architecture and function due to inflammatory- and neutrophil-mediated degradation of mucus layers and structural proteins of the pulmonary connective tissue [1, 2]. Cytokines such as IL-1β [3, 4] and IL-8 [5], the latter itself regulated by IL-1β [6], initiate and maintain this neutrophil-dependant inflammatory cycle in contrast to their normally host-protective roles [7-9]. Anti-inflammatory agents can mitigate tissue destruction to preserve pulmonary function during *P. aeruginosa* pneumonia [10] and CF [11,12].

Newly synthesized IL-1β (pro-IL-1β) is inactive and requires proteolytic processing into a mature active form. Canoniclly, this is carried out by the inflammasome, a macromolecular complex of intracellular pattern recognition receptors and the proteases caspase-1 or caspase-11 [13]. During infection, inflammasomes are formed upon detection of pathogen-associated molecular patterns (PAMPs), including many present in *P. aeruginosa* such as flagellin (FlIc), the type III secretion basal body rod (Pscl), the type IV pilin (PilA), RhsT, exolysin (ExlA), exotoxin A (ExoA), cyclic 3′–5′ diguanylate (c-di-GMP), and lipopolysaccharide (LPS), which are variingly detected by NLRC4, NLRP3, or caspase-11 [7,14-22]. Some pathogens limit inflammation by targeting the inflammasome [23], and *P. aeruginosa* dampens inflammasome activation via the effector ExoU [21].
Research in Context

Evidence before this study

Inflammation is highly damaging during lung infections by the opportunistic pathogen *Pseudomonas aeruginosa*. The proinflammatory cytokine IL-1β is a pivotal contributor, but the mechanisms of its maturation are unclear.

Added value of this study

*Pseudomonas* encodes numerous factors that can potentially redundantly activate the inflammasome, the canonical regulator of IL-1β. Yet the inflammasome is not required for IL-1β activation during *Pseudomonas* infection, leaving the mechanism of activation unclear. This work demonstrates that the *Pseudomonas* LasB protease directly cleaves IL-1β in a manner that activates its activity independently from the inflammasome, leading to pathological inflammation.

Implications of all the available evidence

Our findings show that inhibition of IL-1β conversion by LasB protects against neutrophilic inflammation and destruction of the lung. Adjunctive therapeutics that limit pathological inflammation induced by infection would be beneficial for treating pulmonary infections when used in combination with conventional antibiotics.

Despite the multitude of inflammasome-activating signals that *P. aeruginosa* express, caspsases, NLRP3, and NLRC4 are not essential for pro-IL-1β maturation in macrophages, epithelial cells, or neutrophils infected with *P. aeruginosa* [24,25]. Correspondingly, *P. aeruginosa*-infected caspase-1−/− and caspase-11/−/− mice succumb to a destructive neutrophilic pulmonary inflammation against which IL-1 receptor (IL-1R1)−/− mice are protected [26]. This is at least partially mediated by IL-1α for strains that express ExoU, a minority of clinical isolates that are nonetheless associated with more severe disease; for strains lacking ExoU this is exclusively mediated by IL-1β and not IL-1α [26]. These observations highlight the contribution of IL-1β to *P. aeruginosa* infection but suggest there are mechanisms for its maturation other than the inflammasome.

The pathological cascade of protease dysregulation and activation seen during severe *P. aeruginosa* lung infections provide a possibility for IL-1β maturation by alternative mechanisms. Caspase-8 [27–29], and the neutrophil granule proteases elastase (NE) and proteinase 3 (PR3) [34,30], cleave pro-IL-1β, but this does not always result in maturation to active cytokine [31]. Bronchial secretions, however, also possess abundant protease activity from microbial sources [2]. Here we find that IL-1β is not exclusively matured by host proteases, and that *P. aeruginosa* protease LasB also drives this inflammatory pathway. Targeting this bacterial protease may, therefore, provide supportive therapy to limit inflammatory pathology in pulmonary infection.

2. Materials and methods

Bacterial strains and plasmids. All bacterial strains, plasmids, and primers used in this study are listed in Table 1. lasB and the upstream 260 bp regulatory region in PA01 were cloned into pUC18T-mini-Tn7-hph [32] using Polymerase Incomplete Primer Extension (PIPE) cloning [33] with primers lasB-F, lasB-R, Tn7-F, and Tn7-R. Transformants into Top10 cells were selected on LB agar plates containing 100 μg/mL Hygromycin B (Life Technologies). Stable complementation into PA01 ΔlasB was performed as previously described [32], and transformants selected with 400 μg/mL Hygromycin B. pET-LasB with a C-terminal His-tag was constructed by sequential PIPE cloning with the primers LasB-A, LasB-B, LasB-C, and LasB-D, and proteins were expressed and purified by conventional methods as previously described [34]. pET-pro-IL-1β and the purification of pro-IL-1β have been previously described [34]. pro-IL-1β cleavage experiments were carried out in PBS, 1 mM CaCl₂, 0.01% Tween-20, and examined by SDS-PAGE or IL-1R reporter cells, as detailed [34]. Constructs for the expression of IL-1β mutants were generated by PIPE cloning from pET-pro-IL-1β [34] with the corresponding primers sets in Table 1, and proteins were expressed and purified in the same manner as for pro-IL-1β previously [34]. Bacteria were routinely propagated in Luria broth (LB) medium at 37 °C. For infections, bacterial cultures were grown to late exponential phase (OD₆₀₀ 1.2) then washed and diluted in PBS.

Animal Experiments. Eight-to-ten week old male or female C57Bl/6 and isogenic caspase-1−/− mice (006644 and 016621, Jackson labs) were assigned to experimental groups, each using 15 mice broken into three independent experiments containing five mice each; none were excluded from analysis. Sample size is based on power function of effect size established in our previous studies [34,35]. The effect size (difference in means) and variance in data was expected to be consistent with this prior work, since similar biological processes were being examined. The mouse and bacterial genotypes, infectious doses, and time points was consistent to not confound these comparisons. Microsoft Excel Rand() function was used for all randomization. Mice were anesthetized with ketamine/xylazine intraperitoneally, then 10⁷ CFU PAO1 inoculated intratracheally in 30 μl of 1x PBS, 25 μg/kg lonomastat, and 25 μg/kg Marimastat (2983 and 2631; Tocris). Mice were euthanized 24 h post-infection by CO₂ asphyxiation, and bronchial lavage fluid or lung homogenate were weighed for normalization and dilution plated onto LB agar plates for CFU enumeration, or quantification of cytokines or proteolysis. Bronchial lavage fluid cells were counted on a hemocytometer with cytologic examination on cytospin preparations fixed and stained using Hema 3 (Fisher HealthCare™). Histologic sections were prepared from formalin-fixed and paraffin-embedded lungs, stained with hematoxylin and eosin (H&E). Cytospin and histology slides were imaged on a Hamamatsu Nanozoomer 2.0HT Slide Scanner.

In vitro infection models. Macrophages were generated from femur exudates of wild-type C57Bl/6 or caspase-1/11−/− mice previously [34]. THP-1, HL60, and A549 cells (ATCC TIB-202, CCL-240, and CCL-185) were acquired from ATCC, which regularly authenticate lines by STR profiling. Cells were propagated by standard protocols, and regularly checked for morphology and characteristics typical of their cell type; THP-1 were differentiated 72 h with 200 nM Anakinra (Kineret; Amgen), 100 ng/mL FMK004, FMK007; R&D Systems), 10 μM caspase inhibitors zVAD-fmk, zVAD-fmk, DEVD-fmk, and IETD-fmk (FMK001, FMK005, FMK004, FMK007; R&D Systems), 10 μg/mL complete protease inhibitor cocktail (11697498001; Roche), 1x protease inhibitors AEBSF, Antipain, Aprotinin, Bestatin, EDTA, E-64, Phosphoramidon, Pepstatin, and PMSF (786–207; G-Biosciences). Except when noted otherwise, cells were routinely infected by co-incubation with *P. aeruginosa* at a multiplicity of infection of 10, spun into contact for 3 min at 300 g, and cells or supernatants were harvested for analysis after 2 h.

Cytokine measurements. Relative IL-1 signalling by cells was measured in 50 μl of supernatant from infected or treated cells, then incubated with 1 μM okadaic acid 30 min before transfer onto...
transgenic IL-1R reporter cells (hkb-il1r; Invivogen). Each experiment is normalized to by subtracting background from media only, then making all comparisons relative to wild-type PAO1-infected wildtype cells. Controls of recombinant IL-1α and IL-1β (200-LA, 201-LB; R&D Systems), and neutralising antibodies for each (A15032A, Biolegend and AF-200-NA, R&D Systems) were used following manufacturer protocol (hkb-il1r; Invivogen) to confirm reporter sensitivity, and protein and antibody activity. No cross-reactivity between cytokines and neutralising antibodies was observed. After 18 h, reporter cell supernatants were analysed for secreted alkaline phosphatase activity using HEK-Blue Detection reagent (Invivogen) as previously [35]. Cytokines were quantified by enzyme-linked immunosorbent assay following the manufacturer’s protocol (DY400, DY401, DY410, DY453 DY406; R&D Systems). Expression was examined in cells lysed with RIPA (Millipore). RNA was isolated (Qiagen), cDNA synthesized with SuperScript III and Oligo(dT)20 primers (Invitrogen), and qPCR performed with KAPA SYBR Fast (Kapa Biosystems) with primers for \( \text{il1a} \) and \( \text{il1b} \) and relative expression normalised to \( \text{gapdh} \) and compared by \( \Delta \Delta C_t \) as previously [37].

In vitro transcription/translation was performed with the corresponding primers in Table 1 using pET-

| Table 1 | Bacterial strains, plasmids, and primers used in this study. |
|-----------------|----------------------------------------------------------|
| **Strains** | **Relevant feature(s) or sequence** | **Reference or Source** |
| *P. aeruginosa* | | |
| PAO1 | WT reference strain (NC_002516.2) | [58] |
| PAO1 lasB<sup>+</sup> | lasB transposon insert | [58] |
| PAO1 flic<sup>+</sup> | flic transposon insert | [58] |
| PAO1 lasA<sup>+</sup> | lasA transposon insert | [58] |
| PAO1 psv<sup>+</sup> | psv transposon insert | [58] |
| PAO1 lasB<sup>+</sup>: lasB transposon inserted into mTn7T<sup>+</sup> | This study |
| MDR-P4 | WT strain | G. Sakoulas |
| PA103 | WT strain, ATCC 29260 | ATCC |
| 23712 | WT strain, ATCC 27312 | ATCC |
| 27864 | WT strain, ATCC 27864 | ATCC |
| 10145 | WT strain, ATCC 10145 | ATCC |
| CRN697 | WT strain | G. Sakoulas |
| Hanity | WT strain | G. Sakoulas |

| **Plasmid** | **Relevant feature(s) or sequence** | **Reference or Source** |
| pET-proIL-1β | Vector for expression of recombinant human pro-IL-1β | [34] |
| pET-LasB | Vector for expression of recombinant LasB | This study |
| pUC18T-mTn7T | LasB insertion in mini-Tn7T for complementation | This study |

| **Oligonucleotides** | **Relevant feature(s) or sequence** | **Reference or Source** |
| lasB-F | | |
| lasB-R | | |
| pET-LasB-A | | |
| pET-LasB-B | | |
| pET-LasB-C | | |
| pET-LasB-D | | |
| lasB CT His-1 | | |
| lasB CT His-2 | | |
| Tn7-F | | |
| Tn7-R | | |
| il1b<sup>-</sup>-F | | |
| il1b<sup>-</sup>-R | | |
| gapdh<sup>-</sup>-F | | |
| gapdh<sup>-</sup>-R | | |
| IVTIL1b-term | | |
| IVTIL1b-1 | | |
| IVTIL1b-12 | | |
| IVTIL1b-24 | | |
| IVTIL1b-36 | | |
| IVTIL1b-48 | | |
| IVTIL1b-60 | | |
| IVTIL1b-72 | | |
| IVTIL1b-84 | | |
| IVTIL1b-87 | | |
| IVTIL1b-90 | | |
| IVTIL1b-93 | | |
| IVTIL1b-96 | | |
| IVTIL1b-99 | | |
| IVTIL1b-102 | | |
| IVTIL1b-105 | | |
| IVTIL1b-108 | | |
| IVTIL1b-111 | | |
| IVTIL1b-114 | | |
| IVTIL1b-117 | | |
| IVTIL1b-120 | | |
| IVTIL1b-122 | | |
| IVTIL1b-123 | | |
| IVTIL1b-126 | | |
pro-IL-1β as a template and following the manufacturer’s recommenda-
tions in 10 µl reaction volumes (TNT Coupled Reticulocyte Lysate; Promega). Loading for IL-1R reporter assays was normalised by total
IL-1β product measured by enzyme-linked immunosorbent assay as
above.

**Substrate specificity profiling.** 10 nM LasB was incubated in trip-
llicate with a mixture of 228 synthetic tetradecapeptides (0.5 µM each) in PBS, 2 mM DTT as described previously [38]. After 15, 60, 240 and 1200 min, aliquots were removed, quenched with 6.4 M GuHCl, immediately frozen at −80 °C. Controls were performed with LasB treated with GuHCl prior to peptide exposure. Samples were
acidified to pH<3.0 with 1% formic acid, desalted with C18 LTS tips
(Rainin), and injected into a Q-Exactive Mass Spectrometer (Thermo)
equipped with an Ultimate 3000 HPLC. Peptides separated by reverse
phase chromatography on a C18 column (1.7 µm; 50 cm, 1.0 mm ID, 240 and 1200 min, aliquots were removed, quenched with 6.4 M GuHCl and the cleavage products desalted and analysed by mass spectrometry as described above, except using a 20-min linear gradient from 5% to 50% B and only selecting top 5 peptides for MS/MS. For measurements of protease activity from *in vitro* infections, samples normalised by lung weight were homoge-
nised in PBS 0.01% Tween-20, passed through 0.2 µm-filter, and dilu-
tions incubated with each peptide at 5 µM and the maximum kinetic
velocity calculated as previously [34].

**Ethics.** All animal use was approved by the Institutional Animal Care and Use Committees (IACUC) of UCSD or Emory University fol-
lowing Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and the Office for Laboratory Animal Welfare (OLAW) guidelines.

**Statistics.** Graphical results and statistical tests were performed with GraphPad Prism 8 using 1-way or 2-way t-test or ANOVA as
appropriate. No blinding or exclusion criteria were applied. Tukey post-tests were used to correct for multiple comparisons. Statistical
significance is indicated as (*, P < 0.05; **, P < 0.005; ***, P < 0.0005). All error bars show the mean and the standard deviation (s.d.). Data are representative of at least three independent experiments.

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script nor in the decision to publish. The authors have not been paid to write this article by any agency.

### 3. Results

#### 3.1. IL-1 signalling drives neutrophilic inflammation during *P. aeruginosa* lung infection

Inflammation drives poor clinical outcomes during *P. aeruginosa* lung infection [39]. C57Bl/6 mice infected intratracheally with *P. aerugi-
 nosa* had markedly disrupted airway architecture within 24 h, concurrent with neutrophil infiltration into the lung tissue and bron-
choalveolar lavage fluid (BAL) (Fig. 1a). We examined the contribu-
tion of pro-inflammatory cytokines to this process using the FDA-
approved IL-1 receptor (IL-1R1) antagonist anakinra, which directly
inhibits both IL-1β and IL-1α, but not other critical proinflammatory
cytokines such as KC/CXCL1. IL-6, or TNFα (Fig. 1b). As observed
during human infections, *P. aeruginosa* persisted in the BAL (Fig. 1c) and lung tissue (Fig. 1d) despite significant neutrophil infiltration that
was partially IL-1-dependent (Fig. 1e).

IL-1β is typically released by secretion or cell lysis and requires additional maturation, activities which are all mediated by the inflamma-
some proteases caspases –1 or –11 [13]. CFU and release of IL-1α was unaltered in *P. aeruginosa*-infected caspase-1/11−/− C57Bl/6 mice, but surprisingly, IL-1β release was also only modestly attenu-
ated (Fig. 1f). This pool of extracellular IL-1β has the potential to mediate proinflammatory signalling as an IL-1R1 agonist when the
inhibitory pro-domain has been removed. Neutrophil granular pro-
tases may provide such activation [3,4,18,24], however, since neu-

trophil recruitment is itself IL-1-dependent (Fig. 1a, 1e), and these
neutrophils themselves may later inactivate IL-1β [31], we reasoned that additional proteases initiate the process.

#### 3.2. *P. aeruginosa* induces IL-1β maturation independent of the inflamma-
some

To more specifically measure only IL-1β that is active, we made use of transgenic reporter cells expressing luciferase under the con-
trast, an ionophore that activates the NLRP3 inflammasome, nigeri-
cin, was completely dependent on caspases for the activation of IL-1
signalling. Monoclonal antibodies specific to IL-1R1 or IL-1β, but not IL-1α, inhibited IL-1 signal from caspase-1/11-/- BMM (Fig. 2c). This also suggested no significant contribution from IL-1α in IL-1R1 signalling. The absolute quantity of each cytokine measured by enzyme-linked immunosorbent assay (pro- and mature- forms) remained unchanged (Fig. 2c). Furthermore, _P. aeruginosa_ infection of human cell lines relevant to lung infection (macrophages, THP-1; neutrophils, HL60; type II alveolar epithelial cells, A549) still stimulated IL-1 signalling in the presence of the caspase-1/11-specific inhibitor YVAD-cmk (Fig. 2d). Together, these results indicate that _P. aeruginosa_ stimulates IL-1 signalling through a pool of extracellular IL-1β that is active and matured independently of caspase-1/11.

### 3.3. IL-1β is activated by the _P. aeruginosa_ LasB protease

Proteases contributing to IL-1β activation were evaluated using small molecule inhibitors specific to each protease class. Inhibition of metalloproteases, and not cysteine proteases (e.g. caspases-1, 11, and 8) or serine proteases (e.g. NE and PR3), abrogated IL-1β signalling in _P. aeruginosa_-infected caspase-1/11-/- BMM (Fig. 3a). _P. aeruginosa_ encodes several secreted metalloproteases, and by examining mutants of each (ΔlasA, ΔlasB, ΔaprA), we found LasB to be the most active protease overall as measured by hydrolysis of casein during agar plate growth (Fig. 3b), and was the major contributor to caspase-1/11-independent IL-1β signalling (Fig. 3c). Complementation with the LasB coding sequence under its native promoter restored the ability of _P. aeruginosa_ to induce IL-1β signalling in infected caspase-1/11-/- BMM (Fig. 3d). Furthermore, activation was independent of _il1a_ or _il1b_ expression (Fig. 3e) or IL-1α or IL-1β secretion (Fig. 3f). These data show that LasB induces IL-1 signalling independently of caspase-1/11.

### 3.4. LasB-activated IL-1β is active

Incubation with recombinant LasB was sufficient to convert recombinant human pro-IL-1β into an active form (Fig. 4a). Further examination of pro-IL-1β cleavage by LasB, again using recombinant forms of each protein, showed several intermediate cleavage products which accumulate as a stable product that is degraded no further (Fig. 4b), similar to what occurs upon IL-1β maturation by caspase-1 [40]. Analysis of these fragments by Edman sequencing identified cleavage sites that were all in the N-terminus of pro-IL-1β. Examination of N-terminal truncated IL-1β by _in vitro_ transcription/translation showed a defined region flanking the caspase-1 cleavage site (N-term fragment 117) is sufficient to generate active cytokine (Fig. 4c).

We determined the substrate specificity profile for LasB using a mass spectrometry-based substrate profiling assay previously validated with other microbial proteases [41,42], which showed a distinct...
pathological in

3.5. Metalloprotease inhibitors of LasB prevent IL-1-mediated pathological inflammation

Since IL-1β inhibition protects against lung damage (Fig. 1a, 1b), and because LasB drives IL-1β maturation (Fig. 3c, 4d), we examined whether protease inhibitors active against LasB limit lung injury. Two investigational hydroxamate-based anti-neoplastic metalloprotease inhibitors, marimastat and ilomastat, inhibited LasB cleavage of the IL-1β-derived substrate (Fig. 5a) and P. aeruginosa activation of IL-1β (Fig. 5b) at sub-antimicrobial concentrations (Fig. 5c). During murine pulmonary infection, marimastat and ilomastat each showed therapeutic effects to reduce P. aeruginosa growth (Fig. 5d), neutrophil recruitment (Fig. 5e), IL-1β (Fig. 5f), pulmonary pathology (Fig. 5g), and invasion (Fig. 5h). Together this data suggests that inhibiting LasB, but also including other metalloproteases of the lung such as matrix metalloproteases, can reduce inflammation during infections by P. aeruginosa.

4. Discussion

Opportunistic P. aeruginosa lung infections can destroy tissue structure and impair organ function. Our findings reveal a mechanism by which a bacterial protease, LasB, contributes to pathological inflammation by directly activating IL-1β. LasB is one of the most abundant virulence factors in the lung microenvironment during P. aeruginosa infection and can cleave numerous host factors [43], even exerting broadly anti-inflammatory influences through destructive proteolysis of PAMPs such as flagellin [44], and various cytokines and immune effectors including IFN, IL-6, IL-8, MCP-1, TNF, trappin-2 and RANTES[45-48]. Consequently, LasB-deficient bacteria may preferentially induce a KC, IL-6, and IL-8 dominant inflammatory responses [45], whereas previous reports and our findings show that wild-type LasB-expressing P. aeruginosa induce a strong IL-1β response [49].
Fig. 3. IL-1β is activated by the P. aeruginosa LasB protease. (a) Relative IL-1 signalling by caspase-1/11Δ−/− BMM 2 h post-infection by PAO1 that were previously incubated 1 h with the indicated protease inhibitors classes (Aspartyl, Pepstatin; Cysteine, E64; Metallo, Phosphoramidon; Serine, PMSF; Tryptic, Benzamidine). (b) Visualisation of bacterial proteolytic activity by decreased media opacity on LB agarose plates containing casein. (c) Relative IL-1 signalling by caspase-1/11Δ−/− BMM 2 h post-infection with isogenic mutant strains of PAO1. (d) Relative IL-1 signalling by caspase-1/11Δ−/− BMM 2 h post-infection by PAO1, ΔlasB, or plasmid-complemented ΔlasB. (e) il1a and il1b expression by real-time quantitative PCR and (f) secretion by ELISA. Error bars show mean ± s.d, n = 4, and represent at least 3 independent experiments. Statistical analysis by ANOVA with Tukey post-test, *P < 0.05, **P < 0.005, ***P < 0.0005.

Fig. 4. LasB-activated IL-1β is active. (a) IL-1 signalling activity by 100 ng human pro-IL-1β after 2 h incubation with titrations of recombinant LasB. (b) SDS-PAGE analysis of the kinetics of cleavage and maturation of recombinant human pro-IL-1β (1 μg) by recombinant LasB (50 ng). (c) Signalling activity of recombinant IL-1β N-terminal truncations generated using in vitro transcription/translation from the human il1b gene with coding beginning at the indicated codon, 1 is full-length pro-IL-1β, 117 corresponds to the fragment generated by caspase-1 cleavage. (d) Cleavage of internally-quenched fluorescent IL-1β peptide fragments (amino acids 103−123 of human IL-1β) by recombinant LasB or caspase-1. (e) IceLogo frequency plot showing amino acids significantly enriched (above X-axis) and de-enriched (below X-axis) in the P2 to P2′ positions following incubation of LasB with a mixture of 228 tetradecapeptides. Cleavage occurs between P1 and P1′. Lowercase “n” is norleucine. (f) Cleavage of internally-quenched fluorescent IL-1β peptide fragments by proteases within BAL collected from C57Bl/6 or casp-1/11Δ−/− mice 24 h post-intratracheal infection with 107 CFU of PAO1 or ΔlasB. Error bars show mean ± s.d, n = 4 (a-e), n = 5 (f), and represent at least 3 independent experiments. Statistical analysis by ANOVA with Tukey post-test, *P < 0.05, **P < 0.005, ***P < 0.0005.
LasB activates IL-1β through direct proteolytic removal of its inhibitory amino-terminal pro-domain, bypassing the necessity for host caspases. The LasB and caspase-1 mechanisms for generating mature IL-1β are distinguishable by substrate specificity (a hydrophobic P1' vs aspartic acid P1 site), enzyme class (metalloprotease vs cysteine protease), and cellular source (microbial vs host). LasB activation of pro-IL-1β in both the intra- and extracellular milieu is entirely feasible, given the abundance of intracellular proteins released by pyroptosis and necrosis during infections[13,50] and the abundance of LasB[51]. We recently hypothesized that IL-1β evolved...
as a sensor of diverse proteases [34], a model further supported by the present discovery of a P. aeruginosa protease with this activity.

In lung infection, LasB activation of IL-1β augments neutrophil recruitment and promotes destruction of the pulmonary tissue. IL-1β inhibition protects against this pathology, however, clinical interventions to date have used expensive biologics (e.g. IL-1R1 antagonists) associated with increased risk for severe infections [34,52].

The proteolytic activation of IL-1β may be a more tractable pharmacological target, made possible by disambiguation of the molecular networks involved and, perhaps amenable to the repurposing existing protease inhibitors. Alpha-1-antitrypsin suppresses NE-mediated degradation of the CF lung [53,54], potentially also limiting pro-LasB maturation by NE [30]. This strategy may also act against pro-IL-1β maturation by LasB, which is also inhibited by alpha-1-antitrypsin [55]. Indeed, while inhibiting IL-1 signalling with Anakinra limited inflammation and pathology, mutation or inhibition of LasB also limited bacterial replication, consistent with this protease having other contributions to pathogenesis.

Our results indicate metalloprotease inhibitors such as this protease and α1-antitrypsin may also be beneficial in treating P. aeruginosa pulmonary infections through the inhibition of LasB. These drugs were developed to inhibit matrix metalloproteases, which may separately contribute to inflammation and pathology during P. aeruginosa pulmonary infections and CF [56]. While there may be therapeutic benefit to cross-inhibition of multiple metalloprotease targets, this can give pro- and anti-inflammatory depending on the cell target and model, and most matrix metalloprotease inhibitors have failed clinical trials due to toxicity and off-target effects [57]. Thus, more targeted inhibitors of LasB or IL-1β may be necessary to avoid these issues.

Declaration of Competing Interest

C.N.L. has a research agreement with Antabio during the conduct of this study examining inhibitors of LasB. The remaining authors declare no competing financial interests.

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Data Sharing

The mass spectrometry dataset is available at ftp://massive.ucsd.edu/MSV000081623. Additional materials are available upon request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2020.102984.

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