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The virulence inhibitor INP1855 impairs pathogenicity of *Pseudomonas aeruginosa* and inflammasome activation *in vitro* and *in vivo*.

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INTRODUCTION

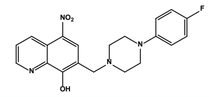
Pseudomonas aeruginosa (P.a) is a major cause of hospitally-acquired and difficult-to-treat infections. With the rise of multidrug-resistant strains and the demise of currently recommended anti-pseudomonal antibiotics, alternative strategies are sorely needed.

A key virulence factor associated with poor clinical outcome and high morbidity in acute infections is the type III secretion system (T3SS).T3SS allows bacteria to inject exotoxins (e.g. ExoU or ExoS) into the host cell cytoplasm, causing cytotoxicity and preventing *P.a.* internalization [1]. In phagocytic cells, T3SS can also deliver flagellin FliC or T3SS rod proteins into the mammalian cytosol, inducing caspase-1 proteolysis via NLRC4 inflammasome activation. Active caspase-1 causes not only cytotoxicity but also the secretion of the IL-1β and IL-18 inflammatory cytokines [2]. These two cytokines repress IL-17 response, thereby impairing *P.a*. clearance *in vivo* [3,4].

OBJECTIVES

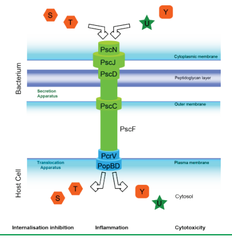
To evaluate the potential interest of the **T3SS inhibitor INP1855**, a small compound identified by high-throughput,

(a) for decreasing *P. aeruginosa* virulence and its deleterious effects on the host cells
(b) for controlling acute pulmonary infection in mice



INP1855
(8-hydroxyquinoline)

STRAINS

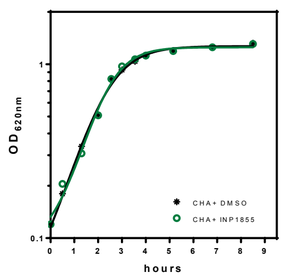


PA103 Wild-type, cytotoxic isolate	**ExoU / ExoT**
CHA Cystic Fibrosis cytotoxic isolate	**ExoS / ExoT**
CHAΔSTY	**NO toxins**
CHAΔpopBD	**NO translocation apparatus**
CHAΔExsA	**NO T3SS**

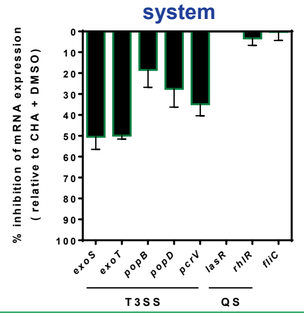
IN VITRO

At the bacterial level

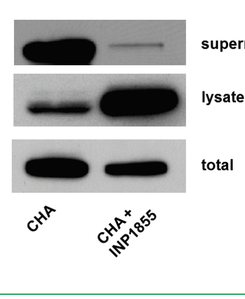
INP1855 is not toxic for bacteria at 200 µM



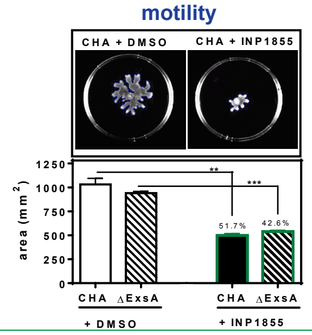
INP1855 ↘ *T3SS* transcription without affecting genes of QS system



INP1855 impairs ExoS secretion



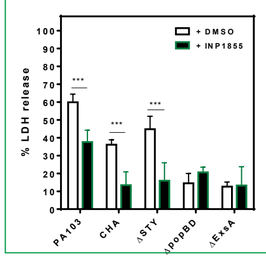
INP1855 ↘ flagellar motility



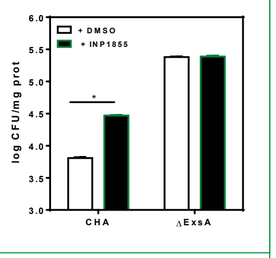
At the host level

INP1855 ↘T3SS-mediated virulence

T3SS- dependent cytotoxicity

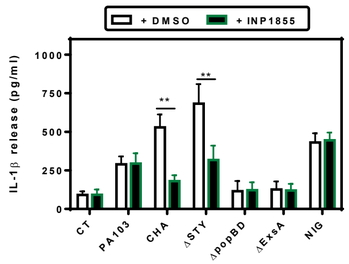


T3SS- dependent phagocytosis inhibition

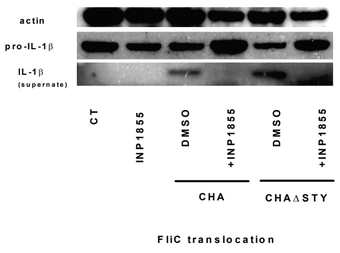


INP1855 ↘T3SS-mediated inflammasome activation

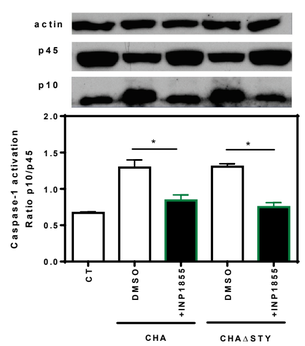
IL-1β secretion



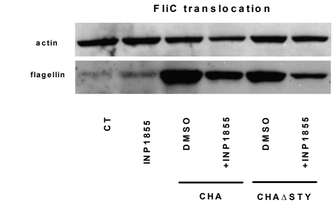
IL-1β and its proform



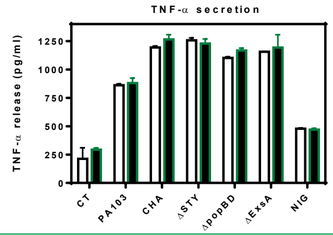
Caspase-1 activation



FliC translocation

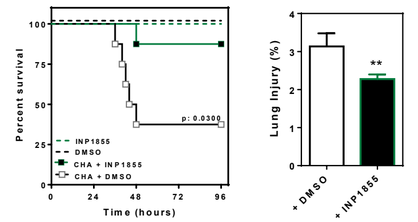


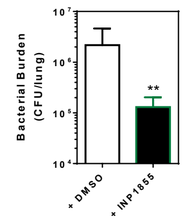
TNF-α secretion

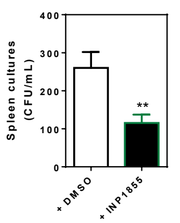


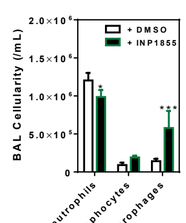
IN VIVO

Acute pulmonary tract infection

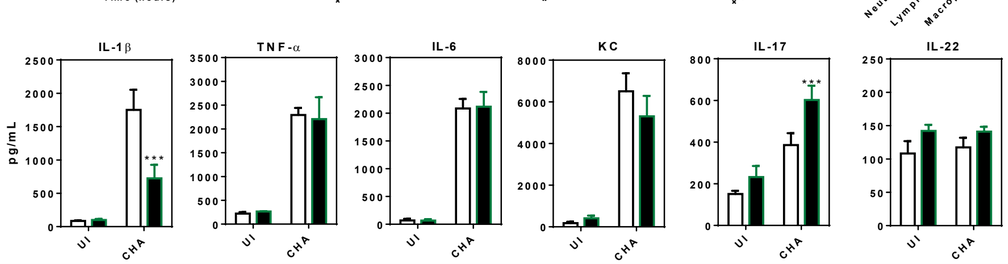






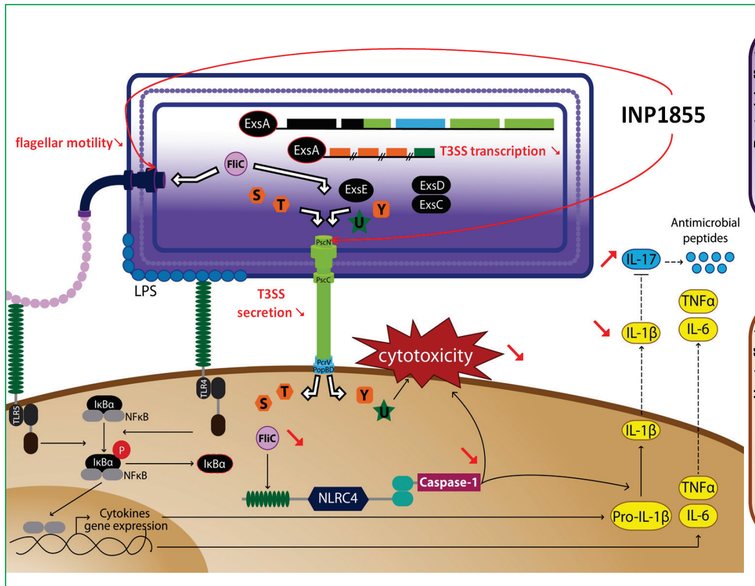


INP1855 ↗ mice survival and bacterial clearance

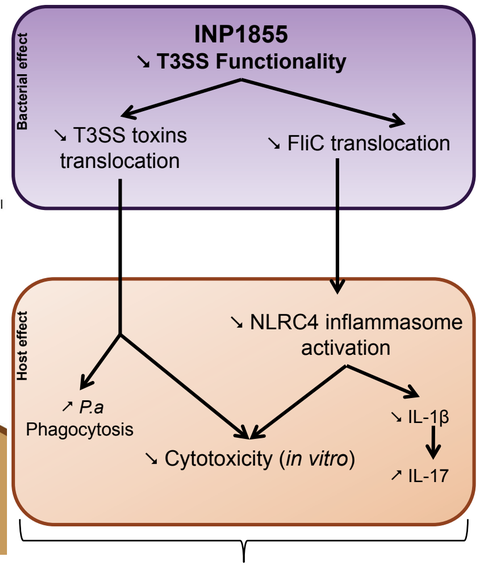


INP1855 ↘ IL-1β and ↗ IL-17 secretion

CONCLUSION



Pictorial view of the model of action of INP1855



↘ Pulmonary lesions and dissemination
↗ Mice survival (*in vivo*)

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[1] Hauser AR *et al* Nat Rev Microbiol 2009;7:654-665
[2] Miao EA and Warren SE, Journal of Clinical Immunology 2010; 30(4): 502-506
[3] Cohen TS and Prince AS, Journal of Clinical Investigation 2013; 23(4):1630-1637
[4] Faure *et al*., Am J Respir Crit Care Med. 2014;189(7):799-811

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