Type Three Secretion System (T3SS)-mediated internalisation and cytotoxicity of *Pseudomonas aeruginosa* by epithelial and phagocytic cells : does inflammasome make the difference?

ID 214

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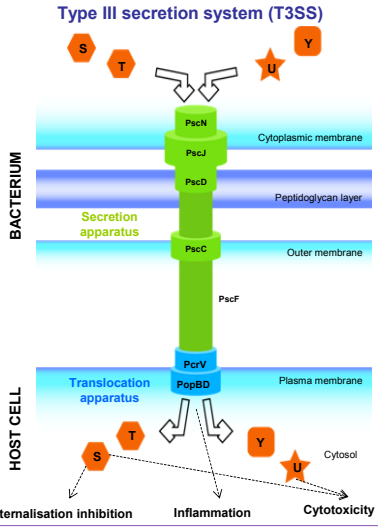
INTRODUCTION and AIMS



T3SS is produced by *P.aeruginosa* (*P.a*) and many other pathogenic Gram-negative bacteria. This system injects effector proteins into the cytosol of host cells. T3SS effectors cause a cell type-dependent cytotoxicity (ExoS and ExoU) [3] and prevent *P.a.* internalization (ExoS, ExoT and ExoY) ([1-2]). T3SS apparatus can also participate to cytotoxicity by delivering flagellin or rod proteins into the mammalian cytosol, which induces caspase-1 proteolysis via NLCR4 inflammasome activation. Active capase-1 induces pyroptosis and release of inflammatory cytokines IL-1β and IL-18 [4]. A role of inflammasome activation in *P.a.* pathogenicity was suggested, related to impairment of bacterial clearance [5].

We ran a systematic study to define the role of T3SS proteins in internalisation, cytotoxicity and inflammasome activation, using epithelial and phagocytic cells, and isogenic strains with specific deletions in T3SS genes.

METHODS

Strains: **CHA** (clinical isolate expressing T3SS), **CHAΔExsA** (deletion of T3SS regulon) **CHAΔS**, **ΔT**, **ΔSTY** (deletion of genes encoding ExoS or ExoT toxins, triple mutant), **CHA ΔpopBD**, **ΔpcrV** (deletion of genes encoding translocation apparatus); **PA103** (cytotoxic strain expressing ExoU phospholipase).

Cells: human alveolar epithelial A549 cells; THP-1 monocytes

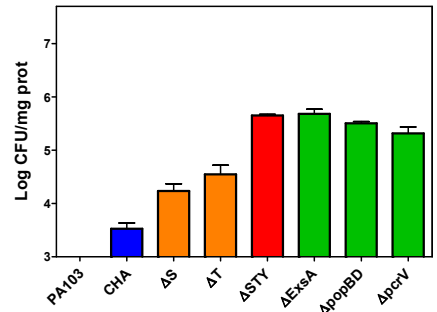
Cell viability: release of the cytosolic enzyme lactate dehydrogenase (LDH) in the culture medium after 7 h (A549 cells) or 5 h (THP-1 monocytes) of incubation *P.a* strains (10 bact./ cell).

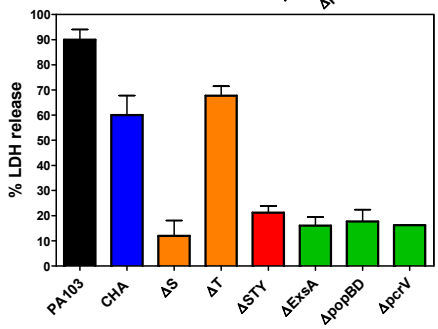
Inflammasome activation: IL-1β release (ELISA); Caspase-1 activation (Western Blot)

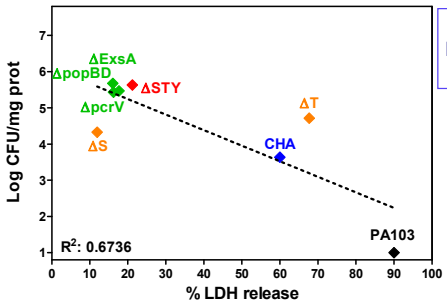
Internalisation assay: bacteria were opsonised with human serum for 1 h and phagocytosis was initiated with 10 bact./ cell for 5 h (A549 cells) or 2 h (THP-1 cells). Non-phagocytised bacteria were eliminated by gentamicin (1 h). Cells were washed and cell-associated CFU were counted.

RESULTS

A549 epithelial cells

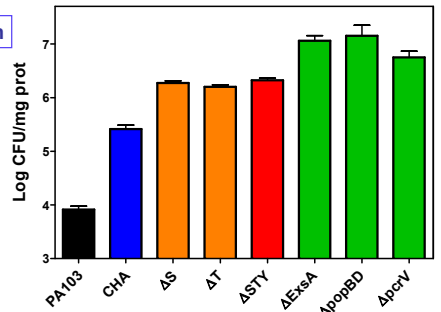


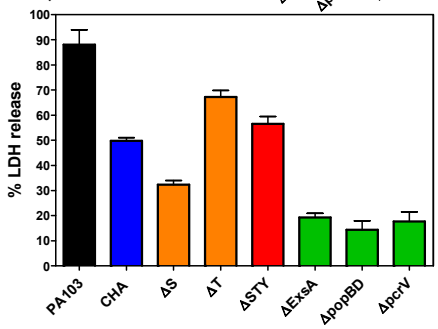


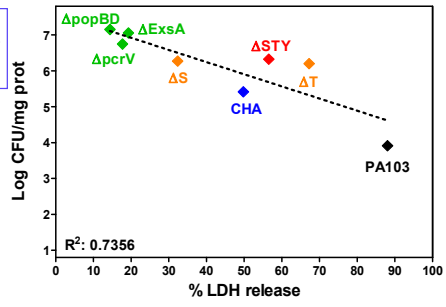


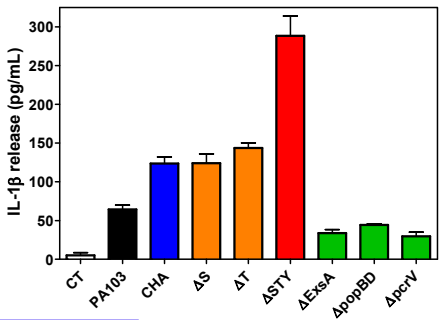
Not detectable

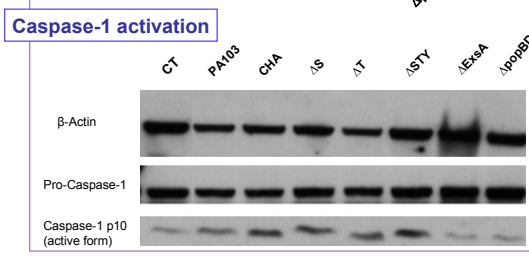
THP-1 monocytes











		PA103	CHA	ΔS	ΔT	ΔSTY	ΔExsA	ΔpopBD	ΔpcrV
A549 epithelial cells	Internalization	-	+/-	+	++	+++	+++	+++	+++
	Cytotoxicity	+++	++	-	++	-	-	-	-
	IL-1β release	-	-	-	-	-	-	-	-
THP-1 monocytes	Internalization	+/-	+	++	++	++	+++	+++	+++
	Cytotoxicity	+++	+	+/-	++	+	-	-	-
	IL-1β release	+	++	++	++	+++	-	-	-

- Internalisation was more efficient in phagocytic cells than in epithelial cells.
- In both cell types, internalisation rate was as follows: [ΔExsA, ΔpopBD, ΔpcrV, ΔSTY] > [ΔT, ΔS] > [CHA] > [PA103].
- Cytotoxicity was inversely correlated to internalisation ([ΔExsA, ΔpopBD, ΔpcrV] < [ΔS] < [ΔT] ≈ [CHA] < [PA103]) and of the same order of magnitude in both cell types, except for **ΔSTY**, which was more cytotoxic for THP-1 cells (56%) than for A549 cells (20%).
- Caspase-1 activation and IL-1β release were induced by CHA, ΔS, ΔT, ΔSTY and to a lower extent PA103, but not by ΔExsA, ΔpopBD, ΔpcrV in THP-1 cells (not detected in A549 cells).

CONCLUSION

In A549 epithelial cells, toxicity is related to toxins injection (especially ExoS) in the cytosol, which also prevents internalisation. In THP-1 phagocytic cells, internalisation remains toxin-dependent while toxicity rather relies on the expression of translocation apparatus. Indeed, ΔSTY (not expressing toxins but still translocation apparatus) remains capable of activating NLCR4 inflammasome, a phagocyte-specific defence mechanism [6], which induces caspase-1 activation and IL-1β maturation. PA103 toxicity remains mainly toxin-dependent, due to rapid membrane-disrupting effects of ExoU.

This poster will be made available for download after the meeting: http://www.facm.ucl.ac.be/posters.htm

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