P0174

Cytotoxicity is correlated with Type Three Secretion System expression and inflammasome activation but not with motility in clinical isolates of *Pseudomonas aeruginosa* from acute infections

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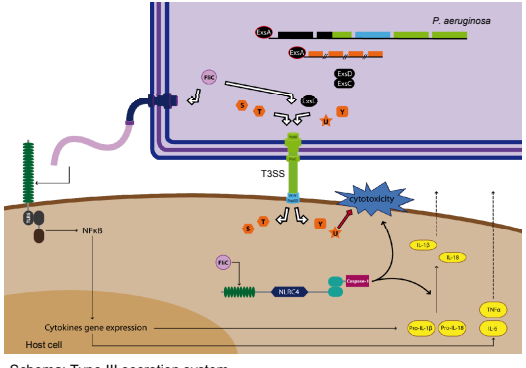
INTRODUCTION

Expression of Type III secretion system (T3SS) in *P.aeruginosa* (*P.a*) is associated with poor clinical outcome and high morbidity in acute infections. T3SS allows bacteria to inject exotoxins (e.g. ExoU or ExoS) into the host cell cytoplasm, causing cytotoxicity and preventing P.a. internalization [1]. T3SS can also deliver flagellin or T3SS rod proteins into the mammalian cytosol, inducing caspase-1 proteolysis via NLCR4 inflammasome activation. Active caspase-1 causes not only cytotoxicity but also the secretion of the IL-1β and IL-18 inflammatory cytokines [2], thereby impairing P.a. clearance [3,4]. Flagellum-mediated motility has also been recently suggested to modulate inflammasome response [5].



Schema: Type III secretion system

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OBJECTIVES

Using strains differing in their expression of T3SS (both laboratory engineered mutants and clinical isolates):

- to compare bacterial motility
- to evaluate their capacity to induce inflammasome activation and cytotoxicity in THP-1 monocytes
- to examine how these parameters are correlated.

MATERIALS & METHODS

Strains: CHA (clinical isolate expressing T3SS) and derivatives thereof (CHAΔSTY [no toxin production]; CHAΔExsA [deletion of T3SS regulon] and CHAΔpopBD [deletion of genes encoding translocation apparatus]); PA103 (cytotoxic strain expressing ExoU); PAO1 (reference strain); 13 clinical strains isolated from patients suffering from acute infections

Cells: THP-1 monocytes

Cell viability: release of the cytosolic enzyme lactate dehydrogenase (LDH) in the culture medium after 5 h of incubation P.a strains (10 bact./ cell)

Inflammasome activation: IL-1beta secretion (ELISA)

Motility: swimming in 0.3% agar

T3SS transcription: Real-time PCR of genes encoding toxins or translocon

Statistical analysis: correlation coefficients determined using JMP version 10.0.2.

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[4] Faure *et al*., Am J Respir Crit Care Med. 2014;189(7):799-811
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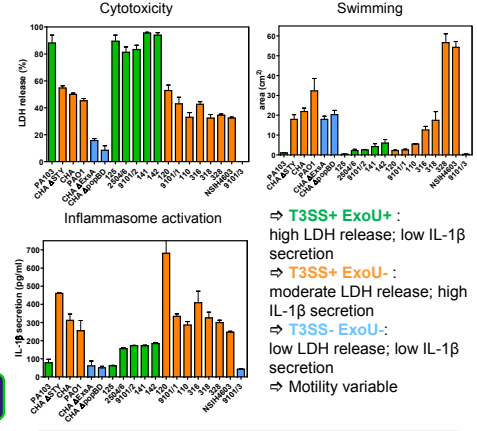
RESULTS

		strains	T3SS mRNA expression [a] exoS	exoU	translocon popB/popD	translocon pcrV	swimming (area [cm²])	activation (IL-1β secretion [pg/mL])	cytotoxicity (LDH release [%])
reference strains	T3SS+ ExoU+	PA103	-	+	+	+	1.0 ± 0.1	78.1 ± 19.3	88.1 ± 18.7
	T3SS+ ExoU-	CHA ΔSTY	-	-	+	+	18.0 ± 3.3	460.5 ± 43.3	54.7 ± 4.3
		CHA	+	-	+	+	23.3 ± 4.5	311.6 ± 34.8	50.0 ± 2.8
		PAO1	+	-	+	+	32.3 ± 8.9	255.0 ± 55.9	45.2 ± 5.1
	T3SS- ExoU-	CHA ΔExsA	-	-	-	-	18.0 ± 2.2	61.4 ± 26.4	15.8 ± 4.6
		CHA ΔpopBD	+	-	-	+	20.3 ± 3.1	50.4 ± 8.4	8.5 ± 9.6
clinical isolates	T3SS+ ExoU+	125 [a]	-	+	+	+	0.4 ± 0.1	61.8 ± 2.1	89.5 ± 12.7
		2504/6 [c]	-	+	+	+	2.2 ± 1.0	156.2 ± 5.4	81.2 ± 10.2
		9101/2 [b]	-	+	+	+	2.5 ± 0.4	172.9 ± 1.8	83.2 ± 8.6
		141 [b]	-	+	+	+	4.2 ± 2.1	171.9 ± 3.1	95.5 ± 1.8
		142 [d]	-	+	+	+	6.0 ± 2.5	184.4 ± 5.9	94.0 ± 5.3
	T3SS+ ExoU-	120 [c]	+	-	+	+	2.2 ± 0.3	682.2 ± 68.0	52.8 ± 11.3
		9101/1 [a]	+	-	+	+	2.5 ± 0.6	334.3 ± 13,3	42.9 ± 13.8
		110 [b]	+	-	+	+	5.5 ± 0.1	286.7 ± 18.0	33.0 ± 9.8
		316 [f]	+	-	+	+	12.6 ± 2.5	409.6 ± 62.8	42.7 ± 5.3
		318 [g]	+	-	+	+	17.5 ± 6.2	325.4 ± 31.8	32.4 ± 7.4
		328 [d]	+	-	+	+	56.6 ± 6.3	299.2 ± 13.8	34.6 ± 2.4
		NSIH 4605 [f]	+	-	+	+	54.3 ± 4.1	230.9 ± 22.7	32.4 ± 2.2
	T3SS- ExoU-	9101/3 [b]	-	-	-	-	0.3 ± 0.1	43.3 ± 1.9	0.7 ± 2.8

*(+): expression detected by RT-PCR; (-) : no expression detected

Origin of the strains: [b] lower respiratory tract ; [c] blood ; [d] wound ; [e] abdominal collection; [f] urines ; [g] eye; [h] upper respiratory tract



⇨ **T3SS+ ExoU+** : high LDH release; low IL-1β secretion

⇨ **T3SS+ ExoU-** : moderate LDH release; high IL-1β secretion

⇨ **T3SS- ExoU-**: low LDH release; low IL-1β secretion

⇨ Motility variable

Main observations & Conclusions

- **T3SS+ ExoU+ strains: high cytotoxicity, causing cell death without inflammasome activation**
- **T3SS+ ExoU- strains (expressing ExoS or no toxins): moderate cytotoxicity, related to inflammasome activation, which induces IL-1β secretion.**
- **Motility: low predictive value.**

⇨**Studying these parameters in clinical strains may help predicting toxin expression. They may also be used for evaluating anti-virulence therapeutic strategies that target T3SS and impair toxin translocation [6] or prevent inflammasome activation (ICAAC 2013; B1055).**

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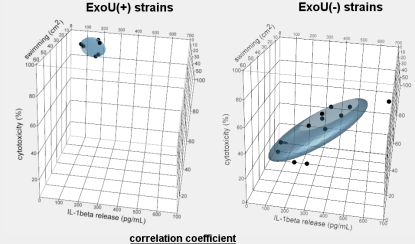

correlation coefficient

parameter	ExoU(+) strains	ExoU(-) strains
IL-1beta release vs cytotoxicity	0.3	0.8
IL-1beta release vs swimming	0.8	-0.1
cytotoxicity vs swimming	0.7	0.1

Correlation between swimming capacity, induction of IL-1beta secretion (inflammasome activation) and induction of LDH release (cytotoxicity) for the 19 strains shown in the Table. Normal contour ellipsoids and correlation coefficients were calculated using JMP version 10.0.2.