**P0174**
**Poster Session I**
**Basic science: pathogenesis of Gram-negative bacteria**

# 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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**Objectives:** Expression of T3SS in *P.a* is associated with poor clinical outcome and high morbidity in acute infections (JID 2001;183:1767-7). T3SS allows bacteria to inject exotoxins (e.g. ExoU or ExoS) into the host cell cytoplasm, causing cytotoxicity and preventing *P.a*. internalization (Nat Rev Microbiol 2009;7:654-65). 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-1beta and IL-18 inflammatory cytokines (J Clin Immunol 2010;3:502-6), thereby impairing *P.a*. clearance (J. Clin Invest 2013;23:1630-7). Flagellum-mediated motility has also been recently suggested to modulate inflammasome response (Infect Immun 2013;81:2043-52). Our aim was to compare inflammasome activation and cytotoxicity caused by *P.a*. strains differing in their expression of T3SS and in motility and to examine how these parameters are correlated.

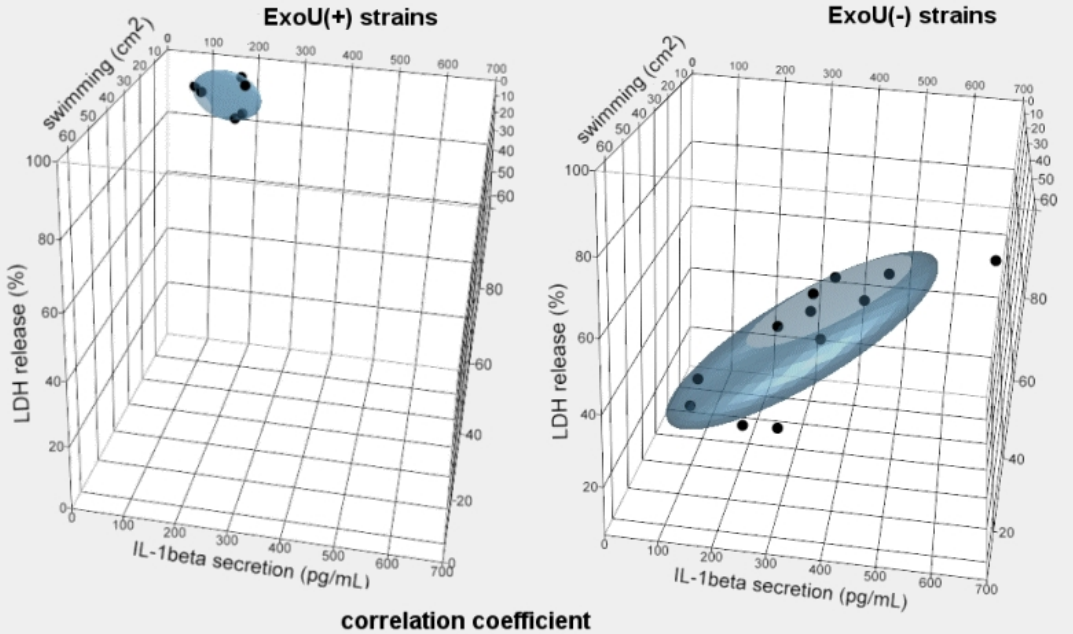
**Methods:** Strains: CHA (clinical isolate expressing T3SS) and derivatives thereof (CHAΔSTY [no toxin production]; CHAΔExsA [deletion of T3SS regulon] and CHAΔpopBD [deletion of genes encoding translocation apparatus]); PA103 (cytotoxic strain expressing ExoU); PAO1 (reference strain); 13 clinical strains isolated from patients suffering from acute infections. Cells: THP-1 monocytes. T3SS transcription: Real-time PCR of genes encoding toxins or translocon. Motility: swimming in 0.3% agar. Inflammasome activation: IL-1beta secretion (ELISA). Cytotoxicity: release of the cytosolic enzyme lactate dehydrogenase (LDH) in culture medium after 5 h of incubation with bacteria (10 bact./ cell). Statistical analysis: correlation coefficients determined using JMP version 10.0.2.

**Results:** The table shows the expression of T3SS genes and swimming capacity of each strain, together with the induced secretion of IL-1beta and release of LDH from THP-1 cells (correlations illustrated in the figure). The 3 strains with no functional T3SS (T3SS- [in grey in the Table]) induced little IL-1 beta secretion and only low or no LDH release. The 6 strains expressing ExoU (T3SS+ExoU+) were barely motile and caused only minimal IL-1beta secretion but a large LDH release. For the 10 strains that did not express ExoU (but either ExoS or no toxins; T3SS+ExoU-), a strong correlation was observed between their capacity to induce IL-1-beta secretion and LDH release, but these parameters were poorly correlated with bacterial motility.

**Conclusion:** Combining the measurement of IL-1beta secretion (inflammasome activation) and of LDH release (cytotoxicity) allows distinguishing between T3SS+ strains expressing ExoU (high cytotoxicity, causing cell death without inflammasome activation) from those expressing ExoS or no toxins (moderate cytotoxicity, related to inflammasome activation, which induces IL-1beta secretion). Motility was of 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 (JID 2002;186:64-73) or prevent inflammasome activation (ICAAC 2013; B1055).

| | | strains | T3SS mRNA expression [a] toxins *exoS* | toxins *exoU* | translocon *popB/popD* | translocon *pcrV* | swimming (area [cm²]) | inflammasome 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 [b] | - | + | + | + | 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 [e] | + | - | + | + | 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 [h] | + | - | + | + | 56.6 ± 6.3 | 299.2 ± 13.8 | 34.6 ± 2.4 |
| | | NSIH 4603 [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 |

[a] (+): 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




| parameter | correlation coefficient ExoU(+) strains | ExoU(-) strains |
|---|---|---|
| IL-1beta secretion vs LDH release | 0.1 | 0.8 |
| IL-1beta secretion vs swimming | 0.8 | -0.1 |
| LDH release vs swimming | 0.5 | 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.