Pseudomonas aeruginosa Exotoxin T inhibits inflammatory responses in wound by blocking the phosphorylation cascade through the NLRC4 inflammasome

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Objective: Chief amongst many cell-associated or secreted virulence factors possessed by *Pseudomonas aeruginosa* (*Pa*) is its Type III Secretion System (T3SS) injectosome which functions as a conduit, allowing *Pa* to directly translocate several effector exotoxins into the target host cytoplasm where they modify host cellular processes and advance *Pa* infection. Most T3SS-expressing *Pa* strains encode either ExoT and ExoU, such as PA103, or ExoS, ExoT and ExoY, such as PAK. ExoT is the only effector toxin that is present in all *Pa* clinical isolates examined thus far, suggesting a more fundamental role for this virulence factor in *Pa* pathogenesis. Although, T3SS is crucial for *Pa* pathogenesis, its recognition by NLRC4 (IPAF) caspase-1 canonical inflammasomes has been shown to lead to inflammatory responses in Bone Marrow derived Macrophages (BMDMs). However, the impact of T3SS and its effectors on inflammatory responses *in vivo* is poorly understood.

Methods: We used BMDM *in vitro* cell culture assays and wound animal models of infection with PA103 and PAK strains to evaluate the impact of T3SS and its effector proteins on host innate immune responses and bacterial fitness *in vivo*.

Results: Our data demonstrate that although T3SS effectors or T3SS apparatus are not required for initial colonization in the wound, *Pa*'s persistence and its ability to cause wound damage is dependent on T3SS and ExoT. We show that T3SS triggers massive inflammatory responses both *in vitro* and *in vivo* through NLRC4 caspase-1 canonical inflammasomes and that ExoT blocks T3SS-induced caspase-1 inflammasome activation and inflammatory responses. We show that infection with ExoT-deleted (ΔT) *Pa* strains with functional T3SS, results in caspase-1 activation, increased IL-1β and IL-18 pro-inflammatory chemokine expression, increased inflammatory leukocytes infiltration and activation at the site of infection, and reduced bacteria number. Complementing ΔT strains with ExoT, restores *Pa*'s ability to block T3SS-induced caspase-1 activation dampen host's inflammatory responses and enhances bacterial colonization both in wound and systemic animal infection models. we demonstrate that T3SS recognition triggers a rapid phosphorylation cascade involving CrkII/Abl → PKC-d → NLRC4, which activates NLRC4 inflammasome, culminating in massive inflammatory responses which limit *P. aeruginosa* infection in wound. CrkII and Abl have not been implicated in inflammasome activation before. We further show that ExoT effectively inhibits NLRC4 inflammasome by blocking the CrkII/Abl-dependent phosphorylation flow to PKC-d/NLRC4 inflammasome.

Conclusions: ExoT, by blocking T3SS-induced inflammatory responses, provides an effective stealth mechanism and allows *Pa* to expand its arsenal of virulence factors through the T3SS potent cytotoxins, (e.g., ExoU or ExoS), which in turn allow it to persistently colonize and propagate its preferred niche, the wound.