Research Article

Type III secretion systems among clinical *Pseudomonas aeruginosa*

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ABSTRACT

Background: *Pseudomonas aeruginosa* is important player among many of the top ten diseases. Its important causative agent among pulmonary infections, burn and wound infections and urinary tract infections may be due to their withstanding or adaptability to survive among different habitats. Many virulence factors are responsible for this array of infections and the important one is Type III secretion systems (T3SSs). They are virulence determinant among *P. aeruginosa* that can orientate the infection style whether it is toxigenesis or invasiveness. **Materials and Methods:** Investigation of T3SS can be achieved by PCR and sequencing. **Results:** The results revealed that T3SS effector proteins are responsible for immune counteraction or voidance (especially interfering with phagocytosis and immaturation of interleukin IL-1B), impairment of many cellular processes, cell shape deformities, and may cause losing of cell integrity in respiratory and wound infections. **Conclusion:** T3SS plays a key role in pathogenesis and immune counteracting in some pseudomonal infection.

KEY WORDS: Acute respiratory distress syndrome, ExoS, Type III secretion system, Wound infection

INTRODUCTION

Pseudomonas aeruginosa

P. aeruginosa is a ubiquitous and common Gram-negative opportunistic pathogen responsible for serious health-care-associated infections annually in the United States. It is one of the Gram-negative airway pathogens that have been assigned a threat level of "serious" by the CDC.[1] It can cause both acute and chronic infections, with substantial morbidity and mortality, in a number of clinical scenarios: acute in intubated patients, those with burns, cancer, immunosuppression, and chronic in cystic fibrosis (CF) and other forms of chronic suppurative lung disease.[2] Epidemic strains of *P. aeruginosa* often dominate within the lungs of individual CF patients, but how they achieve this is poorly understood. One of the ways, strains of *P. aeruginosa* can compete, is by producing chromosomally encoded bacteriocins, called pyocins. Three major classes of pyocin have been identified in *P. aeruginosa*: (i) R-type pyocins resemble non-flexible and contractile tails of bacteriophages. They provoke a depolarization of the cytoplasmic membrane in relation with pore formation. (ii) F-type pyocins also resemble phage tails, but with a flexible and non-contractile rod-like structure. (iii) S-type pyocins are colicin-like, protease-sensitive proteins. They are constituted of two components. The large component carries the killing activity (DNase activity for pyocins S1, S2, S3, AP41; tRNase for pyocin S4; and channel-forming activity for pyocin S5).[3] Type III secretion system (T3SS) is reported to play a key role in the pathogenesis of *P. aeruginosa* infections. Four T3SS effectors (ExoU, ExoS, ExoT, and ExoY) have been identified.[4]

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T3SS

Bacterial secretion systems are machines that accommodated to transport the bacterial proteins, enzymes, and toxins. In general, it divided into one-step secretion systems and two-step secretion systems. One-step secretion system includes transportation from cytosol to outside without needs to stay in periplasm. The type I secretion system of *E. coli* like α-hemolysin (HlyA), T3SS of *Yersinia* (Yop or Ysc) and *Pseudomonas* (Pop or Psc), and type VI secretion system of *Vibrio cholerae*[5-9] were categorized as two-step secretion system includes

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transportation from cytosol to periplasm and from periplasm to outside, and it represented by type II secretion system of *Pseudomonas*, type IV secretion system of *Helicobacter pylori*, and type V secretion system. Type III secretion system (also called T3SS) is the organelle responsible for delivering the bacterial effectors protein into eukaryotic cell and so-called injectisome. It is composed from basal body spanning both bacterial membranes and the periplasm, and an external needle protruding from the bacterial surface.[10,11] T3SS composes of approximately 20 bacterial proteins building up the three main components of T3SS machine: Structural, translocators, and effectors protein. Both structural and translocators are responsible for delivery while the effectors protein performs the virulence trait. Three macromolecular partners associate to form the translocon: two are hydrophobic and one is hydrophilic, and the latter also associates with the T3SS needle. T3SSs are used by plant and animal pathogens, including *Salmonella*, *Shigella*, *Yersinia*, *E. coli,* and *Pseudomonas* mainly to deliver effector proteins into the host cell cytoplasm.[12,13]

Five major families of T3SS can be distinguished: (i) the Ysc family of T3SS, which includes, among others, the Ysc system of *Yersinia*, the Psc system of *P. aeruginosa,* and the Asc system of *Aeromonas salmonicida*, (ii) the Inv-Mxi-Spa family, which includes the *Salmonella enterica* (SPI-1) and *Shigella* spp. Systems, and (iii) the Ssa-Esc family, which includes the systems of *S. enterica* (SPI-2) and of EPEC and EHEC. The fourth and fifth families comprise two different Hrp T3SSs of plant pathogens.[14-16]

ExoS, ExoT, ExoU, and ExoY are the effector protein of pseudomonal T3SS.

Both ExoS and ExoT – sharing the highest homology out of the four known T3SS enzymes – exhibit ADP-ribosyltransferase activity, interfering with manifold signaling pathways in the host cell, such as the Ras-signal transduction.[17,18] ExoS has been implicated in the formation of membrane bleb-niche and avoidance of acidified compartments to allow bacterial multiplication within epithelial cells. ExoU causes direct cytotoxic effects on host cells by its phospholipase A2 activity. Little is known about the role of ExoY during *P. aeruginosa* infection.[19,20]

T3SS Effects on Immune System

In many infections and as immune response to *P. aeruginosa,* epithelial cell and macrophage express antimicrobial peptides (AMPs) to combat the infection. *P. aeruginosa* repress AMP expression by its T3SS through diminishing AMP expression by causing delay in NF-κB, p38, and ERK activation and inhibit reactive oxygen species generation in these cells by its T3SS.[21] It also play a role in intramacrophage life of *P. aeruginosa*, allowing internalized bacteria to evade macrophages.[22] Following the activation of a number of kinase cascades, NF-κB is liberated from its cytosolic binding partners (the IκBs), at which point, it is freely translocated to the nucleus and activates the expression of inflammatory genes. T3SSs activate inflammatory gene expression and the proteolytic cleavage and activation of the inflammatory cytokines IL-1 β and IL-18 by caspase-1 (also called IL-1-converting enzyme). Both of these are potent inducers of inflammation that reflects the culmination of a variety of signaling pathways in the host. Both are also the targets of T3SS effectors.[23] Exoenzyme S (ExoS) induces potent monocyte activation, leading to the production of numerous pro-inflammatory cytokines and chemokines. ExoS bounds a saturable and specific receptor on the surface of monocytic cells. ExoS, LPS, and peptidoglycan were all able to induce tolerance and cross-tolerance to each other, suggesting the involvement of a TLR in ExoS recognition.[24] T3SSs are involved in the perturbation of host cellular immune responses. These pathogens exploit T3SS function to select which cells are recruited/produced by the host to combat infection. They also sabotage the function of the recruited cells to pathogenic advantage. Host immune cells have diverse functions in the control of infection, and their recruitment and coordinated activation are critical for infection control. Pathogens exploit T3SSs to alter this coordination in a variety of ways. Cells responsible for pathogen destruction and processing (antigen-presenting cells), such as neutrophils, macrophages, and dendritic cells (DCs), are critical antibacterial effector cells of the innate immune system.[25] T3SSs are used to interfere with a variety of host cell defenses such as autophagy and phagolysosomal degradation and also subvert the macrophage phagocytic process by preventing delivery of NADPH oxidase and nitric oxide synthase to the phagosome, thus protecting the resident pathogen from damage by reactive oxygen.[26] ExoS has GTPase-activated proteins activity which promotes the inactivation of Rac1 and induces both necrosis and apoptosis in macrophages in a T3SS-dependent manner. In addition, it causes neutrophil cytotoxicity, which was also suggested to be mediated by the T3SS.[27,28]

T3SS Effects Cell Component

P. aeruginosa may induce early T3SS-dependent dephosphorylation and deacetylation of histone H3 in eukaryotic cells.[29] Cytotoxicity induced by ExoU occurs independently of other T3SS effectors and appears to be due to phospholipase activity. The translocation of active ExoU results in destabilization and destruction of intracellular membranes. This destabilization results in necrosis and correlates with

a variety of clinical parameters of disease in infection models including transudation into alveoli, interstitial pathology, and mortality. In addition, T3SS directly activates pattern recognition receptors (PRRs), especially TLR2 and TLR4. ExoS activates TLR2 and TLR4/MD-2/CD14 signaling through its C and N termini, respectively. This activation could be induced in cultured monocytes by exogenously administered ExoS in a manner that required neither effector translocation nor ExoS internalization, suggesting that this effector can act both as a modifier of intracellular signaling.[30,31]

TSS3-Dependent Diseases

P. aeruginosa was implicated in many clinically important diseases. The pathogenesis may be attributed to the arrays of virulence factors of *P. aeruginosa.* TSS3 may be the main virulence factor and the pathogenesis can be attributed to them in the following diseases:

CF

CF is a genetic disease initially linked to the Caucasian population and now recognized in recent studies in much of the rest of the world population.[32,33] CF is produced by mutations in the CF transmembrane conductance regulator (CFTR), a chloride channel at the surface of epithelial and immune cells. As hydration and mucin viscosity in the airways are dependent on the ionic equilibrium across the epithelial barrier, lack of CFTR function reduces airway mucus fluidity, trapping inhaled bacteria.[34] Although CFTR mutations also have negative effects on the function of other organs, such as the pancreas, CF patients receiving nutritional supplementation are able to manage these limitations.[35] During childhood, most CF patients are colonized by both *P. aeruginosa* and *Staphylococcus aureus*. However, later in adulthood, the primary pathogen causing pulmonary infection in CF is *Pseudomonas*. Many studies have addressed why *P. aeruginosa* is the major pathogen in CF. The most commonly accepted hypothesis suggests that physiological abnormalities linked to CFTR mutations confer advantages to *P. aeruginosa* over other pathogens. These advantages include mucus viscosity, excessive production of reactive oxygen species (ROS), impaired autophagy, reduced airway acidity, and accumulation of ceramides, all factors that contribute to the genomic and metabolic plasticity of *P. aeruginosa* and its ability to colonize the airways.[36] Whether the exaggerated inflammatory response seen in CF facilitates the colonization and then chronicity of *P. aeruginosa* infection over other bacteria is not well understood, although recent findings suggest that mutant CFTR directly influences inflammatory signaling, and this favors *P. aeruginosa* over other respiratory pathogens such as *S. aureus* and *Klebsiella pneumoniae*.[36,37] Chronic lower airway infection with *P. aeruginosa* is a major contributor to morbidity and mortality in individuals suffering from the genetic disease CF. Whereas it was long presumed that each patient independently acquired unique strains of *P. aeruginosa* present in their living environment, multiple studies have since demonstrated that shared strains of *P. aeruginosa* exist among individuals with CF. Many of these shared strains, often referred to as clonal or epidemic strains, can be transmitted from one CF individual to another, potentially reaching epidemic status.[38]

The worseness of CF may also be attributed to the NLRC4 inflammasome when induced by flagellin delivered by T3SS. Activation of NLRC4 inflammasome can lead to high level of pro-inflammatory IL-1β which makes the CF exacerbate.[39]

Wound Infection

Wound infections are an emerging medical problem worldwide, frequently neglected in under-resourced countries. *P. aeruginosa* is a common causative agent of wound infection. T3SS was found to mediate inhibition of wound repair in diabetic skin ulcers.[40,41]

Acute respiratory distress syndrome (ARDS)

ARDS is an overwhelming systemic inflammatory process associated with significant morbidity and mortality.[42] *P. aeruginosa* is a predominant cause of nosocomial pneumonia in critically ill patients, which often progresses to ARDS. Upon inoculation into the airway, *P. aeruginosa* infects alveolar epithelial cells and resident macrophages, eliciting release of pro-inflammatory cytokines that recruit immune cells into the lung parenchyma and airspaces. The combined effects of infection and inflammation exacerbate barrier damage, allowing direct infection of, and damage to, the pulmonary vascular endothelium.[43-45] Systemic dissemination of the pathogen along with attendant endotoxemia and cytokine storm can precipitate the onset of sepsis, leading to further pulmonary vascular endothelial barrier disruption. The heterogeneous nature of infection caused by different *P. aeruginosa* isolates is attributable to variability in their cadre of virulence factors. Highly pathogenic isolates express a T3SS. ARDS patient mortality is high among individuals infected with isolates expressing a functional T3SS along with the ExoU effector.[46,47]

CONCLUSION

T3SS plays a key role in pathogenesis and immune counteracting in some pseudomonal infection.

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