Francisella tularensis type VI secretion system comes of age

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Francisella tularensis, a Gram-negative facultative intracellular bacterium, is a causative agent of zoonotic disease tularemia. It is also listed as a category A potential bioterrorism agent. As an intracellular pathogen, F. tularensis evolves several strategies of how to defend the host immune system which include complicated intracellular life cycle, ability to infect a variety of host cell types, high morbidity and mortality, and persistence in the environment.

The pathogenicity of F. tularensis is associated with its ability to survive inside of the phagocytic cells and to escape into the cytoplasm. Following cell entry, F. tularensis is located within a phagosome that begins to mature into a phagolysosome. However, F. tularensis is able to prevent fusion of the phagosome with the lysosome. After that, the bacteria degrade the phagosomal membrane and escape into the host cell cytosol where they replicate. The molecular mechanism how the Francisella escapes the phagosome is not yet fully understood but it has been established that the disruption of genes located in the Francisella pathogenicity island (FPI) leads to the inability of bacteria to escape the phagosome.

Pathogenicity islands (PAI) are the sources of various virulence factors that are crucial for interaction of pathogens with their respective host targets. Generally, the virulence factors repertoire encoded within PAI encompasses several functional groups involving adherence factors, siderophorins, exotoxins, invasion proteins and components of type III and IV secretion systems. The FPI is a 34-kb genomic region that includes ~16–19 open reading frames (ORFs), depending on the particular Francisella subspecies and was first described by Nano et al. This area presenting in all Francisella genomes suggests that it is a part of the core genome. However, in contrast to the rest of the genome the FPI region is characterized by low GC content, indicating horizontal gene transfer. The FPIs share more than 97% nucleotide identity across the different Francisella subspecies; however, while the strains highly virulent for humans exhibit the duplicated FPIs, the low virulent strains own only the single FPI copy. The study of intracellular transcriptome of a highly virulent strain of F. tularensis during its infection cycle within murine macrophages revealed participation of the FPI gene products in both early and late induction events. This finding corroborates the FPI role in early phagocytosis as well as at the end of the cytoplasmic proliferation. The first FPI identified gene was named iglC (intracellular growth locus C), and was found to be needed for Francisella intramacrophage growth. Later on, further genes within the FPI, similarly to iglC, proved their importance for intracellular growth and accordingly were named iglA, iglB, iglD, iglE, iglF, iglG, iglH, iglI and iglJ. For the other group of the FPI ORFs the designation pathogenicity determinant protein (pdp) was adopted and the products of these ORFs were thus denoted PdpA, PdpB, PdpC and PdpD. The intensive bioinformatics search for biologic function of the FPI products revealed for some of them the homology with the components of the type VI secretion systems (T6SS) in Vibrio cholerae (V. cholerae) and Pseudomonas aeruginosa (P. aeruginosa). T6SS is a novel tool in bacterial protein secretion and its gene cluster contains a core of 13 essential genes that are shared in about 25% of sequenced gram-negative bacterial genomes. The typical feature of all T6SSs is the presence of hemolysin co-regulated protein (Hcp) and valine-glycine repeat protein G (VgrG) in culture supernatants. Hcp and VgrG exoproteins exhibit structural similarities to a puncturing device of bacteriophage translocation machinery, therefore, instead of true secretion they are probably released upon mechanical shearing. In the current T6SS model the Hcp tube is topped by VgrG trimer and the existence of this plus-like structure is dependent on the expression...
of icmF, clpV, and dotU genes. Additionally, VipA and VipB, other essential T6SS components, form complexes resembling bacteriophage tail sheath which contraction is crucial for Hcp and VgrG export, as well. The real biological function of Hcp is currently unknown but its structure indicates it might form a channel. As for VgrG protein in the course of V. cholerae infection it was found to cross-link host actin filaments and modulate the host signal transduction. Recently it was observed that a VgrG spike can be further extended by a proline-alanine-alanine-arginine (PAAR) domain-containing protein and just the specific VgrG-PAAR combination are required for assembly of functional T6SS with different effector recruitment.

The functionality of FPI encoded T6SS is still questionable. The problem is that only few FPI genes are related to conserved T6SS components while other important structures such as an AAA+ Clp-like ATPase are missing. The FPI components with the greatest homology to T6SS proteins are IglA and IglB. It has been shown that these proteins mutually interact and this interaction is vital for their stability and virulence. Hence it is assumed that IglA/IglB complex is similar to V. cholerae VipA/VipB complex and form tubular transmembrane structure. The intermediate homology exerts DotU and PdpB/Icm and, finally, Francisella’s VgrG that is a small 17.5 kDa protein shares only limited homology with the VgrG proteins from V. cholerae. On the other hand Francisella’s VgrG protein is also secreted into culture supernatants and into macrophage cytosol. Cell fractionation studies of the FPI proteins localization revealed that IglC together with IglA, IglB and IglD occur in all fractions, including the location outside of bacteria. Furthermore, the IglC secretion to environmental stimuli was found to be IglA and IglG dependent. The structural analyses then indicated overlay of IglC with Hcp3 from P. aeruginosa. All these pieces of information led to current view of IglC as being dominant component of the inner tube. Very recent publication identified additional FPI protein IglG as putative PAAR-like protein which core of the PAAR-like domain is presumably attached to the tip of Francisella T6SS through interaction with VgrG protein. The N-terminal extension of PAAR-like domain was shown to bind another FPI protein IglF. IglF is a secreted protein required for in vitro replication; therefore, IglF might be new Francisella effector protein that the release is IglG dependent.

The latest addition to the Francisella T6SS jigsaw puzzle is IglE protein. The IglE is a small 13.9 kDa lipoprotein encoded within the FPI. Deletion of IglE genes blocks intracellular proliferation of virulent F. tularensis strain as well as leads to in vivo strain attenuation in mice. The defect in intracellular replication and virulence is associated with the loss of IglE lipidation that is responsible for its localization to bacterial outer membrane. Two-hybrid and in vivo co-immunoprecipitation analyses documented that membrane bounded IglE interacts with C-terminus of PdpB/Icm and by this way contributes to channel formation and protein secretion through the Francisella T6SS.

In this issue of Virulence, Bröms et al. used a mutagenesis-based approach to identify amino acids in N-terminal part of IglE that are critical for functioning of Francisella T6SS mediated protein secretion. In contrast to previous studies the authors exploited for the study attenuated live vaccine strain (LVS). First, they in accordance with the previous studies confirmed the outer membrane localization of IglE, its participation in Francisella escape from phagosome, Francisella failure in inflammesome activation and in vivo attenuated phenotype in the mouse model. They also examined the role of IglE in intracellular growth and cytopathogenicity. The previously published data on both the F. tularensis Schu S4 and the F. novicida IglE mutant described the defective bacterial replication in bone-derived macrophages and within J774 cells, respectively. Bröms et al. study exploiting direct injection of the IglE mutant into the cytoplasm of host cells to avoid of phagosome compartment proved efficient cytosolic replication of the mutant strain. Hence, it is evident that IglE protein is important for the phagosomal escape but not for subsequent cytosolic replication. The study of Hara and Hueffer examined by using immune-fluorescence microscopy the localization of FLAG-tagged FPI proteins in infected macrophage-like host cells. The FLAG-tagged IglE protein was found extracellularly in co-location with bacteria and this position was significant for time intervals 30 min and 4 h post-infection. The former interval corresponds with the escape of internalized bacteria from phagosome that supports the Bröms et al. finding. The biologic role of IglE in the latter time interval that is associated with the onset of Francisella proliferation has to be discovered, yet. The major point of this article was the mapping of functional domains within IglE. The authors prepared the series of IglE deletion mutants removing about 20 residues across the entire protein. Of them a region encompassing residues 44 – 125 was found to be necessary for IglE function. In the next step the authors generated a series of frameshift mutants to identify key residues within the extreme N-terminus up to 38 amino acid residue. The prepared mutants were tested for their intramacrophage growth capacity and cytopathogenic effects. The results proved that the residues 33–38 are critical for these IglE functions. To verify whether the loss of IglE function of some of generated IglE mutants reflects the defect in T6SS activity, IglC secretion was
monitored. The acquired data confirmed the strong relationship between the ability of the mutant strains to fully complement the wild IglE role in Francisella pathogenesis and the ability to support IglC secretion. This finding further emphasizes the dependence of efficient T6SS on the presence of functional IglE protein. Additionally, the single alanine mutants were created within lipobox motif covering amino acid residues from 19–22. Again these mutants were tested by battery of cell assays to document their efficiency in infectious process. Surprisingly, despite the loss of lipidation and even low level of the IglE expression the intracellular replication was not abolished. Furthermore, in one of these mutants the C22A the blockade of IglC secretion was detected but the intracellular replication was untouched, again. Hence it can be anticipated that different parts of IglE structure can influence distinct functions.

In conclusion this study described the importance of F. tularensis LVS derived IglE protein for phagosomal escape, cytopathogenic effect and in vivo virulence. The construction of carefully designed mutants then provided information about sequence motifs critical for IglE function. The important finding was that mutated IglE proteins which were not able to complement the function of the wild type IglE variant also exerted non-functional T6SS machinery. This evidence further corroborates involvement of IglE protein in the functional T6SS assembly.

This study together with previous publications also documented that IglE was predominantly located in the outer membrane, in less amount in the inner membrane and even secreted. It is intriguing that the mutations that affected IglE lipidation and hence membrane localization did not influence microbial intracellular replication and cytopathogenicity. The authors explained this phenomenon by dual role of IglE as an outer membrane-localized form or as a non-processed secretion effector. Now the major challenge will be to find the biologic functions for both IglE roles.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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