Review Article

Asian Pacific Journal of Tropical Biomedicine

apjtb.org

doi: 10.4103/2221-1691.372282

Impact Factor® 1.51

# T6SS-5 and the cGAS-STING pathway in *Burkholderia pseudomallei* infection and immunity

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## ABSTRACT

*Burkholderia pseudomallei* is a causative agent of melioidosis that can infect humans and animals in endemic countries, specifically in Southeast Asia and tropical Australia. A fundamental component for the pathogenesis of *Burkholderia pseudomallei* is the capability of the bacterium to enter, survive, replicate, and cause disease in a host cell by inducing the host cell fusion. Cell fusion results in multinucleated-giant cell formation, thus enabling the dissemination of *Burkholderia pseudomallei* intracellularly. cGAS reacts to *Burkholderia pseudomallei* infection by activating the cGAS-STING pathway and subsequently limiting host's aberrant cell division and cellular replication by inducing autophagic cell death. In this review, we discuss the host-pathogen interactions between the type Ⅵ secretion system 5 (T6SS-5) of *Burkholderia pseudomallei* and human cGAS pathway in melioidosis infections. Since T6SS-5 is a main virulent factor in *Burkholderia pseudomallei* and the cGAS pathway is vital for host immune response, elucidating their functions is important for better understanding the pathogenesis of *Burkholderia pseudomallei*.

**KEYWORDS:** *Burkholderia pseudomallei*; Melioidosis; T6SS-5; cGAS-STING; IFN-1

## 1. Introduction

Melioidosis is a neglected tropical disease and can be fatal to humans and animals. This complex disease was first discovered and reported over 100 years ago in 1912 in Burma[1]. This disease is caused by *Burkholderia pseudomallei* (*B. pseudomallei*) and has been proposed by the U.S. Centers for Disease Control and Prevention (CDC) as a Tier 1 agent, with high morbidity and mortality potential as well as could be easily spread[2].

*B. pseudomallei* inhabits natural environments such as soil and water in endemic regions. It is a Gram-negative bacillus and a natural saprophyte that can become dormant and survive in harsh environmental conditions over a long period. Besides, *B. pseudomallei* can adapt to a prolonged nutrient deficiency for up to 10 years and temperature fluctuations between 24℃ to 32℃[3]. This pathogen also can enter, persist, and replicate in both phagocytic and non-phagocytic cells, its intracellular behavior is vital for the pathogenesis and virulence of the disease[4,5].

Even though the mortality rate of melioidosis in Australia appears to be declining with an average rate of 14%, its incidence is still increasing[6]. In northeast Thailand, throughout the year 1997 to 2006, a hospital recorded high mortality rates for admitted melioidosis patients with 42.6% of the annual rate average[7]. Furthermore, in 2018, it has been estimated that the fatality rate of admitted patients for overall melioidosis cases in a public hospital in Thailand was between 30% to 35%[8].

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**How to cite this article:** Semail N, Zuraina NMNN, Ismadi YKM, Mohamad NI, Harun A, Aziah I, et al. T6SS-5 and the cGAS-STING pathway in *Burkholderia pseudomallei* infection and immunity. Asian Pac J Trop Biomed 2023; 13(3): 94-103.

**Article history:** Received 4 January 2023; Revision 21 January 2023; Accepted 23 February 2023; Available online 31 March 2023

Melioidosis cases have been commonly associated with those who had been exposed to contaminated water and soil[6,9–11]. People who work in eco-tourism, agriculture and forestry, construction, and military base have high risks of *B. pseudomallei* infection[12,13]. In Malaysia, 2%-25% of melioidosis cases were reported among those in the forestry, farming, and fishing industry while 3%-18% of the cases were reported in trucking and construction industry[14]. More than 2 000 patients have died because of melioidosis each year based on the mortality rate and reported incidence[13]. This is considerably higher than the death due to tuberculosis disease or dengue fever in Malaysia[13].

The severity of the clinical presentation varies from insignificant and localized infections to fatal chronic infections such as sepsis and formation of abscesses in organs such as the liver, lungs, soft tissue, and spleen. Pneumonia, septic shock, and irregular involvement of neurological problems are among common clinical presentations of melioidosis correlated with high mortality rates even after antibiotic treatments[15,16]. In addition, community-acquired pneumonia and bacteremia are among common clinical presentations and the most frequent diagnosis of melioidosis, respectively[17–20].

The hallmark of *B. pseudomallei*'s virulence is the capability of this bacterium to enter, survive, invade, and embrace the intracellular life cycle of the host[21]. In order to establish a successful infection, survive intracellularly as well as overcome host immune systems, *B. pseudomallei* is equipped with a wide range of virulence factors.

*B. pseudomallei* can infect humans and lead to clinical diseases through numerous virulence factors. Among the virulence factors involved in the *B. pseudomallei* pathogenicity are the capsular polysaccharide, biofilm formation, quorum sensing, flagella, fimbriae, lipopolysaccharide, exoproteins and secretion system genes such as type Ⅱ, type Ⅲ and type Ⅵ secretion systems[11]. Each virulence factor is required for diverse features of intracellular and extracellular of the host, besides the environmental survival of *B. pseudomallei*[22].

Type Ⅵ secretion system 5 (T6SS-5), a needle-like nano-machine, acts as one of the virulent factors in *B. pseudomallei* and most Gram-negative bacteria which particularly involves in host-pathogen interactions[23–25]. This nano-machine permits intercellular spread of the bacteria without encountering extracellular host immune defenses[26]. It is a dependent bacterial killer that functions by injecting a variety of toxins and effectors into the host cell which consequently stimulates immune evasion, inhibits the host cell functions as well as induces autophagic cell death[26]. The T6SS-5 secreted toxins play an important role in establishing host colonization and thus enabling successful infection of the pathogen[27].

After *B. pseudomallei* infection, host defense mechanism reacts by the innate immune system *via* mediating the signaling pathway including cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS) and the downstream signaling adapter stimulator of interferon genes (STING)[24]. Other than bacterial infections and inflammatory diseases, increasing interest in other functional responses of cGAS-STING-mediated signaling has been noted. This review focuses on the correlation between T6SS-5-dependent cell fusion and the cGAS-STING pathway in *B. pseudomallei* infection. A better understanding of *B. pseudomallei* T6SS-5 and the cGAS-STING pathway is important to tackle melioidosis infection and find effective treatment.

## 2. Type Ⅵ secretion system (T6SS)

T6SS is the main virulence determinant in a lot of Gram-negative bacteria, however, the physiological indicators that stimulate the system remain elusive[28]. T6SS is a specialized nano-machine that targets distinct cell types to compete and survive when encountering the host immune mechanisms. This system shares a structural similarity to bacteriophage and could infect numerous cell types by exporting its virulence factors[29]. In many bacterial pathogens, T6SS plays a vital part in pathogenesis involving the transportation or translocation of effector proteins into the host cells[23]. Although the majority of contributions of T6SS-producing bacteria to virulence has not yet been proved or described, it suggests that T6SS is customized to the unique requirements of each bacterium[30].

*B. pseudomallei* genome encodes for six discrete T6SS gene clusters comprising approximately 2.3% of the bacterium's genome, proposing the crucial role of these systems for the capability of *B. pseudomallei* to survive in varied environments[29,31]. The T6SS mechanism consists of a bacteriophage tail-like injectisome and a highly vigorous base plate complex that spans through the inner and outer membranes of bacteria. It is postulated that this secretion system may be driven by a phage tail-like contraction mechanism[32].

Among six T6SS gene clusters in *B. pseudomallei*, only cluster 5 (T6SS-5) contributes to virulence and disease. Two clusters of T6SS might be involved in the perseverance of microbes in environmental niches in which both T6SS-1 and 4 are required for inter-bacterial competition and procurement of metal ions, respectively[29,31,33–35]. Nevertheless, the role of other *B. pseudomallei* T6SS clusters has not yet been studied in detail. The virulence of *B. pseudomallei* T6SS-5 was proven using a Syrian hamster infection model in which the researchers created mutants of each T6SS that had the *Hcp* gene deleted and then examined the pathology in the infected hamsters. Interestingly, only the *Hcp* gene deletion mutant had severely attenuated virulence in the hamsters and consequently resulted in a significantly higher 50% lethal dose[34]. In addition, Chen *et al*. reported that the entire T6SS-5 gene cluster is located on chromosome two of *B. pseudomallei* genome[36]. The significance of each T6SS cluster according to Shalom *et al*. is tabulated in Table 1[31].

**Table 1.** Information of T6SS gene cluster.

| T6SS cluster | Locus tag | G + C content (%) | Significance |
|---|---|---|---|
| T6SS-1 | BPSL3111-BPSL3097 | 66.6 | Required for inter-bacterial species competition and intra-species antagonism |
| T6SS-2 | BPSS0095-BPSS0116 | 63.9 | Detail of its function is still unknown |
| T6SS-3 | BPSS0185-BPSS0167 | 69.6 | Detail of its function is still unknown |
| T6SS-4 | BPSS0515-BPSS0533 | 68.0 | Required for the contact-independent of bacterial competition as well as for the acquisition of metal ions |
| T6SS-5 | BPSS1493- BPSS1511 | 71.7 | Required for intracellular spread as well as in the pathogenesis and virulence of *Burkholderia pseudomallei* |
| T6SS-6 | BPSS2093-BPSS2109 | 70.0 | Detail of its function is still unknown |

## 3. Important role of T6SS–5 in *B. pseudomallei* virulence

T6SS-5 gene cluster plays a fundamental part in the intracellular life cycle of *B. pseudomallei*[29,33]. Besides, T6SS-5 is vital for the pathogenesis and infection in mammalian hosts as well as for systemic melioidosis[4,28,29,34]. T6SS-5 was constantly revealed as a crucial part in intercellular spread and virulence of *B. pseudomallei*, and its entire cluster (BPSS1493 to BPSS1511) is located along the same strand on chromosome two as illustrated in Figure 1[36].

T6SS-5 cluster is comprised of 14 genes encoding the core components for the accumulation of a functional T6SS with four and two accessory subunits encoded for tag and regulator genes, respectively[31,35]. Although the function of these accessory subunits; four tag genes (*tagA*/*B–5*, *tagB–5*, *tagC–5*, and *tagD–5*) and two *virAG* regulator genes, is currently unknown, these proteins are required for transcriptional activation of T6SS-5 and the accurate assembly of the secretion apparatus during infection[37].

Tss core component proteins accumulate into three T6SS sub-complexes: a tubular system consisting of the contractile sheath proteins TssB and TssC surrounding an inner tube formed by the Hcp (tssD) protein sharpened at one end by the TssI (VgrG), an envelope spanning membrane complex (TssM, TssL, and TssJ) and a base plate (TssE, TssF, TssG, and TssK) anchoring tube and sheath to the membrane[35]. tssH, known as clpV, is a sheath cytosolic AAA+ ATPase protein that functions in recycling the contracted TssBC sheath proteins and thus improves the efficiency of T6SS. Tube polymerization and sheath coordination during T6SS expression was initiated by TssA[38,39]. The structure of *B. pseudomallei* T6SS-5 is illustrated in Figure 2.

The expression of *B. pseudomallei* T6SS-5 only occurs when the bacterium is inside the host cell. Wong *et al*. reported that signals for T6SS-5 activation occur in the host cytosol[28]. Chen *et al*. also reported the distinct dissemination of T6SS-5 genes of *B. pseudomallei* inside mammalian cells[36]. Furthermore, this T6SS-5 also has been discovered in systemic animal models as the dominant gene which contributes to respiratory melioidosis[40].

## 4. Cell fusion and multinucleated–giant cell (MNGC) formation induced by the T6SS–5 of *B. pseudomallei*

Intercellular spread of *B. pseudomallei* as well as its closely related non-virulent soil saprophyte *Burkholderia thailandensis* can directly arise after the MNGC formation despite the bacteria's exposure to the extracellular environment. The fusion of plasma membrane and consequent cytoplasmic combination of the cell infected with *B. pseudomallei* with one or more neighboring cells which are induced by the T6SS-5 subsequently results in MNGCs formation[4,33,41–43].

Valine-glycine repeat protein G (VgrG) is essential for cell-cell fusions or MNGC formation and subsequently results in intercellular spread of the bacterial infection[41,44,45]. Cell-penetrating equipment was formed by VgrG to export effector molecules into the targeted hosts[34]. VgrG-5, a novel secreted needle-like spike protein, harbors a conserved *C*-terminal domain amongst *B. pseudomallei*, *Burkholderia mallei*, and *Burkholderia thailandensis* as discovered by bioinformatics analysis[41].

According to Toesca *et al*. among the significant proteins secreted by T6SS-5 mechanisms are Hcp and VgrG[45]. Significantly, Hcp protein is the only T6SS-5 protein expressed in *B. pseudomallei* human infection as the recombinant Hcp protein is very immunogenic and can vigorously react with melioidosis patients' sera[34]. By activating the rest of the T6SS-5 downstream genes, the upstream promoter of *Hcp* gene will drive the superior expression of T6SS-5 machinery inside the mammalian cells[28,36].

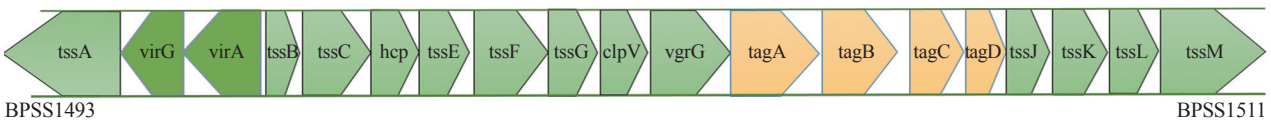


**Figure 1.** Genetic map of the T6SS-5 gene cluster with locus tags of BPSS1493 to BPSS1511 in chromosome two of *Burkholderia pseudomallei*. Green, dark green, and yellow color indicate tss genes, regulatory genes, and tag genes, respectively.

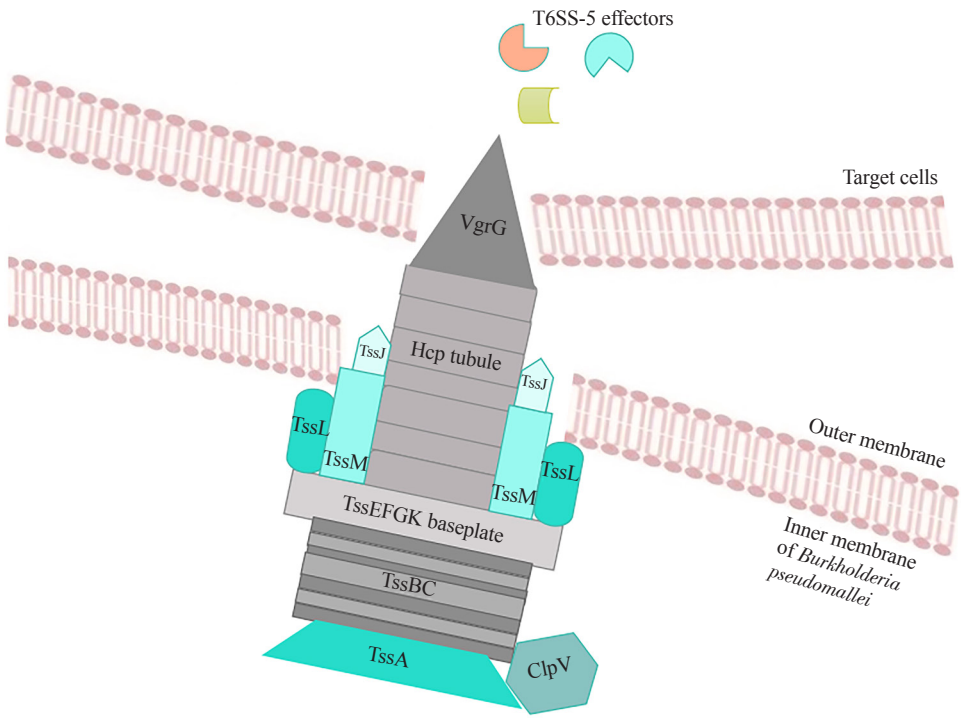


**Figure 2.** Schematic presentation of *Burkholderia pseudomallei* T6SS-5 structure. Shown here is the sheath contraction of Hcp tubule in which VgrG facilitates the penetration and secretion of T6SS-5 effectors into the target cells. The inner Hcp tubule and VgrG tip consist of TssBC contractile sheath and TssA cap protein with TssJ, TssM, and TssL membrane complex and TssEFGK baseplate complex. After the sheath contraction, the tubule structure is disassembled through the action of the ATP-degrading protein clpV.

## 5. The cGAS–STING pathway in innate immunity

The immune system defends the body from diseases by a complex system of biological processes *via* recognition and elimination of invading DNA microbes as well as making host cells inhospitable for their replication[46]. The prompt recognition of microbial invasion *via* immune sensors is crucial for innate immunity[47]. Various pattern recognition receptors have evolved in the mammalian innate immunity to detect damaged cells and pathogens-associated molecular patterns to rapidly trigger the host's defense mechanism[48].

In addition to its function as the life blueprint, DNA also has an important role in the first-line defense system by notifying the host cell of the occurrence of infectious microbial pathogens, foreign cells as well as malignant or damaged cells[49]. Nucleic acid pattern recognition receptor that is known as cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) (cGAMP) synthase (cGAS), together with stimulator of interferon genes (STING) are one of the vital components in the innate immune system[50]. Initially, the STING activation signaling was assumed to be expressed by the immediate sense in the presence of foreign DNA pathogens or damaged cells only. Nevertheless, as research on STING expands, cGAS was then recognized as the upstream protein that is also needed for activation of STING[51,52].

Generally, cGAS can rapidly initiate a downstream immune transcription cascade by promptly detecting DNA in different infectious environments as it is located in the cytoplasm, nucleus, and plasma membrane. Upon recognition of DNA in host cytosolic environment, cGAS generates 2'3'-cGAMP, the second messenger, which results in STING activation. Subsequently, 2'3'-cGAMP binds with STING and is transmitted to the Golgi complex from the endoplasmic reticulum and stimulates signaling cascades such as interferon regulatory factor 3 and TANK-binding kinase 1, leading to the synthesis of inflammatory cytokines, type Ⅰ interferons and other immune mediators[53–56]. The illustration of the cGAS-STING pathway is shown in Figure 3.

Studies on the cGAS-STING pathway have recently become a topic of growing interest. Many studies have collectively elucidated the cGAS-STING signaling pathway mechanisms and provided insight into its roles in innate immunity[46,52,57]. Researchers have determined the important role of this pathway in many pathological and physiological activities, including anti-cancer immunity, host defense against pathogen infections, and in chronic inflammatory and autoimmune diseases[58–61].

The cGAS-STING pathway that is associated with bacterial infections does not always function as a protective mechanism against pathogens, as it occasionally facilitates the survival and replication of the germs[62–64]. It has also been reported to be responsible for protecting against the bacterial infection that causes infectious diseases such as melioidosis, pneumonia, and tuberculosis, as well as against virus infection[47].

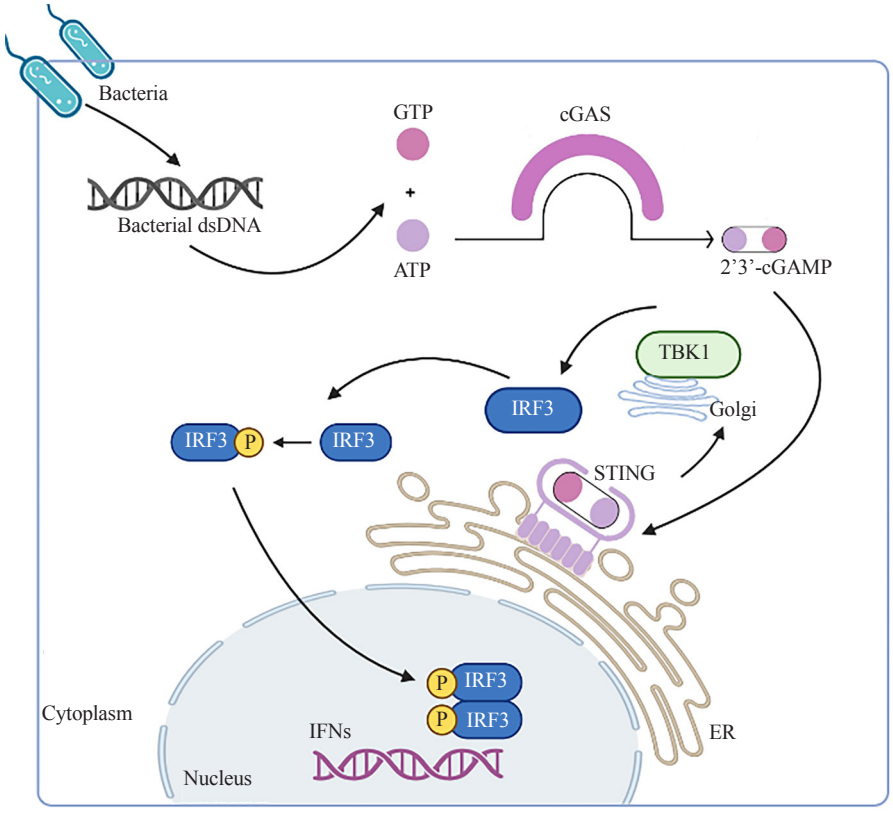


**Figure 3.** The cGAS-STING pathway in innate immunity. Bacterial DNA is sensed by cGAS and subsequently stimulates the catalyzation of ATP and GTP to 2'3'-cGAMP which leads to STING activation. It binds to STING and translocates STING from ER to Golgi and stimulates signaling of TBK1 and IRF3. TBK1 phosphorylates and translocates IRF3 into the nucleus, which induces the synthesis of IFN-1 and other immune mediators. ATP: Adenosine triphosphate; GTP: Guanosine triphosphate; ER: Endoplasmic reticulum; TBK1: TANK-binding kinase 1; IFN-1: Type 1 Interferon; IRF3: Interferon regulatory factor 3.

## 6. The cGAS–STING pathway in *B. pseudomallei* infection

The cGAS-STING pathway in bacterial infections is more varied and complex compared to its function in viral infections. It is commonly involved in bacterial infection in numerous Gram-positive and negative bacteria. Nevertheless, the signaling transduction process and its activation consequence are different in each bacterial species. Besides that, the host's protective and disadvantageous consequences of IFN-1 depends on the species of bacteria and the mode of infection. Furthermore, independent of the IFN-1 response, the activation of STING can also affect the prognosis of disease *via* other mechanisms in different bacterial infections[46].

Despite its defensive role against bacterial infections and inhibition of over-stimulation of the host immune response, the cGAS-STING pathway has been surprisingly found to increase susceptibility for some bacteria species. This is due to the impaired anti-bacterial immunity induced by IFNs which support bacterial pathogens. A surprising pattern of cGAS-STING pathway activation was reported in the infection of *Burkholderia* species. During chronic infection, the formation of cell-cell fusion or MNGC by *B. pseudomallei* has been conventionally considered a Trojan horse-like approach in which the bacteria can escape from host defense mechanism whilst permitting intracellular spreading of the bacteria within the host cell[65].

Contrary to this well-acknowledged prototype, Ku *et al*. discovered that MNGCs are not quiescent things that can be overlooked by the host immune responses[26]. The bacterial T6SS-5-dependent cell fusion triggers the host DNA sensing cGAS-STING pathway activation, and consequently leads to autophagic cell death and cell release[26]. By deploying the caspase system, *B. pseudomallei* affects the immune function, thus causing high morbidity and mortality in melioidosis cases[46].

Ku *et al*. demonstrated that dependent cell-cell fusion or MNGC formation conferred by T6SS-5 in *B. pseudomallei* could lead to formation of micronuclei and genomic instability or DNA damage which then triggers the expression of *IFN–1* gene in a host cell. *IFN–1* gene expression will contribute to the cytosolic DNA sensor cGAS pathway activation in host cells[26]. As a host defense mechanism, the cGAS pathway and the downstream STING act together to mediate innate immune signaling pathways. Apart from its association with pathogen infections and inflammatory diseases, cGAS-STING-mediated signaling is receiving more attention for its other functional response such as its complication with cell death pathways[24]. During cell fusion, the activation of this immune system might affect or contribute to the progression of *Burkholderia* infection in the host[24].

Micronuclei formation serves as a threat to the host, as it potentially constrains cellular transformation by the activation of cGAS-STING pathway and autophagic cell death. Genomic instability or DNA damage due to the formation of micronuclei caused by T6SS-5-driven cell fusion initiates the activation of host cell signaling pathway which results in cell death, as shown in Figure 4. Micronuclei are small membrane-bounded compartments that contain DNA and are isolated from the primary nucleus by a nuclear envelope. Chromosome instability, genome rearrangements, and mutagenesis have all been related to micronuclei for quite some time[66].

Following cGAS-STING pathway activation and an efficient IFN-1 immune response colocalizing with MNGCs formation due to extensive cell fusions, autophagic cell death is induced to minimize the cellular transformation and abnormal cell division as well as to eliminate cytosolic pathogens. Autophagy is a regulated catabolic process that involves a cell self-digestion process *via* the action of autophagosomes together with the enzymes synthesized within a similar cell's lysosome[67,68].

Autophagosome is a double-membrane vesicle that contains damaged DNA and organelles like mitochondria, bulk cytoplasm, and protein aggregates. It engulfs cytoplasmic components which then fuse with lysosomes to degrade and recycle their components. Lysosomal enzymes digest captured materials and release the digested products from the cell as shown in Figure 5. In cGAS-STING signaling, autophagy has dual roles including the initiation of inflammatory immune response and degradation of STING.

## 7. *B. pseudomallei* T6SS–5–dependent cell fusion triggers *IFN–1* gene expression

The secretion of IFN-1 by the cells infected with pathogens causes various consequences on innate and adaptive immunity, either indirectly or directly *via* the stimulation of supplementary mediators[69,70]. IFN-1 has three major functions, which include limiting the spread of infectious agents in infected and neighboring cells by inducing cell-intrinsic antimicrobial states, promoting spontaneous cell killer functions and antigen presentation, as well as hindering cytokine and pro-inflammatory pathways production by modulating innate immune responses. Besides, by acting as an adaptive immune system, IFN-1 stimulates immunological memory as well as high-affinity antigen-specific T and B cell responses[69].

At an early stage of microbial infections, reduced levels of IFN-1 might be needed to induce host cell-mediated immune responses. On the other hand, increased IFN-1 concentrations might inhibit B cell responses or stimulate the synthesis of immunosuppressive particles, as well as decrease the sensitivity of macrophages to IFNγ's activation, which was reported in infections of *Mycobacterium tuberculosis* and *Listeria monocytogenes*[70].

In *B. pseudomallei*, T6SS-5-dependent phenotype during infection in RAW264.7 macrophages has been identified using various T6SS-5 mutants which include ΔclpV, ΔHcp, and ΔvirAG mutants. Ku *et al*. reported that at 16 h after infection, RAW264.7 macrophage infected with T6SS-5 mutants had approximately ten-fold lower expression level of IFN-β compared to the cells infected with *B. pseudomallei*.

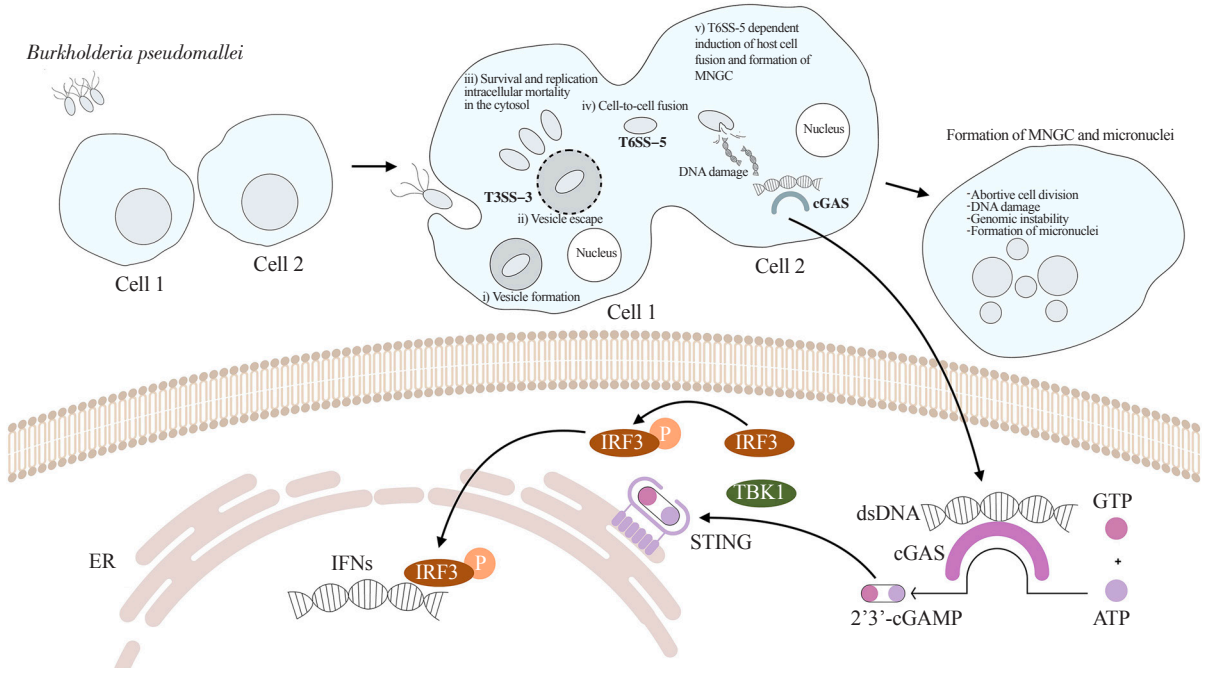


**Figure 4.** T6SS-5-dependent cell fusion triggers *IFN–1* gene expression. Cells infected with T6SS-5 in *Burkholderia pseudomallei* encounter cell-cell fusion which leads to MNGC and micronuclei formation. Subsequent abortive cell division, genomic instability, and DNA damage trigger the immune response and subsequent activation of the cGAS-STING pathway and induction of IFN-1 in the host cell. MNGC: Multinucleated giant cell.

*Burkholderia* defective in T6SS5 induces significantly lower *IFN–1* gene expression, suggesting that the *IFN–1* gene expression is dependent on the cell-cell fusion event. The catastrophic event of cell fusion results in aberrant and abortive mitosis, which leads to DNA damage and genomic instability. Interestingly, instead of producing IFN-1, the cGAS-STING in *B. pseudomallei* leads to transcriptional changes of IFN-1 and subsequent autophagic cell death[26].

The *Burkholderia*'s fusogenic roles of the extended carboxyl-terminal domain (CTD) VgrG-5 are responsible for cell-cell fusion and MNGC formation[45]. Therefore, the MNGCs formation did not occur in the RAW264.7 cells infected with the defective fusion of vgrG5-ΔCTD mutant. Concordantly, a low level of *IFN–β* gene expression in vgrG5-ΔCTD mutant compared to *Burkholderia thailandensis*-infected cells also implies that the *IFN–1* gene expression is regulated by cell-cell fusion[26]. These findings suggested that the cGAS-STING pathway is critical in *Burkholderia* infection for enhanced cytotoxicity and lessening intracellular bacterial loads during cell fusion[65].

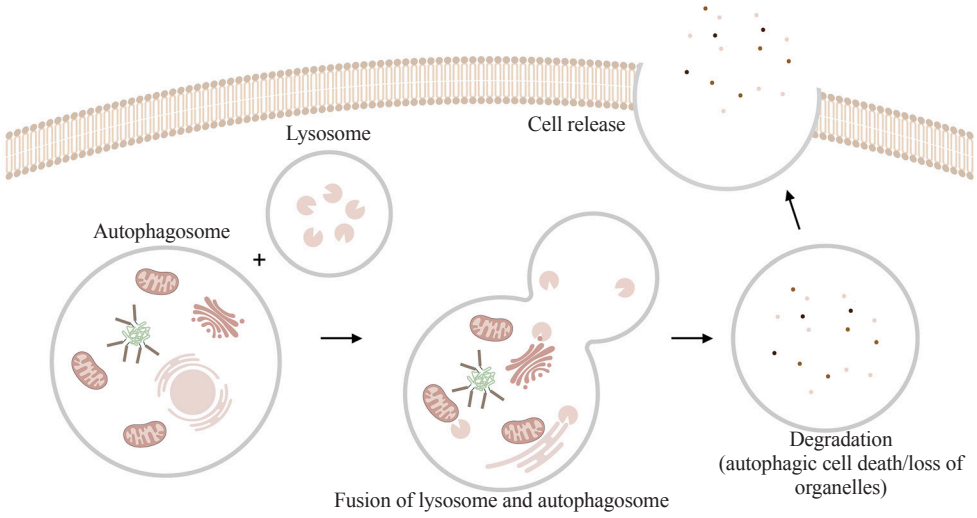


**Figure 5.** Autophagic cell death of the host cell in *Burkholderia pseudomallei* infection after dependent T6SS-5's cell fusion and cGAS-STING signaling. Autophagy and elimination of cytosolic pathogens occur upon activation of the cGAS-STING pathway and an efficient IFN-1 immune response colocalizing with the formation of micronuclei due to extensive cell fusions.

**Table 2.** Summary of the mechanisms involved in *Burkholderia pseudomallei* infection and host cGAS-STING immune response.

| Key point | Mechanisms | Significance |
|---|---|---|
| T6SS-5 in *Burkholderia pseudomallei* virulence | Comprises of bacteriophage tail-like injectisome and base plate complex whose expression only occurs when the pathogen is inside the host cell | A vital part of intercellular spread and virulence of *Burkholderia pseudomallei* in the host cell |
| Cell fusion and MNGC formation induced by *Burkholderia pseudomallei* T6SS-5 | VgrG protein, the needle spike protein, is required as cell-penetrating equipment. Then, the inner Hcp tubule exports effector molecules into the targeted cells. These induce plasma membrane fusion and cytoplasmic combination | Cell fusion leads to MNGC and micronuclei formation of the infected host cells |
| The cGAS-STING pathway in innate immunity | cGAS senses the cytosolic dsDNA which then catalyzes 2'3'-cGAMP and activates STING attached in ER. It binds to STING and translocated to Golgi which then induces the signaling of TBKI and IRF3, leading to IFN-1 production | Involved in bacterial infections, not just for the protection against pathogens but sometimes supporting the survival and replication of some bacteria species |
| The cGAS-STING pathway in *Burkholderia pseudomallei* infection | T6SS-5-dependent cell-cell fusion triggers *IFN–1* gene expression in the host and activates the cGAS-STING pathway | Genomic instability and the formation of micronuclei resulting from T6SS-5-driven cell fusion trigger the host to activate a signaling pathway, leading to cell death |
| *Burkholderia pseudomallei* T6SS-5-dependent cell fusion triggers *IFN–1* gene expression | Smaller cytosolic invasions by pathogens result in an efficient IFN-1 immune response and autophagy that could serve to remove cytosolic pathogens upon cGAS activation. However, extensive cell fusion is a catastrophic event resulting in aberrant and abortive mitosis, which leads to DNA damage and genomic instability | This triggers autophagic cell death instead of a productive type Ⅰ IFN response, with the end goal of limiting cellular transformation |

## 8. Conclusion

This review paper highlights the significance of the association of T6SS-5 gene cluster and the cGAS-STING pathway in *B. pseudomallei* infection. Mechanisms involved in *B. pseudomallei* T6SS-5 infection and host cGAS-STING immune response are summarized in Table 2. It is concluded that the T6SS-5 enhances the competency of *B. pseudomallei* to thrive and survive in targeted host cells. This could elucidate the pathogenesis of *B. pseudomallei* infection in many mammalian species which prompts melioidosis. Unlike other intracellular bacteria, T6SS-5 of *B. pseudomallei* induces host cell fusion and manipulates the host immune response *via* the cGAS-STING pathway. The exclusion and absence of necessary T6SS-5 genes significantly decrease the virulence and acute infection caused by the bacterium in mammalian species. Nevertheless, it is yet to be anticipated whether patients with chronic melioidosis will be more prone to cancer, and if it happened to be so, the cGAS-STING signaling or autophagy pathways mutations in these patients should be considered as unwanted cellular fusions that attenuate the body's natural defense mechanisms. Therefore, more research is needed for a better understanding of the evolutionary T6SS-5 that makes *B. pseudomallei* such a devastating pathogen and for finding new possible solutions to counter this situation.

## Conflict of interest statement

The authors declare that there is no conflict of interest.

## Acknowledgments

We would like to thank Miss Ilia Elena for her time to proofread the manuscript.

## Funding

This study was supported by the Ministry of Higher Education Malaysia for Fundamental Research Grant Scheme with Project Code: FRGS/1/2019/SKK11/USM/02/5.

## Authors' contributions

NS and NZNMN were involved in conceptualization of the review. NS and NZNMN were responsible for writing the manuscript. YKMI contributed to preparing the figures. YKMI, NIM, AH, IA and ZZD reviewed and edited the manuscript. All authors read and approved the final draft of the manuscript.

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## Publisher's note