Comment

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Suppressing host pyroptosis by a ubiquitin-activated phospholipid phosphatase of *Mycobacterium tuberculosis*

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SUMMARY Tuberculosis (TB) is a major public health problem that causes millions of deaths in humans around the world, and the bacterial pathogen *Mycobacterium tuberculosis* (Mtb) is responsible for this disease. Evidence suggested that the inflammasome-pyroptosis pathway is crucial for preventing Mtb infection. Uncertainty exists regarding whether and how these infections can bypass this immune system by Mtb. A recent *Science* article by Chai *et al*. (doi: 10.1126/science.abq0132) revealed a novel role by a eukaryotic-like effector called PtpB during Mtb infection. The PtpB functions as a phospholipid phosphatase suppressing gasdermin D (GSDMD) dependent pyroptosis. And notably, the phospholipid phosphatase activity of PtpB is dependent on binding with mono-ubiquitin (Ub) of the host.

Keywords *Mycobacterium tuberculosis*, phospholipid phosphatase, ubiquitination, pyroptosis

Mycobacterium tuberculosis (Mtb) is a fatal bacterial pathogen that causes tuberculosis (TB) in humans (*1*). It is estimated that one-third of the global population has been infected with Mtb (*2*). In 2021, a total of 1.6 million people died from TB worldwide (*3*). The inflammasome-pyroptosis pathway is critical for immune defense when the host encounters the invasion of pathogenic microorganisms like Mtb (*4,5*). However, it remains unclear whether and how these infections are able to bypass this immune system. Therefore, it is anticipated that understanding how the major effectors of Mtb and other pathogens manipulate the host inflammasome-pyroptosis pathway may lead to the identification of novel anti-TB targets and intervention techniques.

In a recent study, by using a recombinant system of absent in melanoma 2 (AIM2) and NOD-like receptor protein 3 (NLRP3) inflammasomes in HEK293T cells, Chai *et al*. carried out a comprehensive screening of Mtb-encoded eukaryotic-like secretory proteins. They discovered that the Mtb-secreted protein phosphatase PtpB which can inhibit host inflammasome-pyroptosis pathway. The authors present a model that PtpB dephosphorylates host plasma membrane components phosphoinositides phosphatidylinositol-4-monophosphate (PI4P) and phosphatidylinositol-(4,5)-bisphosphate [PI(4,5)P2], thus disrupting N-terminal cleavage fragment of GSDMD (GSDMD-N) dependent inflammatory cytokine release and pyroptosis (Figure 1). And surprisingly, the phosphatase activity of PtpB is activated by binding ubiquitin (Ub) of the host (*6*).

The membrane of host could be a target on the battlefield of pathogen-host interactions. Other groups have reported that in *Legionella pneumophila*, effectors phosphatidylinositol (PI) 3-kinase MavQ and PI 3-phosphatase SidP dynamically remodel the membrane of the host (*7*). And in *Shigella flexneri*, effector IpgB1 binds to acidic phospholipids and regulates actin filament dynamics (*8*). However, whether Mtb could also invade the host by targeting its membrane, and whether there is a link among bacterial effector, host membrane and inflammasome-pyroptosis, if the answer is yes, the underlying mechanism had yet to be addressed. The first highlight in the work of Chai and colleagues is that they answer this question and present the evidence for how Mtb disrupts the membrane components PI4P and PI(4,5)P2, both of them are reported to be important for GSDMD-N mediated pyroptosis (*9,10*). The authors showed that expression of PtpB prominently reduced the membrane localization of GSDMD-N and deletion of *ptpB* of Mtb significantly increased the translocation of GSDMD-N to the plasma membrane of the host.

The second highlight in this work is that they elucidated the novel function mechanism of effector PtpB. Although there are several studies about PtpB before (*11-15*), as a phosphatase, *in vitro* conditions, the phosphatase activity of PtpB is not easily detectable (*16,17*), which implied the activity of PtpB might be regulated *in vivo*. Chai *et al*. first showed that PtpB dramatically decreased the amounts of PI4P and PI(4,5)P2 in the plasma membrane of the host. Furthermore, the

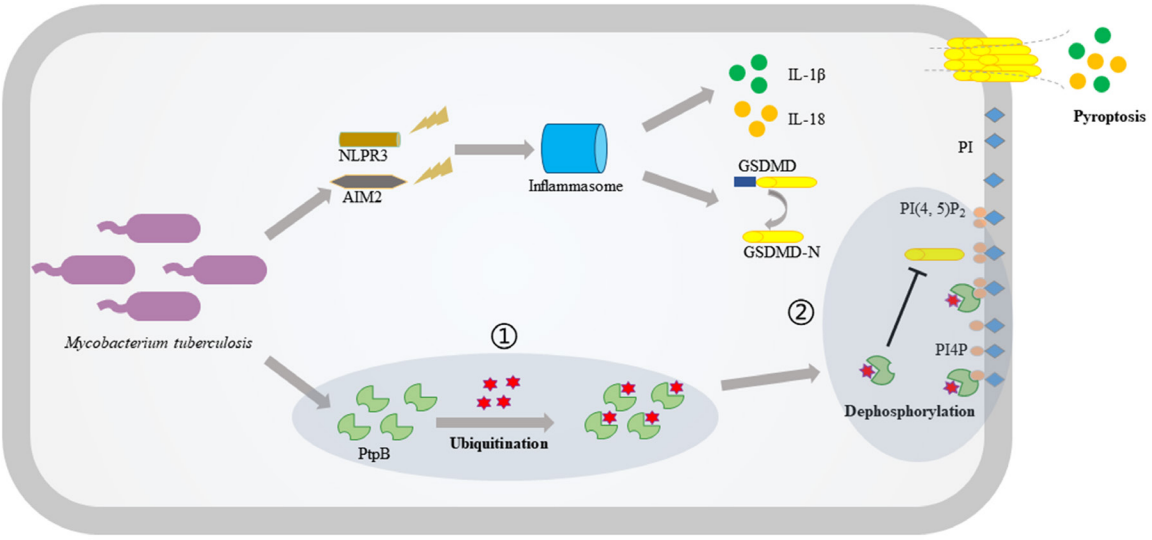

Figure 1. Schematic diagram of PtpB mediated GSMND-dependent pyroptosis during Mtb infection. Firstly, the PtpB secreted by Mtb exploits the Ub of the host to enhance its phospholipid phosphatase. Secondly, the activated PtpB dephosphorylates PI4P and PI(4,5)P2, which disrupts GSMND-N to localize the membrane to form a pore. Thereafter, the release of cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18) from the pore and as well as the pyroptosis are inhibited. The numbers with circles show the two key processes that PtpB is involved in, *i.e.*, ubiquitination and dephosphorylation.

author's structural analysis showed that PtpB possesses an ubiquitin interacting motif (UIM)-like region, next they proved that only mono-Ub, rather than poly-Ub can bind to PtpB and then activates its activity. It is assumed that upon binding with Ub, PtpB would undergo a conformational change to expose its active site for substrate binding. And interestingly, it seems that the activity of UIM-like domain containing enzyme is stringently regulated by Ub, the author's previous work has shown that another secreted tyrosine phosphatase PtpA of Mtb could also suppress innate immunity by binding ubiquitin of the host *via* its UIM-like domain (*18*). Nevertheless, their targets are quietly different, which implied the versatile roles of the homologous phosphatases. Thus, this work presents us with a novel role of Ub activated PtpB by targeting the host's membrane to inhibit pyroptosis.

However, there are still a few unanswered questions. Firstly, it remains unknown why only mono-Ub rather than poly-Ub activates PtpB, and how mono-Ub is added to PtpB, whether this process is achieved by the canonical ubiquitination pathway of the host (*19*), or whether there is a novel enzyme that can ubiquitinate PtpB with mono-Ub, finding the enzyme(s), and the underlying mechanism of the particular ubiquitination process awaits further study in details. Secondly, the authors showed that except for IL-1β and IL-18, PtpB also suppressed macrophages' production of tumor necrosis factor (TNF-α) and IL-6, in a way that was independent of the GSDMD, which is consistent with previous studies that PtpB also inhibits non-inflammasome immune pathways (*14,15*). The increased intracellular survival of Mtb by PtpB could be a combinational outcome of the inflammasome and non-inflammasome immune pathways. Along those lines, it is possible that there is a balance between these two different pathways, and it also raises the question that what is regulator(s) that influence PtpB to function, by inflammasome or non-inflammasome immune pathways? Currently, at least we know the Ub is a determinant of PtpB-mediated inflammasome immune pathways. Whether other regulator(s) exist, and how are they regulated remains to be explored.

From a perspective of development of anti-TB therapeutics, the finding of the novel role of PtpB is valuable and it shows great potential. Firstly, the authors showed that GSDMD could confer robust host protective immune responses against infection. And when Mtb lacks functional PtpB, it cannot counteract host GSDMD-mediated immunity. It is possible that PtpB could be a desirable target for enhancing the immunological efficacy of Bacille Calmette-Guérin (BCG), since it possesses an identical PtpB-encoding gene with Mtb. Secondly, PtpB might be a promising anti-TB target for drug design, because there is no homologous protein of PtpB in humans, which implied excellent selectivity. Based on the two key processes PtpB is involved in, i.e., ubiquitination and dephosphorylation, anti-TB strategies could be developed. Small molecule compounds that block the novel found ubiquitin binding site (Ala240 -Ala242) or key enzyme active site (Cys160) of PtpB might abrogate PtpB's immune system suppression. Thirdly, PtpB could also be an intriguing target by using newly-developed proteolysis targeting chimeras (PROTACs) technology (*20*). Degrades PtpB through the ubiquitin-proteasome system could abolish PtpB mediated pyroptosis inhibition.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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