A novel pathway of immune and host cell evasion by Mycobacterium tuberculosis

Mycobacterium tuberculosis (Mtb), is a pathogenic organism causing infection and a disease phenotype which results in millions of deaths per year. Despite what is known about this organism, researchers and clinicians are still looking for answers as to how this ingenious pathogen evades the host immune response and adapts to its human host, leading to subsequent infection. It is known that immune cells of the host take up Mtb through phagocytosis, however Mtb persists through several mechanisms of manipulation, leading to its survival in the host.

In an interesting finding, researchers from the University of Maryland and the National Institutes of Health in the USA have discovered a novel pathway of host cell manipulation and innate immune evasion by Mtb.

Through manipulation of macrophage functions, Mtb has successfully evolved to evade its host's innate immune response. Macrophages secrete Interleukin-1β (IL-1β) after the activation of the inflammasome complex which is crucial for the host cell defence against Mtb infections. Previous studies have highlighted the importance of IL-1β for host resistance against infections with Mtb, whereby in specific studies using mice, those with a deficient expression for IL-1α or IL-1β or the IL1R1- receptor demonstrated an increased susceptibility

to Mtb infection.

The inflammasome complex consists of the specific Nucleotide binding and oligomerization domain-like receptor (NLR) or AIM2-like receptors (ALR), an adaptor protein ASC in many but not all inflammasomes and finally the protease caspase-1 (Casp1) which will get activated once the full inflammasome complex has formed. Through various mechanisms induced by the complex, the immature, pro-IL-1β gets cleaved to generate the truncated, mature IL-1β which is released by the cell. The inlammasome, upon activation, may also lead to pyroptosis which is regulated by the cleavage of gasdermin D (GSDMD) by activated Casp1 or Casp11. The cleaved GSDMD will produce pores in the cell membrane which will lead to pyroptosis. Pyroptosis. Pyroptosis can be characterized as a Casp1-dependent formation of plasma-membrane pores, leading to cellular lysis and the release of inflammatory intracellular contents.

It has been previously shown that Mtb can cause inhibition of AIM2 inflammasome activation and subsequent pyroptosis. Rastogi et al., has shown in this study, that Mtb is also able to inhibit host cell NLRP3 inflammasome activation and pyroptosis. Through the use of Mtb mutants and stimulated bone marrow-derived macrophages (BMDMs), Rastogi et al., were able to identify PknF, a serine/threonine phosphokinase, as one Mtb component involved in mediating NLRP3 inflammasome inhibition. They successfully demonstrated that PknF is needed for inhibition of the inflammasome which was independent of the *pknF* Mtb mutant inducing more NLRP3-inflammasome activation when compared to wild-type Mtb. The *pknF* deletion mutant of Mtb induced increased production of IL-1β in BMDMs. *The increased production of IL-1β was dependent on NLRP3, the adaptor protein ASC and the protease caspase-1. Infection of BMDMs with the pknF deletion mutant resulted in increased pyroptosis, while the IL-6 production remained unchanged compared to Mtb infected cells, suggesting that the mutant did*

not affect the priming step of inflammasome activation. In contrast, the activation step was affected since potassium efflux, chloride efflux and the generation of reactive oxygen species played a significant role in inflammasome activation and subsequent pyroptosis mediated by the Mtb pknF mutant strain.

PknF belongs to the 11-member family of serine/threonine protein kinases in Mtb and is affiliated with its cellular membrane. Therefore, it is unlikely that there is a direct interaction of PknF with the activation pathway of the inflammasome of the host. It has been shown that PknF is capable of phosphorylating many Mtb proteins but the best described target is the ABC-like transporter protein Rv1747, a protein *important for virulence of Mtb*.

To conclude, Rastogi et al., have identified that the serine/threonine kinase PknF of Mtb *plays an important role in innate immune evasion through inhibition of the NLRP3 inflammasome.* Demonstrating that the Mtb serine/threonine phosphokinase PknF is important for the inhibition of the NLRP3 Inflammasome. In addition, they have *further investigated the Mtb-mediated manipulation of the host cell inflammasome and discovered that Mtb can inhibit NLRP3-inflammasome activation via a mechanism that does not require its ESX-1 type VII secretion system.*

Following the identification of this novel pathway the authors have said, in their own words;

"We show that the activation of a host cell defense complex, the inflammasome, is limited after Mtb infection. Most importantly, we identify a bacterial protein, PknF, that is involved in inflammasome inhibition."

For the investigation of the complex role of IL-1β in the host response to Mtb, the NLRP3-inflammasome activating *pknF* Mtb mutant used in this study may become a powerful tool.

Journal Article: Rastogi et al., 2021. *Mycobacterium tuberculosis inhibits the NLRP3 inflammasome activation via its phosphokinase PknF*. PLOS Pathogens.

Summary by Stefan Botha