concern that people undergoing antibiotic treatment may not respond equally to NR supplementation. It would be interesting to investigate the contribution of the microbiome to NR-induced NAD biosynthesis in the context of varying NR concentrations and different gut microbiota.

This study elegantly demonstrates how metabolic labeling can be used to chip away at our understanding of the complex interactions between the gut microbiome and host in NAD biosynthesis. It stimulates innumerable questions about how the gut microbiome impacts host NAD metabolism and disease. Aging is associated with a decline in NAD levels and immune function and changes in the gut microbiome.[3,6] Understanding the causal relationships between these interlinked associations will be critical in finding therapies that promote healthspan. Using this technique to investigate the contribution of different bacterial strains or patient microbiota on NAD metabolism in different host cells (i.e., immune cells) with different diets, with varying doses of NAD precursor supplementation, and in distinct disease contexts could be instrumental in uncovering the dynamic interactions that underpin this complex host-microbiome symbiotic relationship.

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Putting the p(hosphor) in pyroptosis

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A recent study in *Science* found *Mycobacterium tuberculosis* inhibits pyroptosis of the host cell by secreting a phosphatase (PtpB). PtpB targets the plasma membrane to dephosphorylate PI4P and PI(4,5)P_2, inhibiting recruitment of the pore-forming gasdermin D N-terminal fragment. Pyroptosis inhibition contributes to virulence, as *ptpB*-deficient Mtb is attenuated in mice.

Inflammasomes are multi-protein complexes designed to recognize cytosolic danger/pathogen-associated molecular patterns (D/PAMPs) and activate the innate immune system. Two well-studied sensor components of inflammasome complexes are AIM2, which binds to double-stranded DNA, and NLRP3, which senses intracellular DAMPs (Figure 1).[1] Activated NLRP3 or AIM2 bind to the adaptor protein ASC for subsequent recruitment and autolysis of the zymogen protease, pro-caspase-1 (Figure 1).[1] A major consequence of inflammasome-activated caspase-1 is the cleavage of latent, immature forms of unconventionally secreted cytokines, such as pro-IL1β, and gasdermin D (GSDMD) to generate an N-terminal fragment (GSDMD-NT) that forms pores in the plasma membrane (PM) through which mature IL-1β is secreted. These events can ultimately lead to the rupture of the PM via NINJ1 oligomers in an inflammatory death process termed pyroptosis (Figure 1).[1]

Mycobacterium tuberculosis (Mtb) is a strict human pathogen that constitutes a major burden on global public health.[2] Prior reports demonstrate that, depending on time and context, IL-1β has a protective role in host defense against Mtb in humans and the mouse model of tuberculosis.[2] It is thus counterintuitive that the deletion of mouse inflammasome components important for the activation of the inflammasome complex, such as the adaptor protein ASC, have no significant effect on host resistance after Mtb infection.[2] These data could be interpreted as an absence of function of inflammasome complexes for host protection. Alternatively, one could propose that, being a highly host-adapted pathogen, Mtb has evolved ways to inhibit host cell inflammasome recognition and/or activation and that, therefore, creating inflammasome-response-deficient mice does not make a difference to the host response anymore. Evidence in support

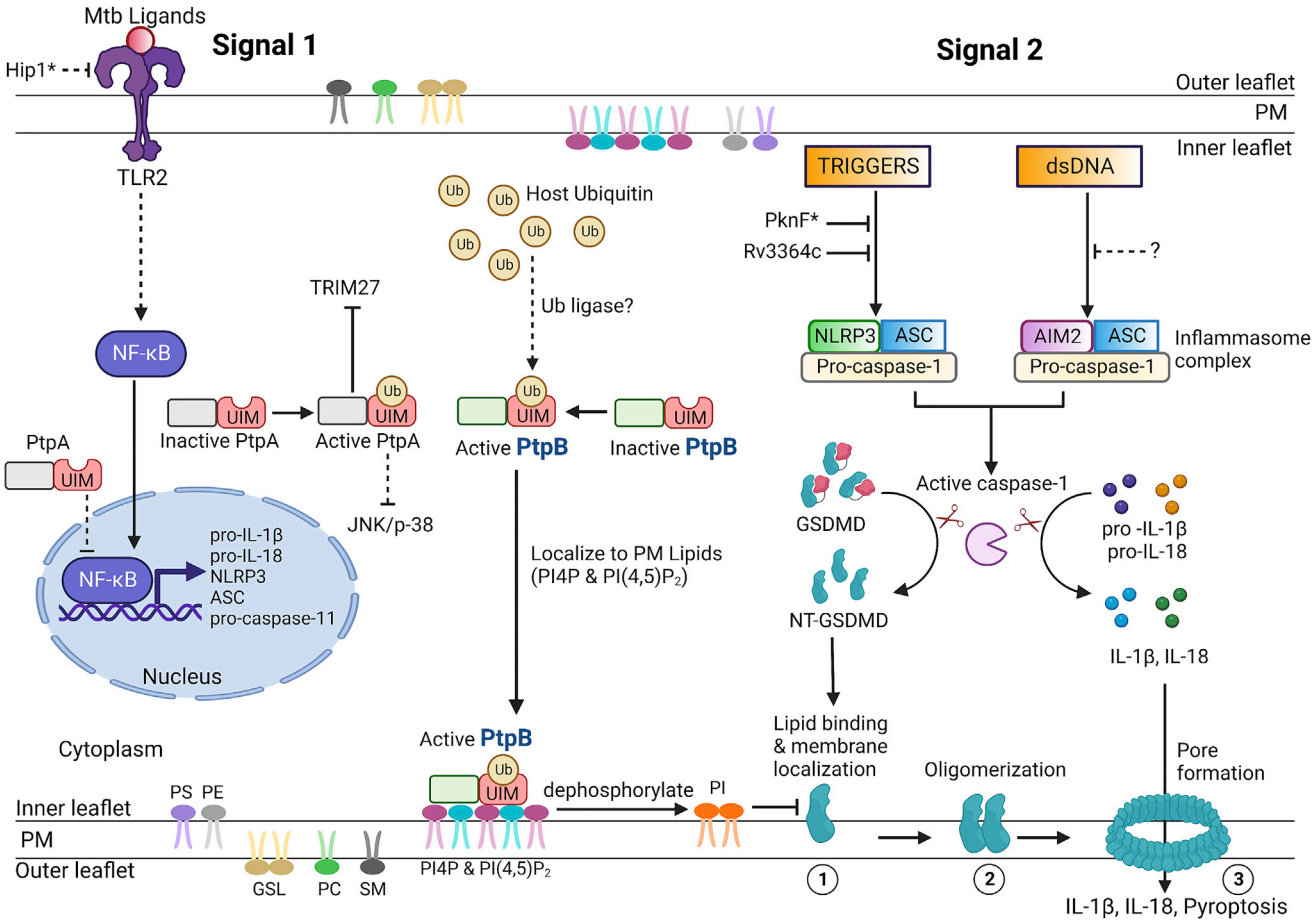

Figure 1. Mycobacterial effectors inhibit different steps of the inflammasome signaling pathway
Several Mtb effectors either secreted (PtpB or Rv3364c) or non-secreted (Hip-1 or PknF, as indicated by *) are involved in evasion of inflammasome-mediated secretion of pro-inflammatory cytokines and pyroptosis. Unknown Mtb effectors are represented by ?. Both PtpA and PtpB are activated by binding of host ubiquitin to a UIM-like domain. In contrast to PtpA, once activated, PtpB localizes to specific PM lipids PI4P and PI(4,5)P_2, thereby dephosphorylating to PI, and thus inhibits the binding and membrane localization of activated GSDMD-NT fragment. Dashed lines, indirect interaction; solid lines, direct interaction; arrowhead, activation; blunt end, inhibition). TLR2, toll-like receptor 2; NF-κB, nuclear factor kappa B; PtpA, protein tyrosine phosphatase A; PtpB, protein tyrosine phosphatase B; PM, plasma membrane; Ub, ubiquitin; UIM, ubiquitin-interacting motif; TRIM27, tripartite motif-containing protein 27; JNK, Jun N-terminal kinase; PknF, protein kinase F; NLRP3, nucleotide-binding domain (NOD)-like receptor protein 3; AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a CARD; GSDMD, gasdermin D; NT- GSDMD, N-terminal gasdermin D fragment; P14P, phosphatidylinositol 4 phosphate; PI(4,5)P_2, phosphatidylinositol 4,5 bisphosphate; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; GSL, glycosphingolipids. Created with https://biorender.com/

of this hypothesis is that Mtb expresses several proteins that are involved in inhibiting host cell inflammasome activation (Figure 1).[2] Hip1 of Mtb limits host cell TLR-2 signaling, which may inhibit typical signal 1 transcriptional and non-transcriptional "priming" events prior to inflammasome triggering.[2] Moreover, Rv3364c indirectly inhibits caspase-1 activation,[2] and Mtb PknF inhibits NLRP3 activation (Figure 1).[3] Mtb also inhibits the AIM2 inflammasome upstream of inflammasome complex formation via an unknown mechanism (Figure 1).[4] Adding to the compendium of bacterial strategies to hide from and inhibit inflammasome signaling, a recent study from Chai et al. in *Science* now adds several Mtb proteins (Rv0153c/PtpB, Rv0561c, Rv0824c/DesA1, Rv0861c/Ercc3, Rv1515c, and Rv1679/FadE16) as putative effectors that limit IL-1β secretion and pyroptosis.[5] The authors performed an elegant screen based on the capacity of a protein out of 201 screened Mtb proteins to suppress inflammasome activation and, more precisely, IL-1β secretion in model HEK293T cells.[5] Chai et al. created a mutant that activates pyroptosis more strongly by deleting the phosphatase B (*ptpB*) in Mtb and demonstrate that this mutant (Δ*ptpB* Mtb) is attenuated in mice. Importantly, the attenuated phenotype of the deletion mutant is dependent on the presence of the host cell inflammasome substrate GSDMD, which is the target of PtpB since in *gsdmd*$^{-/-}$ mice, wild-type Mtb and Δ*ptpB* Mtb have the same virulence. These data support a model in which host cell inflammasome activation can mediate a protective response, but Mtb may inhibit inflammasome activities, namely GSDMD pore formation, to promote virulence. The mechanisms of the protective host inflammasome-dependent immune response in the context of Mtb infections will be of interest in future studies and may help to establish correlates of

protection for vaccine development and novel approaches for targeted host therapeutics.

Another major finding by Chai et al. was the demonstration that PI4P and PI(4,5)P_2 levels in the PM are critical for binding of GSDMD-NT.[5] Previous work shows that GSDMD-NT binds to PI4P and PI(4,5)P_2 but also phosphatidylserine (PS) and cardiolipin (found in mitochondria), suggesting that the lack of PI4P/PI(4,5)P_2 could be compensated by the presence of PS in the PM.[6,7] Why this is not the case in the work by Chai et al. could be due to binding affinities of GSMDM-NT, which seem to be stronger to PI4P/PI(4,5)P_2 compared to PS.[6,7] These differences in binding affinity might be even more pronounced in the context of binding at the PM when compared to the model bilayer systems such as liposome-based assays. Clearly, the work by Chai et al. shows that GSDMD-NT recruitment can be regulated by phosphatases targeting PI4P/PI(4,5)P_2 and inhibitors blocking synthesis of these PIPs. Chai et al. demonstrate this regulation by using a bacterial phosphatase from Mtb, but this may suggest that similar regulation may occur via the expression of host cell phosphatases targeting PI4P/PI(4,5)P_2. Based on the presence of PI4P/PI(4,5)P_2 in other subcellular membranes, whether GSDMD-NT can target other organelles is of interest. Chai et al. demonstrate an effect on PM GSDMD-NT in part by showing that Mtb PtpB does not affect Golgi apparatus function or PI4P levels found at this subcellular site. Nevertheless, there are other compelling organelles that await further investigation for PI4P/PI(4,5)P_2 -dependent targeting of GSDMD-NT. For example, while mitochondria are already known to be targeted by GSDMD-NT likely due to cardiolipin externalization,[1] whether other lipid constituents dictate GSDMD-NT organelle targeting is understudied. Moreover, the potential effect of other host and microbial lipid-handling enzymes in dictating pore location and activity should be investigated. While *Shigella flexeneri* has an effector (IpaH7.8) that targets GSDMD pore formation at the level of degradation of the intact protein,[8] Mtb employs a unique strategy of targeting lipid specificity and abundance to limit host-derived pores.

Chai et al. show that PtpB's phosphatase activity is increased by ubiquitination. Elegant work involving a PtpB point mutant within its unique ubiquitination motif that was unable to be ubiquitinated demonstrated a lack of activity in decreasing host cell PM PI4P/PI(4,5)P_2 levels, and consequently, the mutant protein failed to inhibit pyroptosis induction. This is not the first example of an Mtb protein being ubiquitinated by the host cell; indeed, the sole other secreted phosphatase of Mtb (PtpA) is also ubiquitinated.[9] This ubiquitination is required for activity in host cells that inhibit TRIM27-mediated signaling and pro-inflammatory signaling (Figure 1).[10] Chai et al. show that in contrast to PtpB, PtpA does not localize to the PM. It was previously shown that PtpA also enters the host cell nucleus to inhibit activation of NF-κB promotors via mechanisms independent of its phosphatase activity.[9] Does PtpB also localize to the nucleus and exhibit similar activity? Another intriguing question remaining to be answered is the nature of the host cell ubiquitination system that targets PtpB? Finally, why are the PtpA/B phosphatase activities dependent on host cell ubiquitination? One hypothesis is that this mechanism guarantees that the phosphatases are only active when localized in the host cell cytosol, which might be of advantage if, for example, targeting PI4P/PI(4,5)P_2 on the luminal side of the phagosomal membrane is of a disadvantage to the pathogen.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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