RESEARCH HIGHLIGHT

Gasdermin B in the host–pathogen tug-of-war

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The *Shigella flexneri* effector IpaH7.8 targets gasdermin B for degradation in infected host cells. This bacterial strategy counteracts a host defense mechanism in which the lymphoid cell compartment activates the bactericidal properties of gasdermin B.

It is well appreciated that host cells are not the only beneficiaries of protein post-translational modifications (PTMs). Large-scale quantitative proteomic studies demonstrated that various bacterial pathogens modulate specific host signaling pathways via inducing PTMs. Notable recent examples include the modulation of actin polymerization machinery by *Salmonella enterica*,[1] autophagy receptors by *Mycobacterium tuberculosis*[2] or innate immunity mechanisms by *Shigella flexneri* (*S. flexneri*),[3] among other studies.

Structural and functional mimicry of the host E3 ubiquitin (Ub) ligases represents a powerful way that grants pathogens the ability to leverage substrate protein ubiquitylation for their survival.[4] Many bacterial species contain members of the E3 Ub ligase-like gene family, known as IpaH ligases. For example, *S. flexneri* deploys multiple IpaH ligases to support its fight against cell-autonomous immunity. It contains at least three IpaH effectors on its virulence plasmids and an additional seven effectors encoded by the genomic DNA. IpaH ligases belong to a unique class of E3 Ub ligases, termed as Novel E3 ligases, or NELs, that harbor E2-interacting surface residues, a catalytic cysteine residue utilized for Ub transfer and leucine-rich repeats for substrate protein binding. Prior studies identified several IpaH ligase-mediated host invasion mechanisms, such as easing of *S. flexneri* motility by IpaH9.8 or silencing of NF-κB signaling activation by the IpaH1.4 and IpaH2.5 effectors.[5–7]

In the recent study by Hansen et al.,[8] the authors examine the role of the IpaH7.8 E3 Ub ligase of *S. flexneri* in counteracting host cellular defense mechanisms. The authors apply the Ubiquitin Activated Interaction Traps (UBAIT) approach to identify ubiquitylated proteins specifically catalyzed by the wild-type IpaH7.8 ligase. These experiments showed that the IpaH7.8 effector interacts and ubiquitylates Gasdermin B (GSDMB) in the cell lysate. GSDMB ubiquitylation was also convincingly demonstrated at the endogenous level following infection of host cells by wild-type and not by the IpaH7.8 deletion mutant (Δ*ipah7.8*) *S. flexneri*. An in-depth biochemical investigation revealed that GSDMB is ubiquitylated on multiple surface lysine (Lys) residues, primarily using Lys48-linked Ub chains. Experiments with MG132, a compound that inhibits 26S proteasome, demonstrated an accumulation of GSDMB following *S. flexneri* infection. Consistent with these results, *S. flexneri* infection of host cells led to a significant reduction of the GSDMB protein level in the absence of MG132. These observations led authors to conclude that *S. flexneri* induces proteolytic destruction of GSDMB through a mechanism requiring IpaH7.8-mediated Ub conjugation.

The gasdermin protein family plays an integral role in the immune response by facilitating the delivery of IL-1 family cytokines to the extracellular space or triggering pyroptosis. When activated, these proteins form pores within lipid bilayers, such as the plasma membrane, initiating the release of pro-inflammatory cytokines.[9] In resting cells, the pore-forming N-terminus of gasdermins interacts with the autoinhibitory C-terminus. In response to stimuli, such as cytosolic LPS, caspases cleave the linker region between the termini and initiate activation of gasdermins. According to the current model, binding of the N-terminus to the lipid bilayer alleviates autoinhibition and initiates pore formation.[10] Recent studies also identified a role for GSDMB in cellular immunity. Cytotoxic T lymphocytes and natural killer (NK) cells deliver serine protease granzyme A (GZMA) that cleaves GSDMB in the interdomain linker. Subsequently, active GSDMB promotes pyroptosis and contributes to tumor cell elimination.[11]

In a series of elaborate experiments, the authors demonstrate that GZMA-mediated cleavage of GSDMB is important to restrict *S. flexneri* proliferation and that IpaH7.8 counteracts this mechanism by degrading GSDMB. To that end, cells infected with either the wild-type or Δ*ipah7.8* mutant *S. flexneri* were co-cultured with activated NK cells, which effectively suppressed the proliferation of the Δ*ipah7.8* mutant *S. flexneri* in GSDMB-expressing cells. In contrast, wild-type *S. flexneri* expanded similarly to controls, indicating that IpaH7.8-mediated destruction of GSDMB aids *S. flexneri* proliferation (Fig. 1). Notably, the authors translate this observation in vivo and demonstrate that microbial infection activates GSDMB in the caecum, which is the first evidence to date that speaks about GSDMB microbicidal mode of action. A twist to the story is that GSDMB in this model binds to the lipid component of the Gram-negative bacterial cell wall, suggesting that the activation of pore-forming GSDMB function occurs directly on the bacterial surface. Therefore, this study manifests novel biology relating to the function of GSDMB by targeting a Gram-negative bacterial pathogen.

Several research questions emanate from this study. First, a detailed examination of GSDMB-mediated killing of other cytosolic Gram-negative bacterial pathogens is warranted. Second, what are the structural determinants of GSDMB binding to phospholipids, and whether there are bacterial lipid-specific effectors that may impede GSDMB binding? Third, is there GSDMB-mediated

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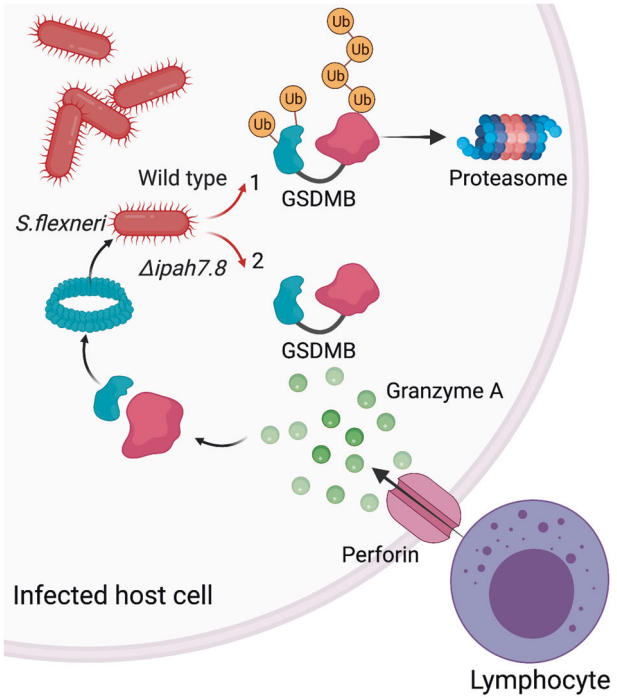

Fig. 1 Activation of gasdermin B bactericidal properties. Wild-type *S. flexneri* delivers the IpaH7.8 effector that ubiquitylates GSDMB and induces its proteasomal degradation (1). Infection of cells by wild-type and not by IpaH7.8-deficient mutant *S. flexneri* (*Δipah7.8*) reduces the GSDMB level (2). Lymphocytes deliver granzyme A to the cytosol of infected cells that cleaves GSDMB in the interdomain linker. Activation of the pore-forming N terminal part of GSDMB (green) facilitates *S. flexneri* killing.

regulation of mitochondrial function since phospholipids are significant constituents of mitochondrial membranes. Could this be a rather general mechanism that modulates mitochondrial function since multiple gasdermins bind phospholipids and cardiolipin?[12] Finally, looking ahead, it would be interesting to explore which lymphoid cell compartment activates GSDMB, the cues leading to lymphocyte engagement, and potential regulatory steps that determine which gasdermin family member is active in the physiological context in vivo.

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COMPETING INTERESTS

Timurs Maculins is an employee of Genentech, Inc and a shareholder in Roche.

ADDITIONAL INFORMATION

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