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Salmonella, the host and its microbiota

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SUMMARY

The intestine is host to a diverse bacterial community whose structure, at the phylum level, is maintained through unknown mechanisms. Acute inflammation triggered by enteric pathogens, such as *Salmonella enterica* serotype Typhimurium (*S. Typhimurium*), is accompanied by changes in the bacterial community structure marked by an outgrowth of the pathogen. Recent studies show that *S. Typhimurium* can harness benefit from the host response to edge out the beneficial bacterial species that dominate in the healthy gut. The elucidation of how *S. Typhimurium* alters the bacterial community structure during gastroenteritis is beginning to provide insights into mechanisms that dictate the balance between the host and its microbiota.

INTRODUCTION

Salmonella enterica serotype Typhimurium (*S. Typhimurium*) is an important food-borne pathogen that in humans causes a self-limited gastroenteritis, characterized by fever, acute intestinal inflammation, diarrhea, and the presence of neutrophils in stool samples [1]. In addition, *S. Typhimurium* is a model organism for studying bacterial genetics and microbial pathogenesis. As the frontier in bacterial pathogenesis research is moving towards understanding the complexity of host-pathogen interaction at the tissue level, studies on the pathogenesis of *S. Typhimurium* gastroenteritis using animal models have helped establish important new concepts that exert a strong influence on the research field. Recent studies on *S. Typhimurium* pathogenesis reveal how tissue-specific host factors and the presence of other bacterial species shape the outcome of host-pathogen interaction in the intestinal lumen. Here, we will review these new paradigms for the interplay between the pathogen, the host and its resident microbial community.

VIRULENCE MECHANISMS

Upon ingestion, *S. Typhimurium* colonizes the terminal ileum and colon, commonly eliciting symptoms of gastroenteritis within less than 24 hours. The signs of disease and the pathological changes in the human terminal ileum and colon can be reproduced in a calf model [2]. Studies in this animal model identified motility and two type III secretion systems as the main *S. Typhimurium* virulence factors important for triggering intestinal

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inflammation [3,4]. Motility and the invasion-associated type III secretion system (T3SS-1) work in concert to enable a fraction of the *S. Typhimurium* population to invade intestinal epithelial cells [5,6]. Acting as a molecular syringe, the T3SS-1 injects proteins, termed effectors, into host cells [7]. Five T3SS-1 effectors, named SipA, SopA, SopB (SigD), SopD and SopE2, act in concert to trigger alterations in the actin cytoskeleton of host cells, thereby promoting epithelial invasion [8] and intestinal inflammation [9]. Once *S. Typhimurium* has crossed the epithelial lining, a second type III secretion (T3SS-2) system enables the pathogen to survive within tissue mononuclear cells (macrophages and dendritic cells) [10]. Finally, *S. Typhimurium* invasion of host tissue is detected by the innate immune surveillance (Figure 1) [11], resulting in the rapid induction of intestinal inflammation, which is largely responsible for the signs of disease [12]. Mechanisms by which *S. Typhimurium* first induces and then benefits from the host inflammatory response have been elucidated using a mouse colitis model [2].

PATTERN RECOGNITION

The innate immune system detects the presence of *S. Typhimurium* in tissue by two distinct mechanisms, each involving a multitude of receptors. The first mechanism, termed pattern recognition, enables the host to distinguish self from bacteria by detecting conserved microbial structures, known as pathogen associated molecular patterns (PAMPs) [13], by humoral proteins, such as complement, or by host cell receptors, such as Toll-like receptors (TLRs).

For example, the O-antigen of the *S. Typhimurium* lipopolysaccharide (LPS) is a PAMP detected by complement component 3 (C3), thereby initiating the alternative pathway of complement activation. The complement fragments C3a and C5a generated during this process are also known as the anaphylatoxins, due to their potency in inducing inflammatory responses [14]. Besides the O-antigen, *S. Typhimurium* LPS contains a lipid A moiety, which is a powerful agonist of TLR4 [15]. Curli, an amyloid fibril present in the extracellular matrix of *S. Typhimurium* biofilms, is the main TLR1/TLR2-ligand detected on intact bacterial cells [16,17]. Finally, motility of *S. Typhimurium* is mediated by flagella, whose major protein subunit, flagellin (FliC), is a PAMP stimulating TLR5 [18]. TLR4, TLR1/TLR2 and TLR5 engage a common adaptor protein, myeloid differentiation primary response gene 88 (MYD88), to initiate mitogen activated protein (MAP) kinase signal transduction pathways that induce expression of pro-inflammatory genes by activating two transcription factors, activator protein 1 (AP-1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (Figure 1).

DETECTION OF PATHOGEN-INDUCED PROCESSES

Although the name suggests a specific association with pathogens, all microbes produce PAMPs regardless of their pathogenic potential. Thus pattern recognition is not sufficient to distinguish a virulent pathogen, such as *S. Typhimurium*, from other microbes with lower disease-causing potential. To mount a response that is commensurate with the threat, the host uses a second mechanism for assessing the pathogenic potential of *S. Typhimurium* by detecting pathogen-induced processes [19].

One pathogen-induced process that marks *S. Typhimurium* for recognition by the host is the T3SS-1-dependent delivery of proteins into the host cell cytosol. T3SS-1-dependent cytosolic access is accompanied by the delivery of conserved components of the T3SS-1 needle complex (PrgJ) [20] and an inadvertent translocation of flagellin into host cells [21]. The presence of PrgJ and flagellin is detected by a nucleotide-binding oligomerization domain (NOD)-like receptor (NLR), termed NLRC4 (NLR family caspase-associated recruitment domain [CARD]-containing protein 4) (Figure 1) [20,22,23]. In turn, NLRC4

forms a complex with caspase-1 and apoptosis-associated speck-like protein containing a CARD (ASC), resulting in cleavage and activation of caspase-1. Upon activation, caspase-1 proteolytically cleaves the precursor forms of interleukin (IL)-1 β and IL-18, into active mature peptides. Alternatively, *S. Typhimurium* can activate caspase-1 through NLRP3 (NLR family pyrin domain-containing protein 3) by an unknown mechanism [24]. A small fraction of *S. Typhimurium* clinical isolates is lysogenized with a bacteriophage carrying the *sopE* gene, which encodes a T3SS-1 effector protein [25]. Translocation of SopE into the host cell cytosol results in caspase-1 activation through an unknown mechanism [26]. Finally, another mechanism for detecting cytosolic access by *S. Typhimurium* is the activation of NOD1 and NOD2, two intracellular sensors of bacterial cell wall fragments [27–29]. NOD1 and NOD2 interact with protein kinase receptor interacting protein-2 (RIP2) to mediate NF- κ B activation. Detection of pathogen-induced processes enables the host to escalate innate responses to levels that adequately address the risk posed by *S. Typhimurium* infection.

THE THREE BRANCHES OF AN ANTIBACTERIAL RESPONSE

Pattern recognition and the detection of pathogen-induced processes by epithelial cells, mononuclear cells (i.e. macrophages and dendritic cells) and complement results in the production of IL-1 β , IL-12, IL-18, IL-23, tumor necrosis factor (TNF)- α , interferon (IFN)- γ and C5a (Figure 1), a cocktail that serves as a starting signal for the orchestration of anti-bacterial responses in tissue. The events set in motion by the release of this pro-inflammatory cocktail culminate in the orchestration of three host defense pathways, including macrophage activation, neutrophil recruitment and the epithelial release of antimicrobials into the intestinal lumen (Figure 2) [30].

In brief, IL-12 and IL-18 induce production of IFN- γ , a cytokine that greatly enhances the ability of macrophages to kill intracellular *S. Typhimurium* [31]. Neutrophil recruitment is orchestrated by several partially redundant mechanisms. IL-23 and IL-1 β synergize in inducing the production of IL-17A by T helper (T_H)17 cells, $\gamma\delta$ T cells, and NKT cells [29,32–34]. In turn, IL-17A acts in concert with IL-1 β and TNF- α to induce the release of CXC chemokines from epithelial cells [34,35]. CXC chemokines and C5a are potent chemoattractants for neutrophils, a cell type that is particularly effective in killing extracellular *S. Typhimurium* [36–38]. Finally, IL-23 contributes to the production of IL-22, a cytokine that stimulates epithelial cells to release antimicrobials, such as lipocalin-2, into the intestinal lumen [39].

In summary, detection of *S. Typhimurium* by the innate immune system activates an antibacterial response consisting of three branches, one directed against intracellular bacteria in tissue, one directed against extracellular bacteria in tissue and one directed against luminal bacteria (Figure 2) [30].

TAKING ONE FOR THE TEAM

The fraction of the *S. Typhimurium* population that enters the tissue is detectable by electron microscopy in mononuclear cells and neutrophils [40]. Resident tissue macrophages represent the preferred intracellular niche of *S. Typhimurium* that supports its growth [41]. However, activation of caspase-1 during *S. Typhimurium* infection triggers a form of pro-inflammatory macrophage cell death [42,43], termed pyroptosis [44] (Figure 1). Release from macrophages by pyroptosis renders *S. Typhimurium* vulnerable to phagocytosis by neutrophils, a cell type capable of killing *S. Typhimurium*, thereby helping to control bacterial numbers in tissue [38]. Alternatively, *S. Typhimurium* can trigger macrophage cell death through a T3SS-1-independent mechanism [45] that involves signaling through TLR4 and its adaptor protein TRIF (TIR-domain-containing adapter-inducing interferon- β) [46].

TRIF physically interacts with receptor-interacting protein (RIP)1 and RIP3 [47], forming a complex that can induce programmed necrosis [48]. Increasing production of IFN- γ during the transition from innate to adaptive immune responses enhances the ability of macrophages to kill intracellular *S. Typhimurium* [31]. Finally, production of specific antibodies by the adaptive immune response further enhances phagocyte-killing mechanisms [49]. As a result, *S. Typhimurium* is beginning to be cleared from intestinal tissues with the onset of adaptive immune responses.

In conclusion, the localization within tissue represents a dead end for *S. Typhimurium*, as the pathogen does not transmit to a susceptible host from this location, but instead is eventually killed by two branches of the host's antibacterial response, macrophage activation and neutrophil recruitment (Figure 2). Interestingly, the fraction of the *S. Typhimurium* population that remains in the intestinal lumen is thriving in the inflamed gut and becomes a sizeable fraction of the intestinal microbiota [50–52]. It has therefore been proposed that induction of an inflammatory response by the invading fraction of the *S. Typhimurium* population, through self-destructive cooperation, empowers the luminal fraction of the *S. Typhimurium* population to bloom in the inflamed gut [53].

FOOD FROM THE FIRE

Interestingly, growth of *S. Typhimurium* is aided by the very inflammatory responses that are aimed at controlling luminal bacteria, including the epithelial release of antimicrobials and the epithelial transmigration of neutrophils into the intestinal lumen (Figure 2). For example, the antimicrobial protein lipocalin-2 is released into the intestinal lumen, where it binds enterobactin, a low molecular weight iron chelator (siderophore) produced by many enteric bacteria [39]. By sequestering enterobactin, lipocalin-2 exerts a bacteriostatic activity on bacteria that depend on this siderophore for iron acquisition [54]. In addition to enterobactin, *S. Typhimurium* produces a glycosylated derivative of enterobactin, termed salmochelin [55], that is no longer bound by lipocalin-2, thereby conferring resistance to this antimicrobial [56]. Salmochelin bestows a growth advantage upon *S. Typhimurium* in the lumen of the inflamed intestine [39], presumably because lipocalin-2 suppresses growth of competing microbes (Figure 3).

In healthy individuals, the gut lumen is thought to be fairly anaerobic, with traces of oxygen being readily consumed by the microbiota. The majority of the microbiota are strictly anaerobic bacteria belonging to the classes Bacteroidetes and Clostridiales that rely on fermentation of amino acids and complex polysaccharides. During infection with *S. Typhimurium*, migration of neutrophils into the intestinal lumen is associated with changes in this microbial community structure [57]. This ultimately results in the enrichment of the pathogen in the gut lumen and a relative depletion of Bacteroidetes and Clostridiales.

One fermentation end product generated by the microbiota is hydrogen sulfide (H_2S), a cytotoxic compound that is converted to thiosulfate ($\text{S}_2\text{O}_3^{2-}$) by the colonic epithelium. During inflammation, neutrophils that transmigrate into the intestinal lumen release reactive oxygen species (ROS) in an attempt to kill bacteria. A by-product of releasing ROS is the oxidation of thiosulfate ($\text{S}_2\text{O}_3^{2-}$) to tetrathionate ($\text{S}_4\text{O}_6^{2-}$) [58]. In contrast to the fermenting microbiota, *S. Typhimurium* can use tetrathionate as a terminal electron acceptor to support its growth by anaerobic respiration, which is more efficient for energy production than fermentation [59]. In addition, *S. Typhimurium* might benefit from inflammation by gaining access to new nutrients because anaerobic tetrathionate respiration facilitates growth on poorly fermentable carbon sources. Collectively, growth by tetrathionate respiration enables *S. Typhimurium* to outgrow competing microbes, resulting in a marked increase in the relative abundance of the pathogen in intestinal contents [58]. In turn, enhanced growth

in the intestinal lumen promotes transmission of *S. Typhimurium* by the fecal oral route [51] (Figure 3).

In summary, the benefit *S. Typhimurium* harnesses from intestinal inflammation is largely based on an improved access to nutrient sources. It has thus been speculated that *S. Typhimurium* virulence evolved predominantly as a means to access nutrient resources in its host [60].

CONCLUSIONS

Research on *S. Typhimurium* pathogenesis has traditionally focused on how virulence factors enable the pathogen to overcome host defenses. However, recent insights into the interplay between *S. Typhimurium*, the host and its microbiota suggest that virulence factors enable the pathogen to elicit help from the host inflammatory response to gain an advantage in its growth competition with the resident microbiota (Figure 3). Thus, the deployment of virulence factors could be seen as an attempt to create a niche in the host in which *S. Typhimurium* can edge out competing microbes to ensure its transmission. Identification of the metabolic pathways that support growth of *S. Typhimurium* over other microbes in the inflamed gut has the potential to identify new targets for intervention, which represents a promising line for future investigation.

HIGHLIGHTS

Non-typhoidal *Salmonella* serotypes trigger innate immune responses by using their virulence factors to invade the intestinal epithelium and survive in macrophages.

The innate immune surveillance system responds to the presence of non-typhoidal *Salmonella* serotypes in tissue with an inflammatory reaction that culminates in macrophage activation, neutrophil recruitment and the epithelial release of antimicrobials.

While inflammatory responses control the pathogen in tissue, neutrophil transepithelial migration and the epithelial release of antimicrobials provide a benefit during their competition with the resident microbiota in the lumen, thereby enhancing transmission of non-typhoidal *Salmonella* serotypes.

In conclusion, the deployment of virulence factors could be seen as an attempt to create a niche in the host in which non-typhoidal *Salmonella* serotypes can edge out competing microbes to ensure their transmission.

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Figure 1. Detection of *S. Typhimurium* by the innate immune systems initiates inflammation

The host senses the presence of *S. Typhimurium* in tissue by detecting PAMPs (e.g. LPS, curli, FliC) or patterns of pathogenesis (e.g. cytosolic access by the T3SS-1) through pathogen recognition receptors located in the cytosol (NOD1, NOD2, NLRC4, and NLRP3), the cell membrane (TLR1/TLR2, TLR4 and TLR5) or the humoral compartment (complement). Signaling through these pathogen recognition receptors results in the production of a pro-inflammatory cocktail (anaphylatoxins, IL-1 β , IL-12, IL-18, IL-23, TNF- α and IFN- γ) that initiates the orchestration of antibacterial responses in tissue.

Salmonella is detected by mononuclear cells/epithelial cells/complement

Figure 2. The three branches of an antibacterial inflammatory response

Detection of *S. Typhimurium* by mononuclear cells, epithelial cells and complement triggers a cytokine storm in host tissue, which culminates in the orchestration of three major antibacterial responses: macrophage activation, neutrophil recruitment and the epithelial release of antimicrobials.

Figure 3. *Salmonella*, the host and its microbiota

S. Typhimurium uses its virulence factors (flagella, T3SS-1 and T3SS-2) to invade the epithelium and survive in mononuclear cells. The ensuing inflammatory response results in the epithelial release of an antimicrobial (lipocalin-2) that sequesters iron chelators (enterobactin) produced by the microbiota, but not an iron chelator (salmochelin) produced by *S. Typhimurium*. ROS generated by neutrophils migrating into the intestinal lumen oxidize an endogenous sulfur compound (thiosulfate) to generate a respiratory electron acceptor (tetrathionate) that enables *S. Typhimurium* to edge out the fermenting microbiota, thereby enhancing transmission of the pathogen.