

# Salmonella effector driven invasion of the gut epithelium: breaking in and setting the house on fire

Stefan A Fattiger<sup>1,2</sup>, Mikael E Sellin<sup>2</sup> and Wolf-Dietrich Hardt<sup>1</sup>



CrossMark

*Salmonella* Typhimurium (*S.Tm*) is a major cause of diarrheal disease. The invasion into intestinal epithelial cells (IECs) is a central step in the infection cycle. It is associated with gut inflammation and thought to benefit *S.Tm* proliferation also in the intestinal lumen. Importantly, it is still not entirely clear how inflammation is elicited and to which extent it links to IEC invasion efficiency *in vivo*. In this review, we summarize recent findings explaining IEC invasion by type-three-secretion-system-1 (TTSS-1) effector proteins and discuss their effects on invasion and gut inflammation. In non-polarized tissue culture cells, the TTSS-1 effectors (mainly SopB/E/E2) elicit large membrane ruffles fueling cooperative invasion, and can directly trigger pro-inflammatory signaling. By contrast, in the murine gut, we observe discreet-invasion (mainly via the TTSS-1 effector SipA) and a prominent pro-inflammatory role of the host?"s epithelial inflammasome(s), which sense pathogen associated molecular patterns (PAMPs). We discuss why it has remained a major challenge to tease apart direct and indirect inflammatory effects of TTSS-1 effectors and explain why further research will be needed to fully determine their inflammation-modulating role(s).

## Addresses

<sup>1</sup> Institute of Microbiology, Department of Biology, ETH Zurich, Zurich, Switzerland

<sup>2</sup> Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

## Corresponding authors:

Fattiger, Stefan A ([stefanfa@ethz.ch](mailto:stefanfa@ethz.ch)), Sellin, Mikael E ([mikael.sellin@imbim.uu.se](mailto:mikael.sellin@imbim.uu.se)), Hardt, Wolf-Dietrich ([hardt@micro.biol.ethz.ch](mailto:hardt@micro.biol.ethz.ch))

Current Opinion in Microbiology 2021, 64:9–18

This review comes from a themed issue on **Host-microbe interactions: bacteria**

Edited by **Vanessa Sperandio** and **Gad M Franke**

For complete overview of the section, please refer to the article collection, "[Host-Microbe Interactions: Bacteria](#)"

Available online 6th September 2021

<https://doi.org/10.1016/j.mib.2021.08.007>

1369-5274/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## Introduction

*Salmonella enterica* is a key global cause of foodborne diarrhea with *Salmonella* Typhimurium (*S.Tm*) as one of the main serotypes affecting humans. *S.Tm* also serves as a prototype for studying the general principles of *Salmonella* infection biology.

*S.Tm* invasion into the gut tissue is intimately linked to diarrhea and gut inflammation, two hallmarks of the disease [1–4]. Gut inflammation provides *S.Tm* with a competitive advantage to outcompete the gut resident microbiota and to establish its niche in the gut lumen [5,6]. Thus, to understand how the pathogen benefits from eliciting enteric disease, we need to assess the host cells which are targeted by the pathogen, the mechanisms of cell invasion and how this invasion process triggers inflammation in the host?"s gut.

Work in primarily calf and mouse infection models has shown that *S.Tm* enters the gut tissue via different routes, including the uptake by M cells or microbe-sampling dendritic cells and the active invasion of absorptive intestinal epithelial cells (IECs) [7]. IEC invasion is best studied, thought to be the main driver of gut inflammation, and hence constitutes the focus of this review. This process strictly depends on the *Salmonella* pathogenicity island 1 (SPI-1), which encodes a needle-like injection structure, called the type-three-secretion-system-1 (TTSS-1), and several effectors for invasion of IECs [7,8]. Upon entry, the infected host cell mounts a first line of pro-inflammatory responses. It is still not entirely clear to which extent pro-inflammatory responses are triggered by signal cascade manipulation via TTSS-1 effectors, or by the host cell?"s pattern recognition receptors (PRRs) that sense pathogen associated molecular patterns (PAMPs) such as peptidoglycan, lipopolysaccharide, flagellin and the TTSS-1 [9,10]. Either way, IEC invasion represents an essential event in the pathogen?"s infection cycle.

Here, we review the recent progress in our understanding of *S.Tm* IEC invasion, with a particular emphasis on the intact host gut. We focus on SPI-1 mediated invasion, since this is best understood at the molecular and cellular level and has been studied using various animal models including mice. We discuss how TTSS-1 effectors collaborate with other *Salmonella* virulence factors, how they contribute to IEC invasion, and their possible roles beyond.

## Molecular basis for *S.Tm* invasion into non-phagocytic cells

Infections, biochemical experiments, and ectopic expression studies using transformed/immortalized cell line cultures have established the canonical view of *S.Tm* invasion into non-phagocytic cells, the molecular basis of which has been reviewed elsewhere [7]. Briefly, TTSS-1

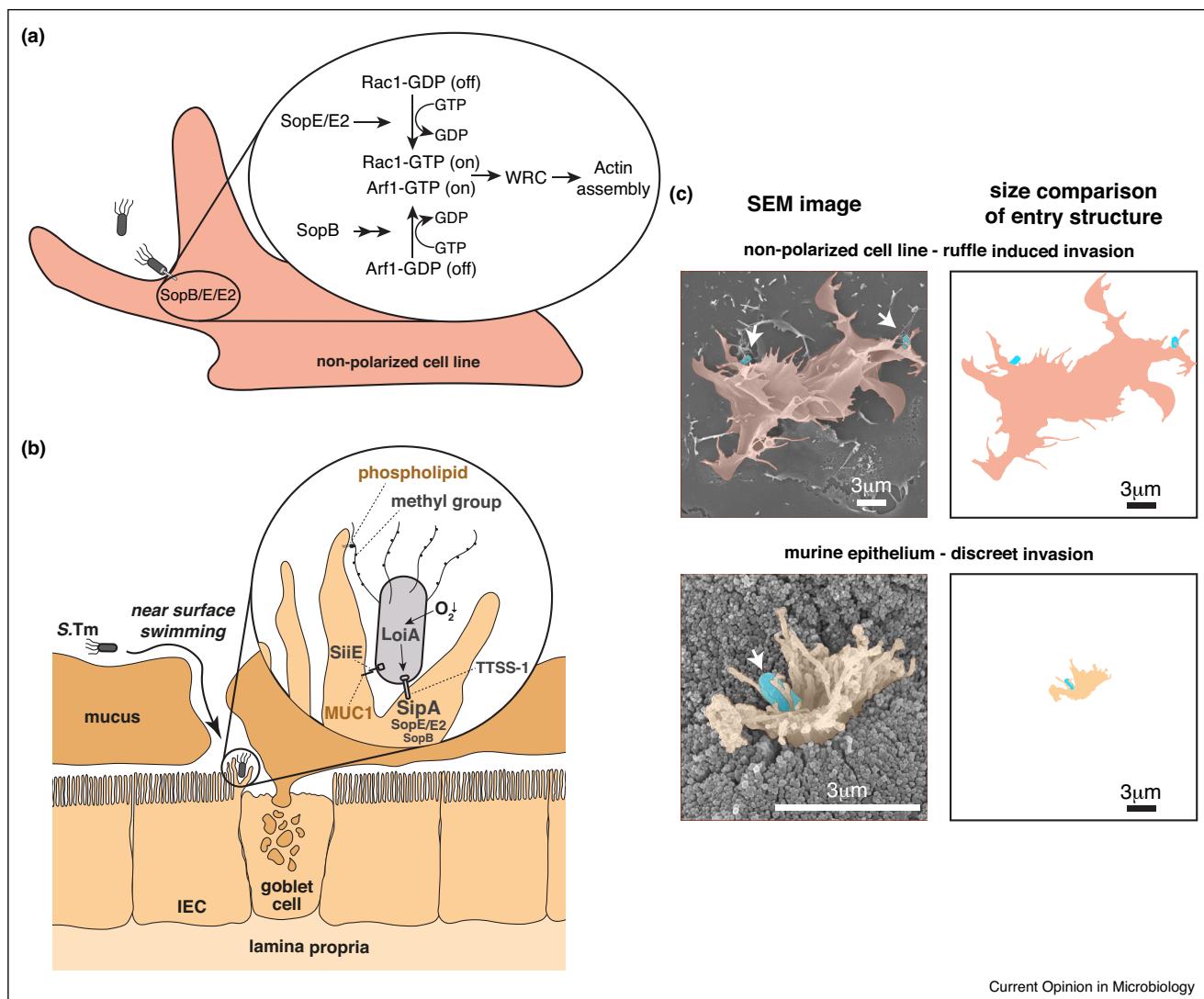
effectors interact with the host actin cytoskeleton to trigger lamellipodia and filopodia-containing surface protrusions (called membrane ruffles) which engulf *S.Tm* into a tight intracellular compartment [11–13]. The size of these ruffles extends far beyond a single *S.Tm*, enabling the simultaneously internalization of several bacteria a phenomenon denoted cooperative invasion [14•,15,16]. The main TTSS-1 effectors driving this type of uptake are SopB, SopE and SopE2, which indirectly promote Arp2/3-dependent actin polymerization through nucleation promoting factors WAVE and WASH [17,18]. SopB is believed to be a lipid phosphatase and/or a phosphotransferase/phosphoisomerase that manipulates phosphoinositide dynamics at the plasma membrane to recruit Arf1 [18–20]. SopE and SopE2 are archetypical members of the WxxxE family of effector proteins [21], mimicking guanine nucleotide exchange factors to activate for example, Rac1 [15,22,23]. Together, Arf1 and Rac1 govern the WAVE Regulatory Complex (WRC; a complex of WAVE and co-factors) to initiate Arp2/3 dependent actin polymerization [24] (Figure 1a). Importantly, the ectopic expression of SopB, SopE or SopE2 is sufficient to elicit pronounced membrane ruffling and/or facilitate the uptake of inert particles and non-invasive bacteria in transformed tissue culture cell models [15,23,25]. In contrast to SopB/E/E2, which target upstream actin-regulatory processes, the TTSS-1 effectors SipA and SipC can directly bind to actin. They feature actin nucleating and bundling activities to target the actin cytoskeleton directly, which supports membrane ruffles, thus invasion [26–29]. While SipC is a translocon component required for TTSS-1 function and invasion, deletion of SipA only has a minor impact on invasion in cultured cell lines. Notably, SipA can drive cell invasion into non-polarized tissue culture cells independent of SopB/E/E2 in a morphologically distinct process which lacks prototypical ruffles [30–33].

This classical view of SPI-1-triggered invasion has been extended with new TTSS-1-independent mechanisms in recent years, since SPI-1-deficient *S.Tm* mutants still feature a residual invasion capacity in cell lines. Rck is the best-studied TTSS-1-independent invasion factor [34]. This is an outer membrane protein that binds to the epidermal growth factor receptor (EGFR) leading to Arp2/3 activation through Rac1 and Akt [35–37]. Since EGFR localizes to the basolateral side of IECs, Rck could potentially promote IEC invasion from the lamina propria compartment [36]. However, a Rck-associated phenotype *in vivo* remains to be shown. PagN is another outer membrane protein, which can drive cell invasion independent of TTSS-1 [38•,39]. Furthermore, a study postulated the existence of additional invasion factors, since a strain simultaneously deficient in TTSS-1 (*invA*), Rck and PagN was still able to invade certain cell lines [40]. Together, this demonstrates that there is still room for discoveries regarding *S.Tm* invasion mechanisms.

Despite the detailed understanding of the molecular mechanisms underlying TTSS-1 effector-triggered invasion by *S.Tm*, the *in vivo* relevance and the associated invasion phenotypes into the gut tissue of an infected host has only received modest attention historically [41–45]. Recent publications partially fill this gap and provide new insights into how *S.Tm* invades IECs *in vivo*. Here, we summarize recent studies of invasion processes *in vivo*, particularly in murine infection models, which have provided the deepest insights. We discuss pre-invasion factors and how TTSS-1 effectors influence invasion, inflammation and intracellular location/survival within IECs. We focus on these early steps of the mucosal infection cycle, since SPI-1 expression has been shown to be promptly downregulated following traversal of the epithelium [46,47].

### Pre-invasion factors prepare *S.Tm* for SPI-1-dependent IEC invasion *in vivo*

Upon arrival in the gut lumen, *S.Tm* integrate a number of environmental inputs to trigger an elaborate signaling cascade that ramps up expression of flagella, TTSS-1 and the SiiE adhesin needed to attack the epithelium [48,49]. Combined, these virulence factors enable *S.Tm* to swim to the epithelium, attach to its surface and invade into IECs. Most parts of the epithelium are covered with a thick layer of Muc2-containing mucus, studded with antimicrobial proteins, secreted IgA and numerous other factors [50] that reduce pathogen access to the epithelial surface. However, this mucus layer has channels and gaps which *S.Tm* exploits via flagella-driven near surface swimming to reach exposed IECs [51]. The flagellum of *S.Tm* can be composed of two antigenically distinct flagellins, FljB and FliC. Compared to FljB, FliC-expressing *S.Tm* are slightly more invasive, which has been explained by a distinct near-surface swimming phenotype with more frequent stops along the surface of cultured cell lines [52]. Furthermore, posttranslational methylation of FliC increases *S.Tm* invasion efficiency [53••]. The methylase FliB adds methyl groups on flagella-surface exposed lysine residues to increase hydrophobicity. In *S.Tm* strains locked for FliC expression (cannot switch to FljB expression), FliB deficiency leads to decreased IEC invasion. It was reasoned that hydrophobicity increases binding efficiency to phosphatidylcholine, the most abundant phospholipid present on host cell membranes (Figure 1b). Attachment is additionally supported by the giant adhesin SiiE encoded on SPI-4, shown to be important for IEC invasion *in vivo* [54••,55]. SiiE co-localizes with MUC1, a cell-surface protein expressed on the apical surface of epithelial cells. During infection of HT29-MTX cell layers, MUC1-deficient cells are more resistant to *S.Tm* as well as *S. Enteritidis* invasion [56••]. The combined data suggest that SiiE promotes invasion by binding to MUC1 glycans (Figure 1b). *S.Tm* genomes encode more than a dozen additional adhesins [57], several of which shown to affect

**Figure 1**

Current Opinion in Microbiology

#### SPI-1-dependent S.Tm invasion of epithelial cells.

**(a)** Effector-driven invasion into non-polarized epithelial cell lines. SopB, SopE and SopE2 are the dominant effectors, which indirectly affect actin assembly through for example, Rac1 and Arf1. **(b)** Recent insights about effector-driven invasion into the absorptive gut epithelium of mice. Expression of SPI-1, methylation of flagellin and the adhesin SiiE prime S.Tm for cell invasion. S.Tm enters IECs through ??discreet-invasion?", where SipA has a decisive role. S.Tm preferentially targets cell?cell junctional zones, such as those of goblet cell-neighboring IECs. **(c)** Scanning electron microscopy images of cell entry structures in non-polarized epithelial cells versus mouse gut epithelium (adapted from Ref. [54\*]). Entry structures differ in terms of morphology and size as indicated by pseudocoloring. Arrow points to S.Tm colored in blue, entry structure colored in red (cell line) or orange (murine gut).

intestinal colonization [58,59], but their specific impact on IEC attachment *in vivo* remains unclear. The TTSS-1 apparatus further promotes attachment by docking into the host cell membrane [60–62].

SPI-1 expression is costly to the pathogen, reducing its growth rate by as much as 50% [63]. To limit these costs, virulence factor expression occurs only at times of need, that is, to initiate gut tissue invasion [64,65]. It is

regulated by a complex network of transcription factors including HilD [66], which also tunes *S.Tm* swimming behaviors [67]. This is illustrated by LoiA (low oxygen induced factor A; encoded on SPI-14), which promotes SPI-1 expression through *hilD* at low oxygen condition as found in the gut lumen. *S.Tm* deficient for LoiA feature reduced invasion efficiency into CaCo-2 cells and are less virulent *in vivo* [68]. In summary, pre-invasion factors including flagellin variants, adhesins, SPI-1-regulating

transcription factors, and the TTSS-1 apparatus itself prime *S.Tm* to invade IECs of the gut mucosa (Figure 1b).

### The SPI-1-driven IEC invasion step in the intact gut epithelium

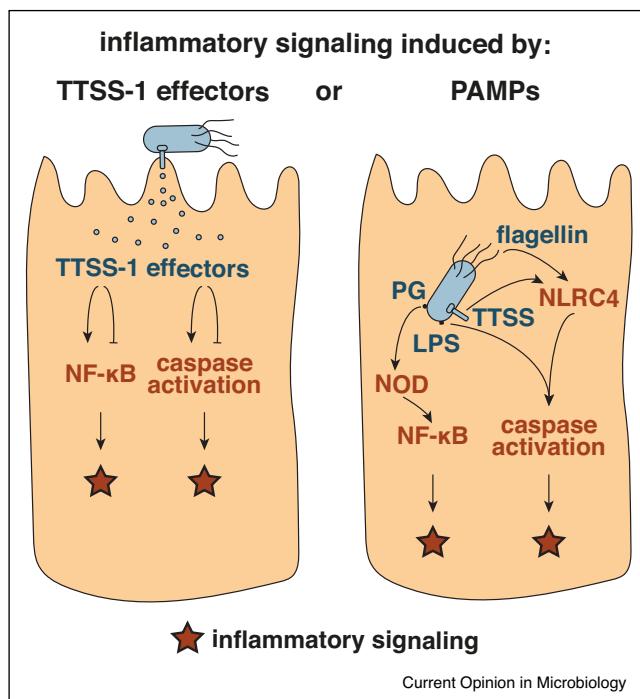
Upon docking to the epithelium, the pre-formed TTSS-1 effectors are delivered by TTSS-1 into the IEC cytosol to fuel invasion. In the infected gut, the individual contributions of the TTSS-1 effectors have been challenging to study, due to their redundancy and *in vivo* complexity. Recently, a neonate mouse model and a specific immune-deficient mouse line (*Nlrc4<sup>-/-</sup>* mice) have provided new insights [54<sup>••</sup>,69,70<sup>••</sup>]. Simultaneous deletion of SopB/E/E2/SipA in *S.Tm* SL1344 [54<sup>••</sup>], or of SopA/B/E2/SipA in *S.Tm* 14028 (which naturally lacks SopE) [70<sup>••</sup>] abolished IEC invasion. In partial similarity to early results from bovine ligated ileal loops [71], in neonate mice, complementation of SopA/B/E2/SipA *S.Tm* 14028 with either SipA, SopE, or SopE2 partially rescued the IEC invasion defect, whereas SopA or SopB failed to do so. Moreover, triple mutants that retain exclusively SipA or SopE2 were still invasion-proficient. This indicates important, but redundant, roles of SipA and SopE2 for *S.Tm* 14028 invasion of neonate IECs [70<sup>••</sup>]. Importantly, it was sufficient to delete only SipA in *S.Tm* SL1344 to detect a pronounced IEC invasion defect in adult mice [54<sup>••</sup>]. This effect was less obvious in neonate mice infected with the corresponding *S.Tm* 14028 mutant [70<sup>••</sup>], suggesting strain specific variations or differences in epithelium maturation stage of neonate versus adult mice. Nevertheless, these two studies demonstrate that SopE/E2 and in particular SipA drive IEC invasion *in vivo* (Figure 1b). This appears surprising, as SipA contributes only slightly to *S.Tm* invasion in non-polarized tissue culture models. Strains that lack SopB/E/E2 rely on SipA in collaboration with SipC for a zipper-like invasion process [30,31]. Based on these observations, *S.Tm* invasion into the mature mouse gut epithelium might proceed without expansive membrane ruffles. Indeed, in line with previous observations in ileal gut-loops from pigs and calves [41,72], IEC invasion by wt *S.Tm* in mice is characterized by smaller finger-like protrusions and does not fuel cooperative invasion, which is a distinct feature of ruffle-mediated entry (Figure 1c, [54<sup>••</sup>]). These observations support that SipA can drive discreet IEC invasion in the mature epithelium of mice (Figure 1b). Moreover, in the complex *in vivo* environment, *S.Tm* exploits cell-cell junctional zones and cells neighboring goblet cells for efficient invasion (Figure 1b) [54<sup>••</sup>]. Differences between mouse epithelium and cell lines can be partially explained by the degree of host cell polarization [54<sup>••</sup>]. Accordingly, it was shown that SipA dependent manipulation of villin located at the brush border of polarized IEC can influence invasion *in vivo* [73].

Overall, the comparison of well-controlled *in vivo* and cell culture experiments demonstrates that the contribution of individual TTSS-1 effectors for invasion is context-dependent. The relative roles of SopB, SopE, SopE2, and SipA are strongly affected by the cellular environment, cell-polarity and cell-maturation. The recent establishment of non-transformed epithelial cell cultures (enteroids, colonoids, and other stem cell derived organoids) as *S.Tm* infection model (established either in 3D or 2D) will be an effective tool to complement *in vivo* work in the future [74,75<sup>•</sup>,76,77<sup>•</sup>,78,79<sup>•</sup>,80<sup>•</sup>]. Organoids offer a simplified, but physiologically relevant model system, in which both near-surface swimming and SPI-1-dependent IEC invasion are closely recapitulated [76,77<sup>•</sup>,79<sup>•</sup>]. Furthermore, the abundance of specific epithelial cell types can be controlled by varying the culture conditions [81,82]. Barcoded *S.Tm* consortium infections will enable investigation of multiple mutants simultaneously under internally controlled conditions and across experimental model systems [14<sup>•</sup>,54<sup>••</sup>]. Hence, we can expect continued progress in our understanding of SPI-1 driven IEC invasion and the specific impact a physiological host cell and tissue context has on this process.

### Pro-inflammatory signaling elicited by the invading pathogen

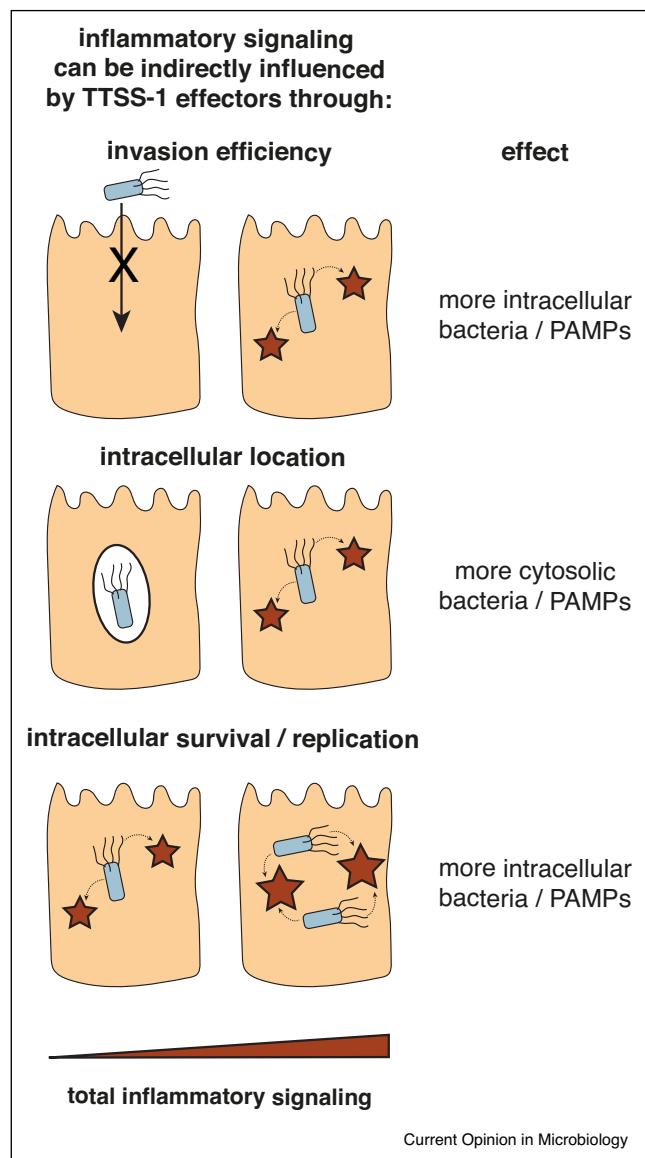
SPI-1 driven IEC invasion leads to inflammatory responses. Since the induction of inflammation is crucial for outcompeting luminal microbiota, it has been suggested that TTSS-1 effectors control the degree of inflammation by interfering directly with pro-inflammatory signaling, cell death pathways and/or tight junctions [10,83–85]. Inflammatory NF-κB signaling can be affected by various TTSS-1 effectors such as SipA, SopB/E/E2 and AvrA [86–89]. Accordingly, a recent study demonstrated that Rab8 GTPase can be targeted by the TTSS-1 effector SopD to interfere with inflammatory signaling [90<sup>••</sup>]. Cell death pathways were shown to be promoted or suppressed by SipA and SopB/E [91–95], and finally, tight junctions were suggested to be targeted by SipA, SopB/E/E2 and AvrA to either increase or decrease inflammation [96–98]. These studies employed mainly tissue culture cell models and arrived at the conclusion that the TTSS-1 effectors are fine-tuning the infected host cell?"s response to the benefit of the pathogen.

Thus, TTSS-1 effectors might control inflammation by directly interfering with multiple host immune responses. However, verifying the role of the proposed mechanisms during gut inflammation *in vivo* remains a major challenge. This is explained by the different processes that can elicit pro-inflammatory responses in the infected cell (Figure 2). It is known that *S.Tm*-related PAMPs such as peptidoglycan, flagellin, TTSS components and LPS can trigger similar inflammatory signaling pathways as the ones which were suggested to be directly manipulated by TTSS-1 effectors (Figure 2) [80<sup>•</sup>,99–105]. Since TTSS-1

**Figure 2**

Both TTSS-1 effectors and PAMPs can induce inflammatory signaling. Examples of inflammatory pathways that can be affected by TTSS-1 effectors and PAMPs.

effectors influence IEC invasion efficiency, intracellular location and survival (discussed below), they will also determine the amount of PAMPs that can be recognized by PRRs within IECs and other cell types of the deeper tissue to elicit pro-inflammatory signaling (Figure 3). Notably, expression of PRRs and other innate immune signaling components can differ markedly between immortalized cell lines and the mature gut tissue [106]. Therefore, it appears plausible that epithelial cell line experiments have tended to particularly identify TTSS-1 effector-driven manipulation of host cell responses due to the partial or complete absence of PAMP-triggered pro-inflammatory cell death pathways. Furthermore, as discussed above, the contribution of TTSS-1 effectors for cell invasion is context-dependent, which implies that cell culture invasion data cannot be used as a proxy for *in vivo* IEC invasion [54<sup>••</sup>,70<sup>••</sup>]. In summary, the points above make it difficult to draw definite conclusions and could explain some of the contradictory results pertaining to links between TTSS-1 effectors and inflammation. To formally prove effector-dependent induction of immune responses in the absence of PAMPs *in vivo*, transgenic mice with cell type-specific inducible expression of TTSS-1 effectors would be required, similarly to the investigations of the *Helicobacter* effector CagA [107].

**Figure 3**

The indirect contribution of TTSS-1 effectors to the degree of inflammation.

TTSS-1 effectors influence invasion efficiency, intracellular location and intracellular survival/replication of S.Tm, which indirectly influence the amount of PAMPs that can be sensed by host immune receptors (red stars). Therefore, TTSS-1 effectors affect the pro-inflammatory signaling by both direct manipulation of the signaling cascades (Figure 2) and indirectly by increasing the density of bacteria/PAMPs within the infected cell.

However, even in such an experimental setup one would need to carefully control for possible contribution of gut luminal PAMPs.

#### TTSS-1 effector expression beyond invasion

Cell culture studies have established the concept that TTSS-1 effectors are expressed beyond invasion to

control host cell machineries and thereby the intracellular fate of *S.Tm*. Host cell internalization leads to the formation of a *Salmonella*-containing-vacuole (SCV) [108], in which the membrane is tightly surrounding the bacteria [12]. This SCV can either grow by fusion with macro- pinosomes or shrink by emanating membrane tubules promoting intracellular growth in the SCV or in the host cytosol, respectively [109•]. SopB influences the integrity of the SCV and together with SopE supports SCV escape into the cytosol [109•,110,111•]. SopE/E2 are additionally associated with early intracellular replication [112]. SipA also promotes initiation of intracellular replication and/or influences *S.Tm* survival and localization within epithelial cells [70•,110,113,114•]. Finally, two recent studies reported new insights about a so far poorly characterized TTSS-1 effector, SopF [115•,116•]. SopF was shown (i) to engage the V-ATPase-ATG16L1 axis thereby inhibiting xenophagy-dependent *S.Tm* restriction, and (ii) to interact with phospholipids and stabilize the SCV.

Importantly, while some of these concepts such as intra-IEC replication and dual location (in SCV and/or cytosolic) were shown to have implications *in vivo* [46,47,69,70•,103,117•], others lack formal *in vivo* validation. Given that most infected IECs have a short lifetime and are promptly expelled into the gut lumen [75•,101–103], some of these TTSS-1 effector-dependent intracellular fate modulations might have a minor impact *in vivo*. This argument is supported by the observation that intracellular SopB expression is mainly detected in expelled epithelial cells in the gall bladder of *S.Tm*-infected mice [91]. However, not all infected IECs are expelled, which would allow intracellular manipulation also over longer time periods at least within a fraction of the invasion foci.

## Conclusions and perspectives

Extensive research has provided a detailed assessment of how *S.Tm* invades IECs with the help of TTSS-1 effectors. It is evident that *S.Tm* has evolved to invade host cells for which it can employ multiple invasion strategies and mechanisms. As SipA is encoded within SPI-1, while SopB, SopE, and SopE2, and the discussed adhesins are encoded elsewhere on the chromosome, it is tempting to speculate that SipA-mediated discreet-invasion represents the primordial IEC invasion mechanism and that initially, PAMP-triggered innate immune responses may have been the main mechanism(s) for eliciting gut inflammation. In this scenario, later acquisition of additional effectors would provide the ability to fine-tune these responses to the pathogen's benefit. This may explain the broader redundancy of effectors and context-dependent differences. These circumstances pose technical challenges to the deciphering of how IEC invasion and the triggering of gut inflammation are interconnected in the host's gut.

## Conflict of interest statement

Nothing declared.

## Acknowledgements

We thank members of the Hardt and Sellin laboratories for helpful discussions. This work was partly supported by grant 310030\_192567 from the Swiss National Science Foundation to W.D.H. and grants from the Swedish Research Council (2018-02223) and the Swedish Foundation for Strategic Research (FFL18-0165) to M.E.S.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Galan JE, Curtiss R 3rd: **Cloning and molecular characterization of genes whose products allow *Salmonella Typhimurium* to penetrate tissue culture cells.** *Proc Natl Acad Sci U S A* 1989, **86**:6383-6387.
  2. Hapfelmeier S, Stecher B, Barthel M, Kremer M, Muller AJ, Heikenwalder M, Stallmach T, Hensel M, Pfeffer K, Akira S, Hardt WD: **The *Salmonella* pathogenicity island (SPI)-2 and SPI-1 type III secretion systems allow *Salmonella* serovar *typhimurium* to trigger colitis via MyD88-dependent and MyD88-independent mechanisms.** *J Immunol* 2005, **174**:1675-1685.
  3. Leung KY, Finlay BB: **Intracellular replication is essential for the virulence of *Salmonella* *Typhimurium*.** *Proc Natl Acad Sci U S A* 1991, **88**:11470-11474.
  4. Watson PR, Paulin SM, Bland AP, Jones PW, Wallis TS: **Characterization of intestinal invasion by *Salmonella* *Typhimurium* and *Salmonella* *dublin* and effect of a mutation in the *invH* gene.** *Infect Immun* 1995, **63**:2743-2754.
  5. Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, Kremer M, Chaffron S, Macpherson AJ, Buer J, Parkhill J et al.: ***Salmonella enterica* serovar *typhimurium* exploits inflammation to compete with the intestinal microbiota.** *PLoS Biol* 2007, **5**:e2177-2189.
  6. Winter SE, Thienennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, Russell JM, Bevins CL, Adams LG, Tsolis RM et al.: **Gut inflammation provides a respiratory electron acceptor for *Salmonella*.** *Nature* 2010, **467**:426-429.
  7. Hume PJ, Singh V, Davidson AC, Koronakis V: **Swiss army pathogen: the *Salmonella* entry toolkit.** *Front Cell Infect Microbiol* 2017, **7**:348.
  8. Kaiser P, Diard M, Stecher B, Hardt WD: **The streptomycin mouse model for *Salmonella* diarrhea: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response.** *Immunol Rev* 2012, **245**:56-83.
  9. Fattiger SA, Sellin ME, Hardt WD: **Epithelial inflammasomes in the defense against *Salmonella* gut infection.** *Curr Opin Microbiol* 2021, **59**:86-94.
  10. Galan JE: ***Salmonella* *Typhimurium* and inflammation: a pathogen-centric affair.** *Nat Rev Microbiol* 2021;1-10.
  11. Finlay BB, Ruschkowski S, Dedhar S: **Cytoskeletal rearrangements accompanying *Salmonella* entry into epithelial cells.** *J Cell Sci* 1991, **99**:283-296.
  12. Fredlund J, Santos JC, Stevenin V, Weiner A, Latour-Lambert P, Rechav K, Mallet A, Krijnse-Locker J, Elbaum M, Enninga J: **The entry of *Salmonella* in a distinct tight compartment revealed at high temporal and ultrastructural resolution.** *Cell Microbiol* 2018, **20**.
  13. Garcia-del Portillo F, Zwick MB, Leung KY, Finlay BB: ***Salmonella* induces the formation of filamentous structures containing lysosomal membrane glycoproteins in epithelial cells.** *Proc Natl Acad Sci U S A* 1993, **90**:10544-10548.

14. Di Martino ML, Ek V, Hardt WD, Eriksson J, Sellin ME: **Barcoded consortium infections resolve cell type-dependent *Salmonella enterica* Serovar Typhimurium entry mechanisms.** *mBio* 2019, 10
- This study compares the contribution of S.Tm TTSS-1 effectors for invasion into epithelial cells versus monocytes and macrophages. The authors use an internally controlled approach in which genetically tagged wild type and effector mutant S.Tm strains are assessed for their invasion efficiency.
15. Hardt WD, Chen LM, Schuebel KE, Bustelo XR, Galan JE: **S. typhimurium encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells.** *Cell* 1998, 93:815-826.
16. Misselwitz B, Barrett N, Kreibich S, Vonaesch P, Andritschke D, Rout S, Weidner K, Sormaz M, Songhet P, Horvath P, Chabria M, Vogel V, Spori DM, Jenny P, Hardt WD: **Near surface swimming of *Salmonella* Typhimurium explains target-site selection and cooperative invasion.** *PLOS Pathogen* 2012, 8:e1002810.
17. Hanisch J, Ehinger J, Ladwein M, Rohde M, Derivery E, Bosse T, Steffen A, Bumann D, Misselwitz B, Hardt WD et al.: **Molecular dissection of *Salmonella*-induced membrane ruffling versus invasion.** *Cell Microbiol* 2010, 12:84-98.
18. Humphreys D, Davidson A, Hume PJ, Koronakis V: ***Salmonella* virulence effector SopE and Host GEF ARNO cooperate to recruit and activate WAVE to trigger bacterial invasion.** *Cell Host Microbe* 2012, 11:129-139.
19. Piscatelli HL, Li M, Zhou D: **Dual 4- and 5-phosphatase activities regulate SopB-dependent phosphoinositide dynamics to promote bacterial entry.** *Cell Microbiol* 2016, 18:705-719.
20. Walpole Glenn FW, Pacheco Jonathan, Chauhan Neha, Abbas Yazan M, Montaño-Rendón Fernando, Liu Zetao, Zhu Hongxian, Brumell John H, Deiters Alexander, Hammond Gerald RV et al.: **Phosphatidylinositol 3-kinase-independent synthesis of PtdIns(3,4)P2 by a phosphotransferase.** *bioRxiv* 2021. 2021.05.25.445663.
21. Alto NM, Shao F, Lazar CS, Brost RL, Chua G, Mattoo S, McMahon SA, Ghosh P, Hughes TR, Boone C, Dixon JE: **Identification of a bacterial type III effector family with G protein mimicry functions.** *Cell* 2006, 124:133-145.
22. Friebel A, Ilchmann H, Aepfelbacher M, Ehrbar K, Machleidt W, Hardt WD: **SopE and SopE2 from *Salmonella* Typhimurium activate different sets of RhoGTPases of the host cell.** *J Biol Chem* 2001, 276:34035-34040.
23. Stender S, Friebel A, Linder S, Rohde M, Mirol S, Hardt WD: **Identification of SopE2 from *Salmonella* typhimurium, a conserved guanine nucleotide exchange factor for Cdc42 of the host cell.** *Mol Microbiol* 2000, 36:1206-1221.
24. Singh V, Davidson AC, Hume PJ, Humphreys D, Koronakis V: **Arf GTPase interplay with Rho GTPases in regulation of the actin cytoskeleton.** *Small GTPases* 2019, 10:411-418.
25. Zhou D, Chen LM, Hernandez L, Shears SB, Galan JE: **A *Salmonella* inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host cell actin cytoskeleton rearrangements and bacterial internalization.** *Mol Microbiol* 2001, 39:248-259.
26. Hayward RD, Koronakis V: **Direct nucleation and bundling of actin by the SipC protein of invasive *Salmonella*.** *EMBO J* 1999, 18:4926-4934.
27. McGhie EJ, Hayward RD, Koronakis V: **Cooperation between actin-binding proteins of invasive *Salmonella*: SipA potentiates SipC nucleation and bundling of actin.** *EMBO J* 2001, 20:2131-2139.
28. McGhie EJ, Hayward RD, Koronakis V: **Control of actin turnover by a salmonella invasion protein.** *Mol Cell* 2004, 13:497-510.
29. Zhou D, Mooseker MS, Galan JE: **Role of the *S. typhimurium* actin-binding protein SipA in bacterial internalization.** *Science* 1999, 283:2092-2095.
30. Andritschke D, Dilling S, Emmenlauer M, Welz T, Schmich F, Misselwitz B, Ramo P, Rottner K, Kerkhoff E, Wada T et al.: **A genome-wide siRNA screen implicates Spire1/2 in SipA-driven *salmonella* typhimurium host cell invasion.** *PLoS One* 2016, 11: e0161965.
31. Cain RJ, Hayward RD, Koronakis V: **Deciphering interplay between *Salmonella* invasion effectors.** *PLoS Pathog* 2008, 4: e1000037.
32. Jepson MA, Kenny B, Leard AD: **Role of sipA in the early stages of *Salmonella* typhimurium entry into epithelial cells.** *Cell Microbiol* 2001, 3:417-426.
33. Perrett CA, Jepson MA: **Regulation of *Salmonella*-induced membrane ruffling by SipA differs in strains lacking other effectors.** *Cell Microbiol* 2009, 11:475-487.
34. Mambu J, Virlogeux-Payant I, Holbert S, Grepinet O, Velge P, Wiedemann A: **An updated view on the Rck invasion of *Salmonella*: still much to discover.** *Front Cell Infect Microbiol* 2017, 7:500.
35. Mijouin L, Rosselin M, Bottreau E, Pizarro-Cerda J, Cossart P, Velge P, Wiedemann A: ***Salmonella enteritidis* Rck-mediated invasion requires activation of Rac1, which is dependent on the class I PI 3-kinases-Akt signaling pathway.** *FASEB J* 2012, 26:1569-1581.
36. Wiedemann A, Mijouin L, Ayoub MA, Barilleau E, Canepa S, Teixeira-Gomes AP, Le Vern Y, Rosselin M, Reiter E, Velge P: **Identification of the epidermal growth factor receptor as the receptor for *Salmonella* Rck-dependent invasion.** *FASEB J* 2016, 30:4180-4191.
37. Wiedemann A, Rosselin M, Mijouin L, Bottreau E, Velge P: **Involvement of c-Src tyrosine kinase upstream of class I phosphatidylinositol (PI) 3-kinases in *Salmonella enteritidis* Rck protein-mediated invasion.** *J Biol Chem* 2012, 287:31148-31154.
38. Barilleau E, Vedrine M, Koczerka M, Burlaud-Gaillard J, Kempf F, Grepinet O, Virlogeux-Payant I, Velge P, Wiedemann A: **Investigation of the invasion mechanism mediated by the outer membrane protein PagN of *Salmonella* Typhimurium.** *BMC Microbiol* 2021, 21:153
- The study investigates TTSS-1-independent invasion via PagN. Using *E. coli* and coated beads, the authors demonstrate that PagN is sufficient to mediate a zipper-like entry involving PI3 kinase signaling, particularly evident in CHO cells.
39. Lambert MA, Smith SG: **The PagN protein of *Salmonella enterica* serovar Typhimurium is an adhesin and invasion.** *BMC Microbiol* 2008, 8:142.
40. Roche SM, Holbert S, Trotter J, Schaeffer S, Georgeault S, Virlogeux-Payant I, Velge P: ***Salmonella* Typhimurium invalidated for the three currently known invasion factors keeps its ability to invade several cell models.** *Front Cell Infect Microbiol* 2018, 8:273.
41. Frost AJ, Bland AP, Wallis TS: **The early dynamic response of the calf ileal epithelium to *Salmonella* typhimurium.** *Vet Pathol* 1997, 34:369-386.
42. Jepson MA, Clark MA: **Studying M cells and their role in infection.** *Trends Microbiol* 1998, 6:359-365.
43. Meyerholz DK, Stabel TJ: **Comparison of early ileal invasion by *Salmonella enterica* serovars Choleraesuis and Typhimurium.** *Vet Pathol* 2003, 40:371-375.
44. Santos RL, Zhang S, Tsolis RM, Baumler AJ, Adams LG: **Morphologic and molecular characterization of *Salmonella* typhimurium infection in neonatal calves.** *Vet Pathol* 2002, 39:200-215.
45. Takeuchi A: **Electron microscope studies of experimental *Salmonella* infection. I. Penetration into the intestinal epithelium by *Salmonella* Typhimurium.** *Am J Pathol* 1967, 50:109-136.
46. Hausmann A, Bock D, Geiser P, Berthold DL, Fattinger SA, Furter M, Bouman JA, Barthel-Scherrer M, Lang CM, Bakkeren E et al.: **Intestinal epithelial NAIP/NLRc4 restricts systemic dissemination of the adapted pathogen *Salmonella* Typhimurium due to site-specific bacterial PAMP expression.** *Mucosal Immunol* 2020, 13:530-544.

47. Laughlin RC, Knodler LA, Barhoumi R, Payne HR, Wu J, Gomez G, Pugh R, Lawhon SD, Baumler AJ, Steele-Mortimer O, Adams LG: **Spatial segregation of virulence gene expression during acute enteric infection with *Salmonella enterica* serovar Typhimurium.** *mBio* 2014, **5**:e00946-13.
48. Ellermeier JR, Slauch JM: **Adaptation to the host environment: regulation of the SPI1 type III secretion system in *Salmonella enterica* serovar Typhimurium.** *Curr Opin Microbiol* 2007, **10**:24-29.
49. Erhardt M, Dersch P: **Regulatory principles governing *Salmonella* and *Yersinia* virulence.** *Front Microbiol* 2015, **6**:949.
50. Johansson ME, Hansson GC: **Immunological aspects of intestinal mucus and mucins.** *Nat Rev Immunol* 2016, **16**:639-649.
51. Furter M, Sellin ME, Hansson GC, Hardt WD: **Mucus architecture and near-surface swimming affect distinct salmonella typhimurium infection patterns along the murine intestinal tract.** *Cell Rep* 2019, **27**:2665-2678 e3.
52. Horstmann JA, Zschieschang E, Truschel T, de Diego J, Lunelli M, Rohde M, May T, Strowig T, Stradal T, Kolbe M, Erhardt M: **Flagellin phase-dependent swimming on epithelial cell surfaces contributes to productive *Salmonella* gut colonization.** *Cell Microbiol* 2017, **19**.
53. Horstmann JA, Lunelli M, Cazzola H, Heidemann J, Kuhne C, Steffen P, Szefs S, Rossi C, Lokareddy RK, Wang C et al.: **Methylation of *Salmonella* Typhimurium flagella promotes bacterial adhesion and host cell invasion.** *Nat Commun* 2020, **11**:2013.
- In this study, the authors investigate how lysine methylation of S.Tm flagellin by the methylase FlIB impacts IEC attachment and invasion efficiency in cell line cultures and intestinal colonization in mice. Methylation of flagellin facilitates attachment to the host cell surface and thereby promotes invasion.
54. Fattinger SA, Bock D, Di Martino ML, Deuring S, Samperio • Ventayol P, Ek V, Furter M, Kreibich S, Bosia F, Muller-Hauser AA et al.: ***Salmonella* Typhimurium discreet-invasion of the murine gut absorptive epithelium.** *PLoS Pathog* 2020, **16**:e1008503.
- This study compares S.Tm invasion of epithelial cell lines versus the murine gut epithelium with regards to TTSS-1 effectors and entry structure morphology. The study concludes that the SPI-1 effector SipA drives discreet-invasion into the absorptive gut epithelium of mice, which contrasts to the largely SopB/E/E2-dependent invasion via expansive membrane ruffles observed in cell line models.
55. Gerlach RG, Jackel D, Stecher B, Wagner C, Lupas A, Hardt WD, Hensel M: ***Salmonella* pathogenicity island 4 encodes a giant non-fimbrial adhesin and the cognate type 1 secretion system.** *Cell Microbiol* 2007, **9**:1834-1850.
56. Li X, Bleumink-Pluym NMC, Luijckx YMCA, Wubbolts RW, van Putten JPM, Strijbis K: **MUC1 is a receptor for the *Salmonella* SiiE adhesin that enables apical invasion into enterocytes.** *PLoS Pathog* 2019, **15**:e1007566.
- This study demonstrates that the SiiE adhesin of *Salmonella* binds to MUC1 on the apical surface of IECs during the pre-invasion step. Host cells deficient for MUC1 are less prone to be invaded by S.Tm.
57. Hansmeier N, Miskiewicz K, Elpers L, Liss V, Hensel M, Sterzenbach T: **Functional expression of the entire adhesome of *Salmonella enterica* serotype Typhimurium.** *Sci Rep* 2017, **7**:10326.
58. Chessa D, Winter MG, Jakomin M, Baumler AJ: ***Salmonella enterica* serotype Typhimurium Std fimbriae bind terminal alpha(1,2)fucose residues in the cecal mucosa.** *Mol Microbiol* 2009, **71**:864-875.
59. Weening EH, Barker JD, Laarakker MC, Humphries AD, Tsolis RM, Baumler AJ: **The *Salmonella enterica* serotype Typhimurium lpf, bcf, stb, stc, std, and sth fimbrial operons are required for intestinal persistence in mice.** *Infect Immun* 2005, **73**:3358-3366.
60. Lara-Tejero M, Galan JE: ***Salmonella enterica* serovar typhimurium pathogenicity island 1-encoded type III secretion system translocases mediate intimate attachment to non-phagocytic cells.** *Infect Immun* 2009, **77**:2635-2642.
61. Misselwitz B, Kreibich SK, Rout S, Stecher B, Periaswamy B, Hardt WD: ***Salmonella enterica* serovar Typhimurium binds to HeLa cells via Fim-mediated reversible adhesion and irreversible type three secretion system 1-mediated docking.** *Infect Immun* 2011, **79**:330-341.
62. Santos AJ, Meinecke M, Fessler MB, Holden DW, Boucrot E: **Preferential invasion of mitotic cells by *Salmonella* reveals that cell surface cholesterol is maximal during metaphase.** *J Cell Sci* 2013, **126**:2990-2996.
63. Sturm A, Heinemann M, Arnoldini M, Benecke A, Ackermann M, Benz M, Dormann J, Hardt WD: **The cost of virulence: retarded growth of *Salmonella* Typhimurium cells expressing type III secretion system 1.** *PLoS Pathog* 2011, **7**:e1002143.
64. Diard M, Garcia V, Maier L, Remus-Emsermann MN, Regoes RR, Ackermann M, Hardt WD: **Stabilization of cooperative virulence by the expression of an avirulent phenotype.** *Nature* 2013, **494**:353-356.
65. Palmer AD, Slauch JM: **Mechanisms of *Salmonella* pathogenesis in animal models.** *Hum Ecol Risk Assess* 2017, **23**:1877-1892.
66. Lou L, Zhang P, Piao R, Wang Y: ***Salmonella* pathogenicity island 1 (SPI-1) and its complex regulatory network.** *Front Cell Infect Microbiol* 2019, **9**:270.
67. Cooper KG, Chong A, Kari L, Jeffrey B, Starr T, Martens C, McClurg M, Posada VR, Laughlin RC, Whitfield-Cargile C et al.: **Regulatory protein HilD stimulates *Salmonella* Typhimurium invasiveness by promoting smooth swimming via the methyl-accepting chemotaxis protein McpC.** *Nat Commun* 2021, **12**:348.
68. Jiang L, Feng L, Yang B, Zhang W, Wang P, Jiang X, Wang L: **Signal transduction pathway mediated by the novel regulator LoiA for low oxygen tension induced *Salmonella* Typhimurium invasion.** *PLoS Pathog* 2017, **13**:e1006429.
69. Zhang K, Dupont A, Torow N, Gohde F, Leschner S, Lienenklaus S, Weiss S, Brinkmann MM, Kuhnel M, Hensel M et al.: **Age-dependent enterocyte invasion and microcolony formation by *Salmonella*.** *PLoS Pathog* 2014, **10**:e1004385.
70. Zhang K, Riba A, Nietschke M, Torow N, Reznik U, Putz A, Fulde M, Dupont A, Hensel M, Hornef M: **Minimal SPI1-T3SS effector requirement for *Salmonella* enterocyte invasion and intracellular proliferation in vivo.** *PLoS Pathog* 2018, **14**:e1006925.
- This study investigates TTSS-1-dependent S.Tm invasion into the gut epithelium of neonate mice. The authors find that the effectors SopE2 and SipA play a particularly important role for IEC invasion and that these effectors, together with SopB, impact the intracellular replication niche.
71. Raffatellu M, Wilson RP, Chessa D, Andrews-Polymeris H, Tran QT, Lawhon S, Khare S, Adams LG, Baumler AJ: **SipA, SopA, SopB, SopD, and SopE2 contribute to *Salmonella enterica* serotype typhimurium invasion of epithelial cells.** *Infect Immun* 2005, **73**:146-154.
72. Meyerholz DK, Stabel TJ, Ackermann MR, Carlson SA, Jones BD, Pohlenz J: **Early epithelial invasion by *Salmonella enterica* serovar Typhimurium DT104 in the swine ileum.** *Vet Pathol* 2002, **39**:712-720.
73. Lhocine N, Arena ET, Bomme P, Uebelmann F, Prevost MC, Robine S, Sansonetti PJ: **Apical invasion of intestinal epithelial cells by *Salmonella* Typhimurium requires villin to remodel the brush border actin cytoskeleton.** *Cell Host Microbe* 2015, **17**:164-177.
74. Crowley SM, Han X, Allaire JM, Stahl M, Rauch I, Knodler LA, Vallance BA: **Intestinal restriction of *Salmonella* Typhimurium requires caspase-1 and caspase-11 epithelial intrinsic inflammasomes.** *PLoS Pathog* 2020, **16**:e1008498.
75. Fattinger SA, Geiser P, Samperio Ventayol P, Di Martino ML, Furter M, Felmy B, Bakkeren E, Hausmann A, Barthel-Scherr M, Gui E et al.: **Epithelium-autonomous NAIP/NLRC4 prevents TNF-driven inflammatory destruction of the gut epithelial barrier in *Salmonella*-infected mice.** *Mucosal Immunol* 2021, **14**:615-629.
- This study investigates the epithelial NAIP/NLRC4 response over time during S.Tm infection of mice. The authors demonstrate that the

- epithelium-autonomous NAIP/NLRC4 dependent expulsion of infected cells limits S.Tm transversal into the lamina propria, which otherwise induces a TNF hyper-response that destroys the epithelial barrier.
76. Forbester JL, Goulding D, Vallier L, Hannan N, Hale C, Pickard D, Mukhopadhyay S, Dougan G: **Interaction of *Salmonella enterica* Serovar Typhimurium with intestinal organoids derived from human induced pluripotent stem cells.** *Infect Immun* 2015, **83**:2926-2934.
77. Geiser P, Di Martino ML, Samperio Ventayol P, Eriksson J, Sima E, Al-Saffar AK, Ahl D, Phillipson M, Webb DL, Sundbom M et al.: ***Salmonella enterica* Serovar Typhimurium exploits cycling through epithelial cells to colonize human and murine enteroids.** *mBio* 2021, **12**.
- This study uses three dimensional murine and human enteroids as a model to study the infection cycle of S.Tm. The study shows that S.Tm growth in the enteroid lumen is fueled by TTSS-1-dependent epithelial invasion followed by reemergence of intracellular S.Tm from expelled IECs. The results are complementary to Chong et al. [114•].
78. Holly MK, Han X, Zhao EJ, Crowley SM, Allaire JM, Knodler LA, Vallance BA, Smith JG: ***Salmonella enterica* infection of murine and human enteroid-derived monolayers elicits differential activation of epithelium-intrinsic inflammasomes.** *Infect Immun* 2020, **88**.
79. Lawrence AE, Abuaita BH, Berger RP, Hill DR, Huang S, Yadagiri VK, Bons B, Fields C, Wobus CE, Spence JR et al.: ***Salmonella enterica* Serovar Typhimurium SPI-1 and SPI-2 shape the global transcriptional landscape in a human intestinal organoid model system.** *mBio* 2021, **12**.
- In this study, human intestinal organoids were infected with S.Tm to explore the host transcriptional response. The results suggest that pro-inflammatory gene expression is largely independent of TTSS-1 mediated invasion, but that invasion affects cell cycle and DNA repair responses.
80. Samperio Ventayol P, Geiser P, Di Martino ML, Florbrant A, Fattinger SA, Walder N, Sima E, Shao F, Gekara NO, Sundbom M et al.: **Bacterial detection by NAIP/NLRC4 elicits prompt contractions of intestinal epithelial cell layers.** *Proc Natl Acad Sci U S A* 2021, **118**.
- The authors show that non-transformed enteroids contract upon TTSS-1-dependent S.Tm invasion, which densifies the IEC packing at the site of invasion. This response is induced by the epithelial NAIP/NLRC4 inflammasome and does not rely on other cell types.
81. Fujii M, Matano M, Toshimitsu K, Takano A, Mikami Y, Nishikori S, Sugimoto S, Sato T: **Human intestinal organoids maintain self-renewal capacity and cellular diversity in niche-inspired culture condition.** *Cell Stem Cell* 2018, **23**:787-793 e6.
82. Yin X, Farin HF, van Es JH, Clevers H, Langer R, Karp JM: **Niche-independent high-purity cultures of Lgr5+ intestinal stem cells and their progeny.** *Nat Methods* 2014, **11**:106-112.
83. Agbor TA, McCormick BA: ***Salmonella* effectors: important players modulating host cell function during infection.** *Cell Microbiol* 2011, **13**:1858-1869.
84. McGhie EJ, Brawn LC, Hume PJ, Humphreys D, Koronakis V: ***Salmonella* takes control: effector-driven manipulation of the host.** *Curr Opin Microbiol* 2009, **12**:117-124.
85. Srikanth CV, Mercado-Lubo R, Hallstrom K, McCormick BA: ***Salmonella* effector proteins and host-cell responses.** *Cell Mol Life Sci* 2011, **68**:3687-3697.
86. Keestra AM, Winter MG, Auburger JJ, Frassle SP, Xavier MN, Winter SE, Kim A, Poon V, Ravesloot MM, Waldenmaier JF et al.: **Manipulation of small Rho GTPases is a pathogen-induced process detected by NOD1.** *Nature* 2013, **496**:233-237.
87. Keestra AM, Winter MG, Klein-Douwel D, Xavier MN, Winter SE, Kim A, Tsolis RM, Baumler AJ: **A *Salmonella* virulence factor activates the NOD1/NOD2 signaling pathway.** *mBio* 2011, **2**.
88. Sun H, Kamanova J, Lara-Tejero M, Galan JE: ***Salmonella* stimulates pro-inflammatory signalling through p21-activated kinases bypassing innate immune receptors.** *Nat Microbiol* 2018, **3**:1122-1130.
89. Ye Z, Petrof EO, Boone D, Claud EC, Sun J: ***Salmonella* effector AvrA regulation of colonic epithelial cell inflammation by deubiquitination.** *Am J Pathol* 2007, **171**:882-892.
90. Lian H, Jiang K, Tong M, Chen Z, Liu X, Galan JE, Gao X: **The Salmonella effector protein SopD targets Rab8 to positively and negatively modulate the inflammatory response.** *Nat Microbiol* 2021, **6**:658-671.
- The study explores the inflammatory-regulating effect of the S.Tm effector SopD. The results show that SopD interacts with Rab8 and can work as both an agonist and antagonist for pro-inflammatory host cell responses, with possible implications *in vivo*.
91. Finn CE, Chong A, Cooper KG, Starr T, Steele-Mortimer O: **A second wave of *Salmonella* T3SS1 activity prolongs the lifespan of infected epithelial cells.** *PLoS Pathog* 2017, **13**:e1006354.
92. Hu GQ, Yang YJ, Qin XX, Qi S, Zhang J, Yu SX, Du CT, Chen W: ***Salmonella* outer protein B suppresses colitis development via protecting cell from necroptosis.** *Front Cell Infect Microbiol* 2019, **9**:87.
93. Muller AJ, Hoffmann C, Galle M, Van Den Broeke A, Heikenwalder M, Falter L, Misselwitz B, Kremer M, Beyerart R, Hardt WD: **The *S. typhimurium* effector SopE induces caspase-1 activation in stromal cells to initiate gut inflammation.** *Cell Host Microbe* 2009, **6**:125-136.
94. Ruan H, Zhang Z, Tian L, Wang S, Hu S, Qiao JJ: **The *Salmonella* effector SopB prevents ROS-induced apoptosis of epithelial cells by retarding TRAF6 recruitment to mitochondria.** *Biochem Biophys Res Commun* 2016, **478**:618-623.
95. Srikanth CV, Wall DM, Maldonado-Contreras A, Shi H, Zhou D, Demma Z, Mumy KL, McCormick BA: ***Salmonella* pathogenesis and processing of secreted effectors by caspase-3.** *Science* 2010, **330**:390-393.
96. Boyle EC, Brown NF, Finlay BB: ***Salmonella enterica* serovar Typhimurium effectors SopB, SopE, SopE2 and SipA disrupt tight junction structure and function.** *Cell Microbiol* 2006, **8**:1946-1957.
97. Liao AP, Petrof EO, Kuppireddi S, Zhao Y, Xia Y, Claud EC, Sun J: ***Salmonella* type III effector AvrA stabilizes cell tight junctions to inhibit inflammation in intestinal epithelial cells.** *PLoS One* 2008, **3**:e2369.
98. Zhang Y, Wu S, Ma J, Xia Y, Ai X, Sun J: **Bacterial protein AvrA stabilizes intestinal epithelial tight junctions via blockage of the C-Jun N-terminal kinase pathway.** *Tissue Barriers* 2015, **3**:e972849.
99. Hisamatsu T, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, Podolsky DK: **CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells.** *Gastroenterology* 2003, **124**:993-1000.
100. Inohara N, Ogura Y, Chen FF, Muto A, Nunez G: **Human Nod1 confers responsiveness to bacterial lipopolysaccharides.** *J Biol Chem* 2001, **276**:2551-2554.
101. Knodler LA, Crowley SM, Sham HP, Yang H, Wrande M, Ma C, Ernst RK, Steele-Mortimer O, Celli J, Vallance BA: **Noncanonical inflammasome activation of caspase-4/caspase-11 mediates epithelial defenses against enteric bacterial pathogens.** *Cell Host Microbe* 2014, **16**:249-256.
102. Rauch I, Deets KA, Ji DX, von Moltke J, Tenthorey JL, Lee AY, Philip NH, Ayres JS, Brodsky IE, Gronert K, Vance RE: **NAIP-NLRC4 inflammasomes coordinate intestinal epithelial cell expulsion with eicosanoid and IL-18 release via activation of caspase-1 and -8.** *Immunity* 2017, **46**:649-659.
103. Sellin ME, Muller AA, Felmy B, Dolowschiak T, Diard M, Tardivel A, Maslowski KM, Hardt WD: **Epithelium-intrinsic NAIP/NLRC4 inflammasome drives infected enterocyte expulsion to restrict *Salmonella* replication in the intestinal mucosa.** *Cell Host Microbe* 2014, **16**:237-248.
104. Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, Hu L, Shao F: **Inflammatory caspases are innate immune receptors for intracellular LPS.** *Nature* 2014, **514**:187-192.
105. von Moltke J, Trinidad NJ, Moayeri M, Kintzer AF, Wang SB, van Rooijen N, Brown CR, Krantz BA, Leppala SH, Gronert K, Vance RE: **Rapid induction of inflammatory lipid mediators by the inflammasome *in vivo*.** *Nature* 2012, **490**:107-111.

106. Hausmann A, Russo G, Grossmann J, Zund M, Schwank G, Aebersold R, Liu Y, Sellin ME, Hardt WD: **Germ-free and microbiota-associated mice yield small intestinal epithelial organoids with equivalent and robust transcriptome/proteome expression phenotypes.** *Cell Microbiol* 2020, **22**: e13191.
107. Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A, Higashi H, Musashi M, Iwabuchi K, Suzuki M et al.: **Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse.** *Proc Natl Acad Sci U S A* 2008, **105**:1003-1008.
108. Steele-Mortimer O: **The *Salmonella*-containing vacuole: moving with the times.** *Curr Opin Microbiol* 2008, **11**:38-45.
109. Stevenin V, Chang YY, Le Toquin Y, Duchateau M, Gianetto QG, Luk CH, Salles A, Sohst V, Matondo M, Reiling N, Enninga J: **Dynamic growth and shrinkage of the *Salmonella*-containing vacuole determines the intracellular pathogen niche.** *Cell Rep* 2019, **29**:3958-3973.e7
- This study investigates intracellular S.Tm fate upon cell entry by live microscopy. The authors show that the sizing of the SCV by tubule formation or fusion with infection associated macropinosomes determines if S.Tm stays intravacuolar or escapes into the host cytosol.
110. Klein JA, Grenz JR, Slauch JM, Knodler LA: **Controlled activity of the *Salmonella* invasion-associated injectisome reveals its intracellular role in the cytosolic population.** *mBio* 2017, **8**.
111. Roder J, Hensel M: **Presence of SopE and mode of infection result in increased *Salmonella*-containing vacuole damage and cytosolic release during host cell infection by *Salmonella enterica*.** *Cell Microbiol* 2020, **22**:e13155
- The authors investigate with fluorescence reporter strains how pathogen and host cell-related factors contribute to the intracellular lifestyle of S.Tm. They conclude that intracellular fate is highly dependent on the host cell invasion mechanism and the TTSS-1 effector composition.
112. Vonaesch P, Sellin ME, Cardini S, Singh V, Barthel M, Hardt WD: **The *Salmonella* Typhimurium effector protein SopE transiently localizes to the early SCV and contributes to intracellular replication.** *Cell Microbiol* 2014, **16**:1723-1735.
113. Brawn LC, Hayward RD, Koronakis V: ***Salmonella* SPI1 effector SipA persists after entry and cooperates with a SPI2 effector to regulate phagosome maturation and intracellular replication.** *Cell Host Microbe* 2007, **1**:63-75.
114. Chong A, Starr T, Finn CE, Steele-Mortimer O: **A role for the *Salmonella* type III secretion system 1 in bacterial adaptation to the cytosol of epithelial cells.** *Mol Microbiol* 2019, **112**:1270-1283
- This study investigates the role of SipA expression in intracellular cytosolic S.Tm and shows that TTSS-1 effector SipA contributes to the early survival and initiation of replication upon S.Tm escape into the host cell cytosol.
115. Lau N, Haeberle AL, O'Keeffe BJ, Latomanski EA, Celli J, Newton HJ, Knodler LA: **SopF, a phosphoinositide binding effector, promotes the stability of the nascent *Salmonella*-containing vacuole.** *PLoS Pathog* 2019, **15**:e1007959
- Complementary to Xu et al. [116•], this study explores the role of the TTSS-1 effector SopF. The authors identify that SopF binds to phosphoinositides in eukaryotic membranes and maintains SCV integrity upon S.Tm host cell entry.
116. Xu Y, Zhou P, Cheng S, Lu Q, Nowak K, Hopp AK, Li L, Shi X, Zhou Z, Gao W et al.: **A bacterial effector reveals the V-ATPase-ATG16L1 axis that initiates xenophagy.** *Cell* 2019, **178**:552-566.e20
- Complementary to Lau et al. [115•], this study explores the role of the TTSS-1 effector SopF. The study shows that SopF blocks antibacterial autophagy, which promotes intracellular survival of S.Tm.
117. Chong A, Cooper KG, Kari L, Nilsson OR, Hillman C, Fleming BA, Wang Q, Nair V, Steele-Mortimer O: **Cytosolic replication in epithelial cells fuels intestinal expansion and chronic fecal shedding of *Salmonella* Typhimurium.** *Cell Host Microbe* 2021, **29**:1177-1185.e6
- The results from this study in mice show that S.Tm invasion into the epithelium, cytosolic escape, and subsequent IEC expulsion contributes to S.Tm intestinal colonization *in vivo*. The results are complementary to Geiser et al. [77•].