Salmonella Typhimurium and inflammation: a pathogen-centric affair

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Abstract | Microbial infections are controlled by host inflammatory responses that are initiated by innate immune receptors after recognition of conserved microbial products. As inflammation can also lead to disease, tissues that are exposed to microbial products such as the intestinal epithelium are subject to stringent regulatory mechanisms to prevent indiscriminate signalling through innate immune receptors. The enteric pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium, which requires intestinal inflammation to sustain its replication in the intestinal tract, uses effector proteins of its type III secretion systems to trigger an inflammatory response without the engagement of innate immune receptors. Furthermore, S. Typhimurium uses a different set of effectors to restrict the inflammatory response to preserve host homeostasis. The S. Typhimurium–host interface is a remarkable example of the unique balance that emerges from the co-evolution of a pathogen and its host.

Serovars
Types of *Salmonella enterica* based on their surface antigenic composition.

Innate immune receptors
Surface receptors in immune cells that can recognize conserved bacterial products and stimulate an inflammatory response.

Bacterial-associated molecular patterns
Conserved bacterial molecules that can stimulate innate immune receptors.

Dysbiosis
A condition in which the composition of the resident intestinal microbiota is altered in a manner that leads to disruption of intestinal physiology.

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Salmonella enterica subsp. *enterica* is a major public health concern, and it is estimated to cause more than 300,000 deaths annually, mostly in developing countries[1,2]. On the basis of its surface antigenic composition, *S. enterica* subsp. *enterica* is classified into hundreds of serovars[3,4]. Some serovars (for example, *S. enterica* subsp. *enterica* serovar Typhi and *S. enterica* subsp. *enterica* serovar Paratyphi) are adapted to their human host, where they cause a systemic infection known as typhoid or paratyphoid fever, respectively, and they are therefore referred to as 'typhoidal serovars'[5–7]. Other serovars, such as *S. enterica* subsp. *enterica* serovar Typhimurium, have a broad host range, and in humans they most often cause self-limiting gastroenteritis and are referred to as 'non-typhoidal serovars'[8]. Intestinal inflammation is central for the disease that follows infection with non-typhoidal salmonellae[9].

In the context of infectious diseases, inflammation is often seen as a central host response that is aimed at the expulsion of an invading pathogen. Indeed, the inflammatory response is the most prominent outcome of the stimulation of innate immune receptors that have evolved to detect bacterial-associated molecular patterns, which are abundantly displayed by bacterial pathogens[10–12]. However, in the case of S. Typhimurium infections, the inflammatory response is essential for this pathogen to colonize the intestinal tract[13,14]. It is well established that the resident intestinal microbiota restricts infection by bacterial pathogens[15–17], although the mechanisms by which the resident microbiota exerts this effect are incompletely understood. It has been shown that the dysbiosis that is caused by intestinal inflammation results in a breakdown of the colonization barrier[13,18,19]. Moreover, intestinal inflammation results in the availability of nutrients that are otherwise not accessible in the uninflamed gut[10,13]. Therefore, the stimulation of intestinal inflammation enables *S.* Typhimurium to compete with the resident microbiota and secure carbon sources and electron acceptors essential to sustain its metabolism and its replication in the gut[13,14,20,21]. Consequently, in the case of *S.* Typhimurium, the inflammatory response can be viewed as a pathogen-stimulated host response that secures *S.* Typhimurium replication rather than simply a host response aimed at the expulsion of the pathogen. In this Review, I discuss the mechanisms by which *S.* Typhimurium triggers inflammation in the intestinal tract through the activities of effector proteins delivered by its type III secretion systems (T3SSs). In addition, I explore the T3SS-dependent mechanisms that are aimed at recovering host homeostasis after the inflammatory response. For other aspects of the biology of *Salmonella* in the intestinal tract, including its interaction with the resident microbiota, readers are referred to other reviews[22,23].

Infection with *S.* Typhimurium

Non-typhoidal salmonellae such as *S.* Typhimurium are most often acquired through the consumption of contaminated food or water[24]. Although the acidity of the stomach constitutes an effective barrier against this pathogen[25], consumption of a large enough inoculum or contaminated food with buffering capacity may result in a productive infection leading to overt disease. After its oral acquisition, *S.* Typhimurium travels down the

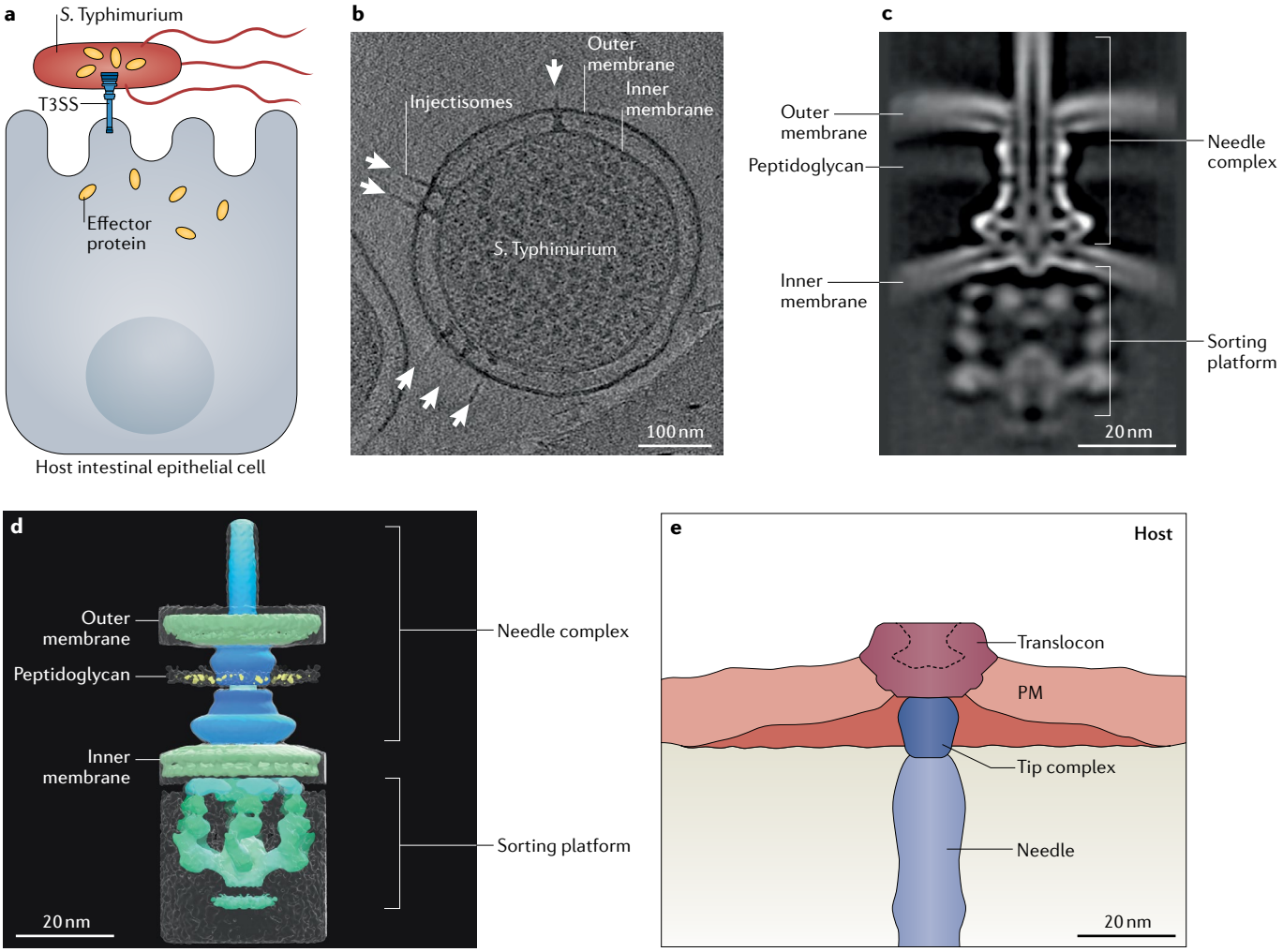

Fig. 1 | **The type III protein secretion system. a** | Diagram depicting *Salmonella enterica* subsp. *enterica* serovar Typhimurium delivering effector proteins through its type III secretion system (T3SS) into the host epithelial cell. This T3SS is encoded by *S.* Typhimurium within its pathogenicity island 1 (not shown). **b** | Electron micrograph of a *S.* Typhimurium cell showing multiple T3SS injectisomes (indicated by the arrows). **c**,**d** | Cryo-electron microscopy images of the T3SS in situ. A central section (part **c**) and a 3D surface rendering (part **d**) of the T3SS injectisome are shown. **e** | Cross section of the interface between *S.* Typhimurium and host cells as revealed by cryo-electron tomography. PM, host-cell plasma membrane. Parts **b**, **c** and **d** reprinted with permission from REF.[108], Elsevier. Part **e** reprinted from REF.[109], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

Type III secretion systems (T3SSs). Complex molecular machines evolved by many bacterial pathogens to modulate host-cell processes through the delivery of bacterially encoded effector proteins directly into the target host cells.

Injectisomes
A name used to refer to the entire type III protein secretion nanomachine that injects effector proteins into host cells.

Flagella
A bacterial organelle that serves to propel the bacteria through liquid environments.

intestinal tract, reaching the large intestine, where most of its replication is thought to occur. Much of what is known about *S.* Typhimurium pathogenesis has been learned with the mouse model of infection[26]. The disease presentation in mice is substantially different from human disease, as *S.* Typhimurium causes systemic infection in this animal model. Nevertheless, at least some of the basic concepts learned from this model system are likely to be applicable to the understanding of human disease. After reaching the large intestine, *S.* Typhimurium uses its flagella and chemotactic systems to reach a location close to the intestinal epithelium[27]. Contact with the intestinal epithelium leads to the activation of the T3SS, which is encoded within *Salmonella* pathogenicity island 1 (SPI-1)[28,29] (FIG. 1), resulting in the delivery of several bacterial effector proteins with the capacity to modulate various host processes (see REFS[30,31]). The main outcome of this initial interaction is the stimulation of host-cell responses that lead to the internalization of bacteria and the transcriptional reprogramming of the infected cell, and ultimately inflammation[32–36] (see below; FIG. 2). More specifically, the activation of host Rho-family GTPases, in particular RAC1, by the bacterial effector proteins SopE, SopE2 and SopB leads to actin cytoskeleton rearrangements and macropinocytosis, which promotes bacterial internalization[37–40]. Other effectors, such as the actin nucleator SipA, also contribute to the internalization process[41]. Once internalized in a membrane-bound compartment, *S.* Typhimurium modulates vesicle trafficking through the activities of effectors that are secreted mainly by a second T3SS, which is encoded within its pathogenicity island 2 (SPI-2). The expression of this T3SS is stimulated by the conditions of the intracellular host environment, such as low magnesium concentrations and low pH[42]. Modulation of vesicle trafficking enables *S.* Typhimurium to avoid innate immune responses and

Salmonella pathogenicity island 1
(SPI-1). A discrete region of the *Salmonella enterica* genome that encodes several genes associated with pathogenesis, including one of its type III secretion systems.

Rho-family GTPases
A family of low molecular weight signalling proteins with intrinsic GTPase activity that regulate several cellular processes.

Macropinocytosis
A process by which cells can take up extracellular material.

to build an intracellular niche that is permissive for its survival and replication. However, the bulk of the bacterial load in the intestine derives not from the intracellular pool but from the expansion of the luminal pool of bacteria[13]. Indeed, the stimulation of the inflammatory response initiated by the T3SS effectors (see below) and subsequently amplified by the engagement of the innate immune system enables *S.* Typhimurium to overcome the rather stringent colonization resistance mechanisms from the resident microbiota. Intestinal inflammation results in dysbiosis and the depletion of resident bacterial species that antagonize the replication of luminal *S.* Typhimurium at least in part by competing for essential nutrients. In addition, the inflammatory response enables *S.* Typhimurium to have access to nutrients and electron acceptors that are otherwise unavailable in uninflamed tissues and that are necessary to support pathogen replication (for reviews see REFS[22,43]). Ultimately, the acquired immune response mounted by the infected host results in the elimination of the pathogen and the recovery of the host's homeostasis. Although in mice *S.* Typhimurium quickly becomes systemic and most often leads to death[26], in most other healthy hosts, infections with non-typhoidal salmonellae are self-limiting and do not become systemic[44]. However, in recent years, the emergence of variants of non-typhoidal salmonellae capable of causing systemic disease in humans have been reported[45].

Stimulating intestinal inflammation

Bypassing innate immune receptors. Unlike most other tissues, where the presence of bacterial products capable of stimulating innate immune receptors can trigger inflammation, the intestinal tract presents a challenge to those pathogens that rely on the inflammatory response to sustain their replication. The presence

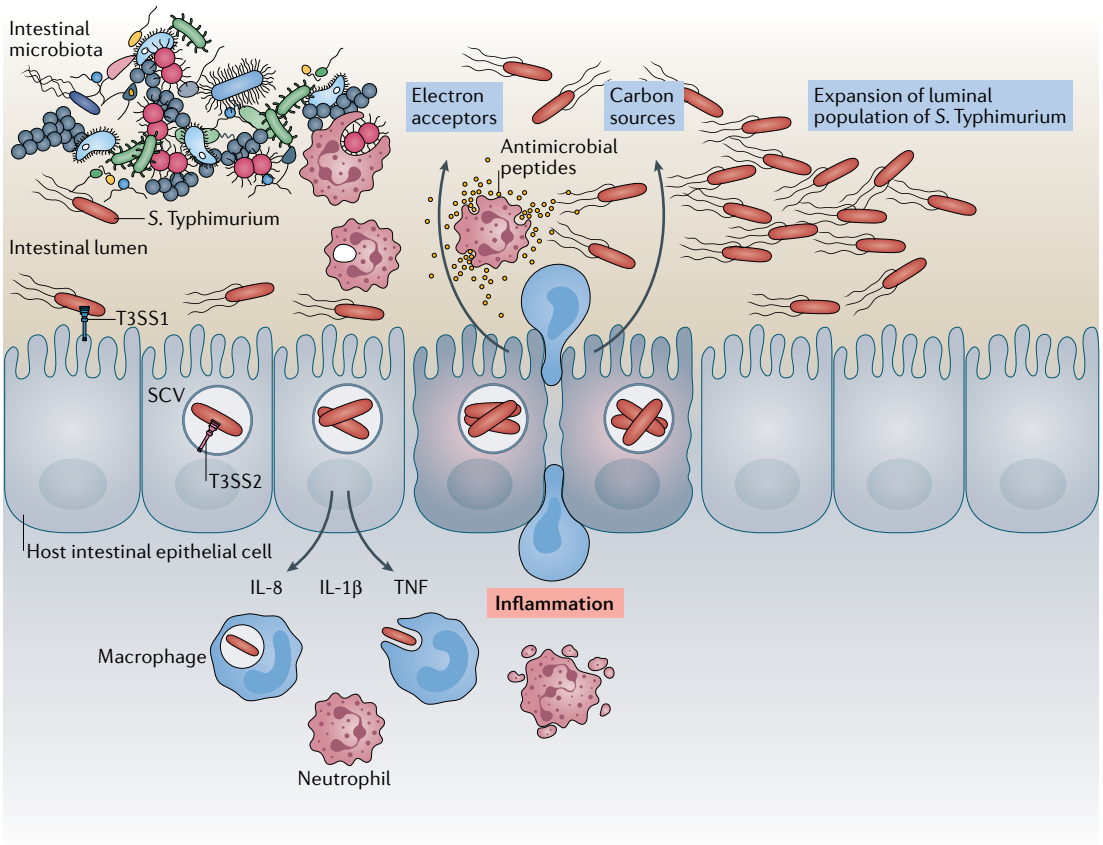

Fig. 2 | **Model for the interaction of *S.* Typhimurium with the intestinal epithelium.** After gaining access to the host via the oral route, *Salmonella enterica* subsp. *enterica* serovar Typhimurium reaches the large intestine, where the pathogen makes contact with the intestinal epithelium. This results in the activation of the type III secretion system encoded within its pathogenicity island 1 (T3SS1). Effector proteins delivered by this system trigger host-cell responses that result in bacterial internalization and the formation of a *Salmonella*-containing vacuole (SCV). The intracellular environment provides the cues for *S.* Typhimurium to express another type III protein secretion system encoded within its pathogenicity island 2 (T3SS2), which enables the pathogen to avoid innate immune defence mechanisms and replicate within cells. The production of proinflammatory cytokines, such as interleukin-8 (IL-8), tumour necrosis factor (TNF) and IL-1β, by the infected cells starts a cascade of events that lead to the recruitment of inflammatory cells, including macrophages and neutrophils. The tissue inflammatory response alters the intestinal lumen environment with the presence of immune cells and increased levels of antimicrobial peptides, which results in the depletion of the resident microbiota. Furthermore, the inflamed tissues provide nutrients and electron acceptors that fuel the replication of the luminal population of *S.* Typhimurium.

Guanine nucleotide exchange factors
(GEFs). Proteins that can activate GTPases by stimulating the release of GDP to allow the binding of GTP.

Inflammasome
A cytoplasmic signalling platform that leads to the activation of caspase 1 or caspase 11 and the subsequent stimulation of inflammation.

in the intestinal tract of an abundance of microbial products derived from the resident microbiota with the potential to stimulate innate immune receptors requires the intestinal epithelium be subject to stringent negative regulatory mechanisms that can prevent the pathology that could result from the indiscriminate firing of these receptors[11,46–50]. Misregulation of those mechanisms can result in chronic inflammatory conditions such as Crohn's disease or inflammatory bowel disease. Consequently, to initiate an inflammatory response in the gut, *S.* Typhimurium cannot rely on the stimulation of innate immune receptors by conserved bacterial products (for example, lipopolysaccharide, peptidoglycan or flagellin) that, like many other bacteria, it possesses in abundance. Rather, it triggers inflammation by bypassing those receptors (FIG. 3). Given its central role in pathogenesis, the mechanisms by which *S.* Typhimurium trigger intestinal inflammation have been a long-standing question in the field and, at times, have been the subject of some controversy. More than two decades ago, before the role of innate immune receptors had been established, it was shown that *S.* Typhimurium could stimulate mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) signalling in cultured intestinal epithelial cells, and that stimulation of these responses resulted in the production of proinflammatory cytokines[35,51]. More importantly, it was shown that stimulation of these responses was strictly dependent on the activity of the T3SS encoded within SPI-1 (REFS[35,51]). These findings were the first indication that *S.* Typhimurium has evolved specific adaptations to trigger inflammation in the intestinal tract. However, later, when the sensing mechanisms of the innate immune system had been revealed, studies showed that the transcriptional responses stimulated by *S.* Typhimurium in cultured epithelial cells resembled those stimulated by innate immune receptors[36]. The requirement of a functional SPI-1 T3SS to stimulate these responses presumably eliminated the possibility that the proinflammatory responses were triggered by conserved agonists of innate immune receptors (such as lipopolysaccharide, peptidoglycan or flagellin) that are abundantly present in *S.* Typhimurium. However, several studies suggested that components of the SPI-1 T3SS itself (for example, the needle and inner rod components) may be recognized by innate immune receptors[52,53]. These observations raised the possibility that the inflammatory responses that followed *S.* Typhimurium infection could be the result of the recognition of the T3SS machinery by the innate immune system. However, the ability of *S.* Typhimurium to stimulate inflammatory signalling was strictly dependent on the function of three specific effector proteins of the SPI-1 T3SS: SopE, SopE2 and SopB[36,39] (see below). Consequently, a mutant lacking these three effectors was unable to trigger inflammatory signalling. As this mutant encodes a wild-type SPI-1 T3SS machinery, these findings in principle ruled out the hypothesis that the inflammatory response results from the recognition of components of the secretion machinery by innate immune receptors. However, these observations led to a conundrum: how could *S.* Typhimurium through the delivery of its effector proteins SopE, SopE2 and SopB trigger 'innate immune-like' signalling without engaging innate immune receptors? The answer to this conundrum would require a better understanding of the mechanisms by which the SPI-1 T3SS effector proteins stimulate these responses (FIG. 3).

Effector-mediated inflammatory signalling. The SPI-1 T3SS effectors SopE and SopE2 are guanine nucleotide exchange factors (GEFs) for the Rho-family GTPases RAC1 and CDC42 (REFS[37,54]). SopB, which is a phosphoinositide phosphatase[55], can also activate Rho-family GTPases although indirectly through the induction of phosphoinositide fluxes that result in the activation of endogenous GEFs for these Rho-family GTPases[39]. By activating RAC1, these effectors mediate actin cytoskeleton rearrangements that lead to bacterial internalization into host cells[39]. In addition, by activating CDC42, these effectors also stimulate MAPK and NF-κB signalling that ultimately results in the production of proinflammatory cytokines[35,39,51]. Although these findings provided major insight into the mechanisms by which *S.* Typhimurium triggers inflammation, these observations could not explain the similarities between the transcriptional responses induced by *S.* Typhimurium with those induced by the stimulation of innate immune receptors as no connection between CDC42 and canonical innate immune signalling mechanisms had been reported. Subsequent studies proposed that the activation of RAC1 by the *S.* Typhimurium effectors per se through unknown mechanisms is sensed as a 'danger-associated molecular pattern' by the innate immune receptor nucleotide-binding oligomerization domain-containing 1 (NOD1), leading to NF-κB activation and the proinflammatory transcriptional response[56]. However, this proposal was not consistent with previous observations indicating that removal of CDC42 abolished *S.* Typhimurium stimulation of inflammatory signalling in cultured cells, even though the absence of CDC42 does not affect the ability of *S.* Typhimurium to activate RAC1 or to gain access to host cells[39]. These observations were also inconsistent with previous reports indicating that removal of RIP2 (REF.[36]) or caspase 1 and caspase 11 (REF.[57]), which are crucial components of the NOD1–inflammasome pathway[36,57], do not affect the ability of *S.* Typhimurium to stimulate intestinal inflammation in mice. These issues were finally clarified when it was shown that stimulation of CDC42 by the *S.* Typhimurium T3SS effector proteins SopE, SopE2 and SopB leads to the activation of the CDC42-effector p21-activated kinase 1 (PAK1) and the subsequent formation of a non-canonical signalling complex composed of PAK1, tumour necrosis factor-associated factor 6 (TRAF6) and transforming growth factor-β (TGFβ)-activating kinase 1 (TAK1)[58]. Removal of PAK1, TRAF6 or TAK1 from various cell lines abrogated the ability of *S.* Typhimurium to stimulate inflammatory signalling. Furthermore, oral administration of a highly specific inhibitor of all group 1 PAKs (PAK1, PAK2 and PAK3) substantially reduced the inflammatory response and replication of *S.* Typhimurium in the mouse intestinal tract without affecting its ability to invade cells[58]. It is well documented that TRAF6 and TAK1 are crucial

components of a signal transduction hub downstream of multiple Toll-like receptors[59,60]. These observations provided a mechanistic explanation for the similarities between the *S.* Typhimurium-induced proinflammatory transcriptional responses and those that generally follow the stimulation of innate immune receptors. Therefore, by engaging innate immune signalling pathways downstream of the actual receptors, *S.* Typhimurium

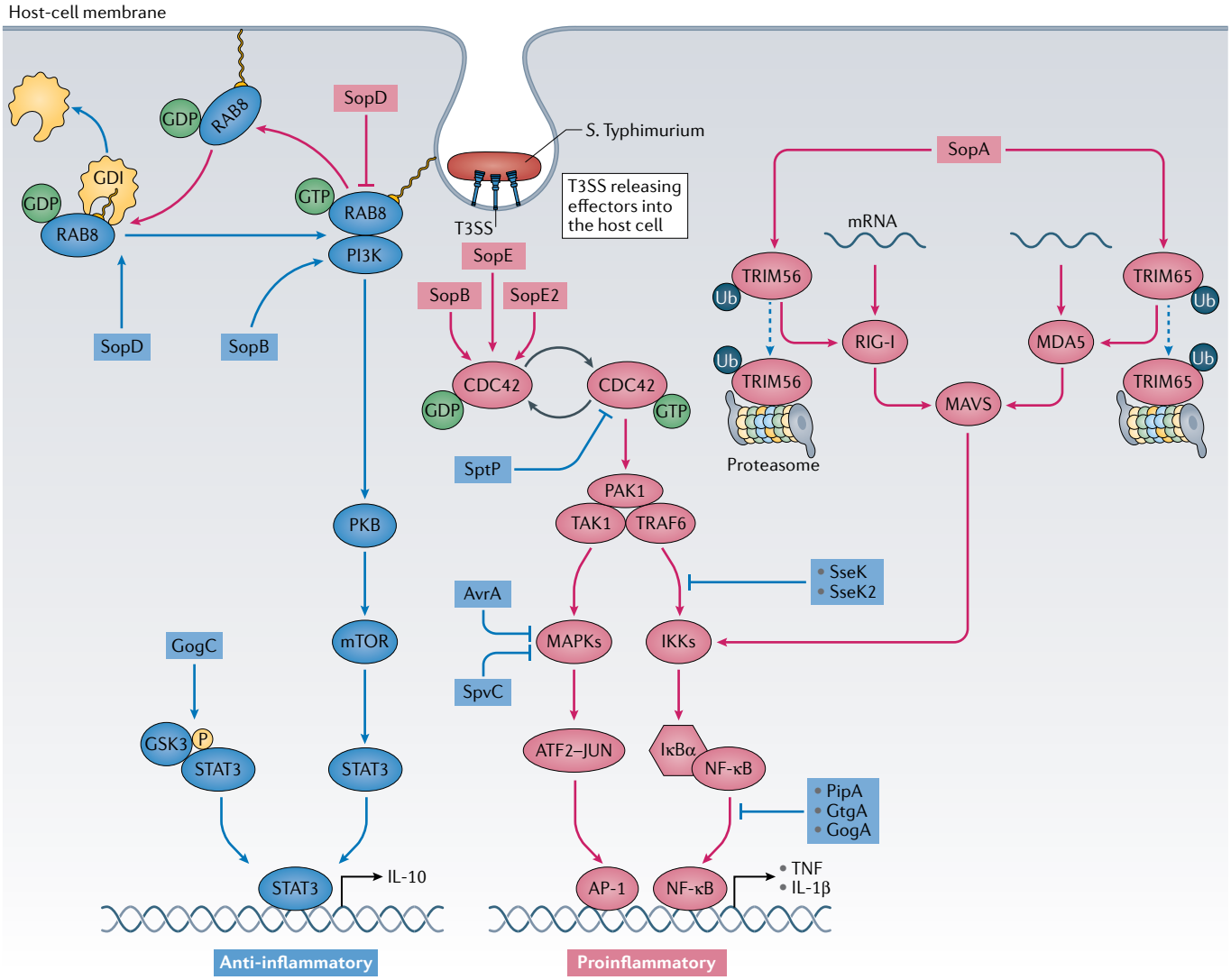

Fig. 3 | **Induction of proinflammatory and anti-inflammatory signalling pathways by *S.* Typhimurium.** Contact of *Salmonella enterica* subsp. *enterica* serovar Typhimurium with intestinal epithelial cells leads to the activation of the type III secretion system (T3SS) encoded within its pathogenicity island 1 (SPI-1) and the delivery of a battery of effector proteins that stimulate inflammatory signalling (depicted in red). More specifically, the effector proteins SopE, SopE2 and SopB activate CDC42, leading to the formation of a non-canonical signalling complex formed by p21-activated kinase 1 (PAK1), transforming growth factor β-activating kinase 1 (TAK1) and tumour necrosis factor (TNF)-associated factor 6 (TRAF6), ultimately resulting in nuclear factor-κB (NF-κB) activation and proinflammatory cytokine production. The SPI-1 T3SS effector protein SopA, which is an E3 ubiquitin ligase, also contributes to inflammation by activating the retinoic acid-inducible gene I protein (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) signalling pathway through the ubiquitylation (Ub) of its regulators, TRIM56 and TRIM65. The SPI-1 T3SS effector SopD, which is a GTPase-activating protein (GAP) for RAB8, stimulates inflammation by antagonizing an anti-inflammatory pathway that is dependent on this Rab GTPase. Later in infection and to help the host recover homeostasis, *S.* Typhimurium delivers a battery of effector proteins of its SPI-1 and SPI-2 T3SSs that antagonize inflammatory signalling. Those effectors (depicted in blue) include SptP, which is a GAP for CDC42; PipA, GtgA and GogA, which proteolytically target the NF-κB transcription factors RELA and RELB; SseK1 and SseK2, which antagonize NF-κB signalling by transferring *N*-acetylglucosamine to death domain-containing proteins in this signalling pathway; AvrA, which interferes with mitogen-activated protein kinase (MAPK) signalling by acetylating MAPK kinase 4 (MKK4) and MKK7; and SpvC, which is a phosphothreonine lyase that targets the MAPKs extracellular signal-regulated kinase 1 (ERK1), ERK2 and p38. Other effectors help host recovery by directly simulating anti-inflammatory signalling. These include (depicted in blue) SopB, which with its phosphoinositide phosphatase activity stimulates phosphoinositide 3-kinase (PI3K)-dependent anti-inflammatory signalling; SopD, which stimulates the same anti-inflammatory signalling pathway by removing RAB8 from its cognate GDP-dissociation inhibitor (GDI); and GogC, which stimulates signal transducer and activator of transcription (STAT)-dependent signalling and the production of anti-inflammatory cytokines such as interleukin-10 (IL-10). Furthermore, the ubiquitylation of TRIM56 and TRIM65, which initially results in the activation of RIG-I and MDA5 proinflammatory signalling, eventually targets these regulators for degradation in the proteasome, thus turning off this signalling pathway. PKB, protein kinase B.

stimulates a response that shares great similarity with the responses stimulated by the activation of canonical innate immune receptors, while avoiding the negative regulatory mechanisms that prevent the activation of these receptors in the intestinal tract (FIG. 3).

Blocking the inflammatory response in the intestine by inhibiting PAKs resulted in a substantial reduction in the number of *S.* Typhimurium bacteria in the mouse intestinal tract[58], which is consistent with the requirement of intestinal inflammation for bacterial replication in the intestinal lumen. However, this inhibiting effect was not observed in animals that had been pretreated with streptomycin to deplete the resident microbiota. These results are consistent with previous observations indicating that in the absence of the competing microbiota *S.* Typhimurium does not need intestinal inflammation to sustain its replication[13]. By contrast, blocking PAKs in the intestinal epithelium resulted in an increase in bacterial load in systemic tissues[58]. These observations indicate that while intestinal inflammation is critically important for the replication of *S.* Typhimurium within the intestine, this response is also central for the host to anatomically restrict the pathogen and prevent its access to deeper tissues.

Although SopE, SopE2 and SopB are essential for the initiation of the inflammatory response following infection, two other effector proteins amplify the response. One of these effectors is SopA, which was originally identified as an effector protein that is required for the efficient stimulation of intestinal inflammation in a cow model of infection[61]. Subsequent studies showed that SopA is a HECT-type E3 ubiquitin ligase that preferentially uses the host's ubiquitin-conjugating enzyme H5A (UBCH5A), UBCH5C and UBCH7 of the ubiquitylation machinery[62]. The similarity with eukaryotic HECT ubiquitin ligases was later corroborated by its crystal structure, which showed that, despite very little sequence similarity, SopA shares structural architectural features with its eukaryotic counterparts[62]. Functional and biochemical studies showed that SopA exerts its proinflammatory activity by ubiquitylating the TRIM-family ubiquitin ligases TRIM56 and TRIM65, stimulating downstream signalling[63]. TRIM proteins are a large family of E3 ubiquitin ligases that have been implicated in various functions[64–66]. More specifically, TRIM56 modulates innate immune responses by ubiquitylating and activating stimulator of interferon genes (STING), a major component of the retinoic acid-inducible gene I protein (RIG-I) signalling pathway that leads to inflammation[67]. By contrast, TRIM65 interacts with melanoma differentiation-associated protein 5 (MDA5)[63], a member of the RIG-I-like receptor (RLR) protein family[68,69], stimulating downstream signalling that also results in interferon-β expression and inflammation. RLRs such as RIG-I itself and MDA5 are essential components of microbial RNA-sensing pathways[68]. However, it is unclear whether the ability of SopA to modulate RLR signalling is enhanced by the presence of microbial nucleic acids. Nevertheless, it has been reported that during infection of non-phagocytic cells, the mRNA from *S.* Typhimurium can be sensed by the RIG-I pathway[70]. It has also been reported that the SopA-mediated ubiquitylation of TRIM56 and TRIM65 leads to their degradation[71]. Although this activity would be incompatible with the well-documented proinflammatory role of SopA, it is possible that it may contribute to the recovery of host homeostasis subsequent to the inflammatory response (see below).

Like SopA, the *S. Typhimurium* T3SS effector protein SopD synergizes with other effectors to stimulate inflammation[72,73]. Recent studies have uncovered the mechanisms by which this effector protein stimulates the inflammatory response[74]. Because inflammation can lead to tissue damage, innate immune receptors are often linked to anti-inflammatory pathways that help the recovery of host homeostasis[11,46–50]. One such anti-inflammatory pathway that functions downstream of cell surface-localized Toll-like receptors is strictly dependent on RAB8. This signalling mechanism results in the activation of phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB; also known as AKT), which ultimately promotes an anti-inflammatory programme[75–77]. In addition, the SPI-1 T3SS effector SopB, which is a phosphoinositide phosphatase, can also activate this RAB8-dependent anti-inflammatory pathway by fluxing phosphoinositides and thereby activating PI3K and PKB (see below)[74]. SopD antagonizes this anti-inflammatory response by directly targeting RAB8 as a specific GTPase-activating protein[74]. Therefore, by inhibiting an anti-inflammatory pathway, SopD effectively functions as a proinflammatory effector protein. Consequently, similarly to SopE, SopE2 and SopB, the effector proteins SopA and SopD can also stimulate inflammation by targeting innate immune inflammatory signalling without the need to engage innate immune receptors.

The role of the inflammasome

Inflammasomes are cytosolic signalling platforms that can sense and coordinate the response to the presence of pathogen-associated molecules in the host-cell cytoplasm[78–80]. Depending on their mechanisms of activation, they are classified as canonical and non-canonical. Canonical inflammasomes, which include the NLRP1, NLRP3, NLRC4, pyrin and AIM2 inflammasomes, are generally activated by conserved microbial products, resulting in the activation of caspase 1. The non-canonical inflammasome is activated by the direct sensing of lipopolysaccharide by caspase 11 in mice or the human orthologues caspase 4 and caspase 5. Activation of both types of inflammasomes leads to similar types of responses, which include the stimulation of the production of proinflammatory cytokines and a form of programmed cell death known as pyroptosis (extensively reviewed in REFS[81–83]). The ability of *S.* Typhimurium to stimulate pyroptosis in macrophages through the activation of this signalling platform in a T3SS-dependent manner has been long recognized[84,85]. In addition, inflammasome signalling has also been shown in intestinal epithelial cells, where it has a role during *S.* Typhimurium infection[86,87]. The mechanisms by which *S.* Typhimurium activates the inflammasome through the activity of its SPI-1 T3SS are not fully understood and are most likely to be multifactorial.

Pyroptosis
A form of cell death that leads to inflammation.

As a functional SPI-1 T3SS (although not its effectors) is required for *S.* Typhimurium to activate the inflammasome, it is likely that the deployment of the T3SS translocon on the eukaryotic cell membrane leads to ion fluxes that may be the activation trigger. When overexpressed in cells, the needle filament and inner rod components of the T3SS have been shown to activate the inflammasome[52,53]. However, as these components are essential for the function of the T3SS, it has been challenging to ascertain the physiological significance of these cell culture observations as mutations of these components would affect T3SS function and therefore deployment of the translocon. Flagellin, the building subunit of the flagellar filament, has also been shown to activate the inflammasome[88]. Although it is clear that the inflammasome is important in controlling *S.* Typhimurium systemic infection[86,87], its specific contribution to the stimulation of intestinal inflammation seems to be secondary, at least in the context of the mouse model of infection. Consistent with this notion, the ability of *S.* Typhimurium to stimulate intestinal inflammation is unaltered in mice simultaneously deficient in caspase 1 and caspase 11 (REF.[57]) or RIP2 (REF.[36]), which are essential components of inflammasome signalling. It has been reported that activation of the inflammasome leads to the extrusion of intestinal epithelial cells harbouring *S.* Typhimurium[89]. Therefore, rather that contributing to inflammation, activation of the inflammasome may help the host to recover homeostasis after the inflammatory response triggered by *S.* Typhimurium by reducing bacterial numbers through epithelial shedding.

Actively promoting cell homeostasis

Pathogens that have a sustained long-standing association with their hosts have evolved specific mechanisms not just to ensure their replication but also to preserve the host's homeostasis. This concept may seem counterintuitive at first, as research tends to emphasize mechanisms of pathogenesis. This is particularly the case for inflammation, as microbial factors aimed at decreasing the inflammatory response to preserve host homeostasis are often viewed as 'virulence factors' that are aimed at thwarting the host's defence response. This concept is eloquently illustrated by the repertoire of *S.* Typhimurium T3SS effectors that counter the activities of proinflammatory effector proteins (see below) (FIG. 3; TABLE 1). Removal of the antagonistic effectors results in increased disease and virulence[90–92], which demonstrates that preservation of host homeostasis through virulence limitation is central to the ecology and the evolution of this pathogen.

Table 1 | **Proinflammatory and anti-inflammatory type III secretion effectors in *Salmonella enterica* subsp. *enterica* serovar Typhimurium**

Effector type	Effector	Function
Proinflammatory effector	SopE, SopE2	GEFs for Rho-family GTPases[37,54] (stimulate NF-κB through non-canonical PAK1–TRAF6–TAK1 signalling[58])
	SopB	Phosphoinositide phosphatase[55] (activates endogenous GEFs for Rho-family GTPases[39])
	SopA	E3 ubiquitin ligase[62] (activates RIG-I and MDA5 signalling through ubiquitylation of TRIM56 and TRIM65 (REF.[63]))
	SopD	GAP for RAB8 (neutralizes a RAB8-dependent anti-inflammatory pathway)[74]
Anti-inflammatory effector	SptP	GAP for Rho-family GTPases[93]
	SopB	Phosphoinositide phosphatase[55] (activates PI3K-dependent anti-inflammatory pathways[74])
	SopA	E3 ubiquitin ligase[62] (antagonizes RIG-I and MDA5 signalling through ubiquitin-mediated degradation of TRIM56 and TRIM65 (REF.[71]))
	SopD	GDI-dissociation factor for RAB8 (activates a RAB8-dependent anti-inflammatory pathway)[74]
	GogC (also known as SteE, SarA or PagJ)	Stimulates STAT3-dependent anti-inflammatory signalling[103,104]
	SpvD	Inhibits RELA nuclear translocation[96]
	PipA, GtgA, GogA	Proteases for NF-κB transcription factors RELA and RELB (inhibit NF-κB-dependent transcription)[92]
	AvrA	Acetylates MKK4 and MKK7 (inhibits JNK signalling)[97,98]
	SpvC	Phosphothreonine lyase for ERK1, ERK2 and p38 (inhibits MAPK signalling)[99,100]
	SseK1, SseK2	*N*-Acetylglucosamine transferase for DEAD-domain containing proteins (inhibits NF-κB signalling)[94,95]

ERK, extracellular signal-regulated kinase; GAP, GTPase-activating protein; GDI, GDP-dissociation inhibitor; GEF, guanine nucleotide exchange factor; JNK, JUN amino-terminal kinase; MAPK, mitogen-activated protein kinase; MDA5, melanoma differentiation-associated protein 5; MKK, mitogen-activated protein kinase kinase; NF-κB, nuclear factor-κB; PAK1, p21-activated kinase 1; PI3K, phosphoinositide 3-kinase; RIG-I, retinoic acid-inducible gene I protein; STAT3, signal transducer and activator of transcription 3; TAK1, transforming growth factor β-activating kinase 1; TRAF6, tumour necrosis factor-associated factor 6; TRIM, tripartite motif-containing protein.

Antagonistic effectors use at least two general mechanisms to antagonize the inflammatory response: they directly counter signalling pathways that have been triggered by agonistic, proinflammatory effectors; and they actively stimulate anti-inflammatory pathways. Among the first group is the SPI-1 T3SS effector SptP, which is a GTPase-activating protein for the Rho-family GTPases CDC42, RAC1 and Rho[93], thus opposing the proinflammatory activity the effectors SopE and SopE2, which are GEFs for the same Rho-family GTPases[37,54]. By limiting the activation of CDC42 in particular, SptP limits the inflammatory response and helps the host to recover homeostasis[93]. Another subset of effectors, PipA, GtgA and GogA, proteolytically target the NF-κB transcription factors RELA and RELB, effectively limiting the inflammatory response to *S.* Typhimurium[92]. Consistent with their biochemical activity, removal of these three effectors from *S.* Typhimurium resulted in a substantial increase in intestinal inflammation and increased lethality in a mouse model of infection[92]. Similarly, the effector proteins SseK1 and SseK3 inactivate NF-κB signalling by transferring *N*-acetylglucosamine to specific arginine residues in the death domains of several key proteins in this signalling pathway[94,95]. Additional examples of effectors that directly target inflammatory signalling components in a negative regulatory manner include SpvD, AvrA and SpvC. SpvD inhibits NF-κB activation by interfering with the nuclear translocation of RELA through interactions with the exportin XPO2, which mediates nuclear–cytoplasmic recycling of importins[96]. AvrA suppresses JUN amino-terminal kinase (JNK) signalling through acetylation of the upstream kinases MAPK kinase 4 (MKK4) and MKK7 (REFS[97,98]). By contrast, SpvC is a phosphothreonine lyase that directly targets extracellular signal-regulated kinase 1 (ERK1), ERK2 and p38 by irreversibly removing phosphate groups from phosphothreonine residues[99,100]. As predicted by their biochemical activities, *S.* Typhimurium mutants lacking either AvrA or SpvC induced more pronounced intestinal inflammation in a mouse model of infection[90,91].

The second group of effectors help to preserve host homeostasis by actively stimulating anti-inflammatory pathways. For example, by fluxing phosphoinositides with its phosphoinositide phosphatase enzymatic activity, SopB stimulates the activation of a RAB8-dependent PI3K–PKB–mTOR signalling pathway that functions downstream of Toll-like receptors[74]. This pathway leads to the production of the anti-inflammatory cytokine interleukin-10 (IL-10), thus promoting host recovery[75–77]. Interestingly, the same pathway is targeted by the effector protein SopD but through a completely different mechanism[74]. SopD stimulates the dissociation of RAB8 from its cognate GDP-dissociation inhibitor (GDI), which leads to the GTP loading of this GTPase and the subsequent stimulation of the PI3K–PKB–mTOR anti-inflammatory pathway. The activity of SopD is equivalent to that of eukaryotic GDI-displacement factors, which activate Rab GTPases by removing them from their cognate GDIs, thus targeting them to the membrane for recycling and activation[101,102]. Therefore, in the case of SopD, both proinflammatory (see above) and anti-inflammatory activities are exerted by the same effector.

Another example of this group of effectors is GogC (also known as SteE, SarA or PagJ), which targets signal transducer and activator of transcription 3 (STAT3)[103,104]. This signalling protein is involved in many cell biological processes, including signalling pathways that direct the recovery of homeostasis after an inflammatory response[105,106]. *S.* Typhimurium is a potent activator of this signalling pathway, which is required for its efficient intracellular growth[107]. The STAT3 activation mechanism is non-canonical as it does not require Janus kinases. Instead, the mechanism requires the host kinase GSK3, which phosphorylates GogC, leading to the formation of a GogC–STAT3 complex and the activation of this signalling cascade[103,104].

Concluding remarks

The mechanisms by which *S.* Typhimurium triggers intestinal inflammation through the action of its T3SS effectors are now well understood. The studies of these mechanisms have provided insight not only into the pathogenesis of non-typhoidal *Salmonella* infection but also into the underlying mechanisms that lead to some chronic intestinal inflammatory illnesses such as Crohn's disease or inflammatory bowel disease. Given the central role of intestinal inflammation in the pathogenesis of *Salmonella* infections, it is possible that knowledge of the detailed mechanisms by which this pathogen modulates inflammation could be useful for the development of novel anti-infectants targeting relevant host pathways or bacterial effector proteins. It was shown that oral administration of an inhibitor of host PAKs, which are essential for the initiation of the inflammatory response to *S.* Typhimurium, effectively blocked the ability of this pathogen to trigger intestinal inflammation and replicate within the intestinal tract[58]. However, addition of the inhibitor resulted in increased bacterial replication in systemic tissues. These findings illustrate the challenge of targeting a host response that is required for both pathogen replication and host defence. The mechanisms by which *S.* Typhimurium modulates the inflammatory response are a remarkable example of the complex adaptations that emerge from long-standing host–pathogen associations.

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