UC Irvine
UC Irvine Previously Published Works

Title

Exploiting host immunity: the Salmonella paradigm

Permalink

Journal

ISSN

Authors

Behnsen, Judith
Perez-Lopez, Araceli
Nuccio, Sean-Paul
et al.

Publication Date

DOI

Peer reviewed

Exploiting host immunity: the *Salmonella* paradigm

Judith Behnsen[1,2], **Araceli Perez-Lopez**[1,2], **Sean-Paul Nuccio**[1], and **Manuela Raffatellu**[1,2,*]

[1]Department of Microbiology and Molecular Genetics, University of California Irvine School of Medicine, Irvine, CA 92697-4025

[2]Institute for Immunology, University of California Irvine School of Medicine, Irvine, CA 92697-4025

Abstract

Pathogens have evolved clever strategies to evade and in some cases exploit the attacks of an activated immune system. *Salmonella enterica* is one such pathogen, exploiting multiple aspects of host defense to promote its replication in the host. Here we review recent findings on the mechanisms by which *Salmonella* establishes systemic and chronic infection, including strategies involving manipulation of innate immune signaling and inflammatory forms of cell death, as well as immune evasion by establishing residency in M2 macrophages. We also examine recent evidence showing that the oxidative environment and the high levels of antimicrobial proteins produced in response to localized *Salmonella* gastrointestinal infection enable the pathogen to successfully outcompete the resident gut microbiota.

Keywords

Salmonella; immunity; mucosal immunity; immune evasion; inflammation

Introduction

The immune response has the important function of defending the host from pathogens and potentially harmful commensals (e.g. pathobionts). Nevertheless, pathogens cause disease, implying that they can at least temporarily overcome host immune defenses to establish an infection. Over the years, a number of studies have put forward the concept that the immune response can actually be of benefit to harmful microbes, as various aspects can be exploited by pathogens to enhance their colonization and replication within the host.

A prime example of an infectious agent that exploits the host's immune response is the pathogen *Salmonella enterica* (hereafter referred to as *Salmonella*), a facultatively

*to whom correspondence should be addressed: Manuela Raffatellu MD, Department of Microbiology and Molecular Genetics and Institute for Immunology, University of California, Irvine, Irvine, CA 92697 USA, manuelar@uci.edu Fax: 949-824-8598 Tel: 949-824-0359.

intracellular bacterium responsible for an estimated 90 million cases of human gastroenteritis [1] and 20 million cases of human typhoid fever [2] per year. Although the disease elicited by *Salmonella* is dependent upon characteristics of the pathogen's serological variety (serovar) as well as characteristics of the host, infections are generally either localized to the gastrointestinal tract or systemically disseminative [3, 4]. For humans, localized gastrointestinal infections of *Salmonella* are caused by the many hundreds of "non-typhoidal" serovars within the species, including the model serovar Typhimurium. The disease elicited by these serovars is usually self-limiting and is characterized by inflammatory diarrhea with a massive recruitment of neutrophils to the site of infection [5]. Nevertheless, despite the robust innate immune response, non-typhoidal *Salmonella* serovars colonize the intestine to high numbers and are shed in the feces of infected individuals for up to a month. Consistent with these clinical observations, several studies have now shown that *Salmonella* exploits intestinal inflammation to compete with the resident microbiota and to thrive in the inflamed gut [6–12].

Although most *Salmonella* serovars elicit gastroenteritis in humans, and all are invasive with respect to the intestinal mucosa, a relative few "typhoidal" serovars have evolved to exclusively cause disseminated, life-threatening infections [3, 4]. Such is the case for serovar Typhi, the causative agent of typhoid fever in humans, a systemic disease characterized by fever and enlargement of the spleen and liver (hepatosplenomegaly) [13]. Survival in extraintestinal sites involves a complex interplay between *Salmonella* and immune cells, primarily macrophages, which are permissive for the pathogen's replication and constitute a niche that promotes *Salmonella* persistence within the host.

Recent advances have provided new insights into how *Salmonella* exploits and evades immunity to promote its colonization and replication within the host. Here we review emerging evidence on the mechanisms by which *Salmonella* circumvents innate immunity to disseminate and establish infection at systemic sites. We further examine recent findings describing approaches by which *Salmonella* co-opts the immune response in the inflamed gut to thrive and compete with the resident microbiota. Overall, our goal is to convey general concepts on how *Salmonella* exploits host immunity to its own advantage.

Strategies towards establishing systemic infection

Infection of the gastrointestinal tract and of systemic sites such as the spleen and the liver involves multifaceted interactions between *Salmonella* and macrophages. Macrophages are efficient in the phagocytosis and killing of bacteria through mechanisms including acidification, the production of antimicrobial peptides and toxic free radicals, cell death by pyroptosis, and the recruitment of other mediators of immunity by releasing proinflammatory cytokines [14]. Even so, *Salmonella* successfully manipulates and employs these phagocytes to promote its own survival and to further establish infection. Below we describe some of the mechanisms by which *Salmonella* interacts with macrophages and other host cells, and how it exploits innate immunity to propagate within the host.

Manipulation of TLR and NLR signaling

Macrophages can detect pathogens through recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) located in the cellular and vacuolar membranes or in the cytosol. Membrane-associated PRRs include Toll-like receptors (TLRs), whereas cytosolic PRRs include Nod-like receptors (NLRs). Both TLRs and NLRs mediate recognition of *Salmonella*, and aspects of their activation are exploited to further establish infection (Figure 1).

Host-beneficial TLR recognition of *Salmonella* by macrophages is achieved primarily through TLR2 and TLR4, as mice lacking either or both of these PRRs exhibit increased bacterial burdens in the mesenteric lymph nodes (MLNs) following oral infection [15]. However, stimulation of macrophages with TLR agonists leads to increased phagocytosis, and accompanying increased intracellular replication of *Salmonella* [16]. Arpaia et al found that mice with genetic deletion of three TLR loci (TLR2, TLR4 and TLR9) were less susceptible to death by *Salmonella* infection than are TLR2$^{-/-}$/TLR4$^{-/-}$ and TLR4$^{-/-}$/ TLR9$^{-/-}$ mice, indicating that *Salmonella* requires robust TLR signaling for intracellular replication in immunocompetent hosts [17]. The authors went on to show that TLR-deficient macrophages fail to acidify; this acidification was found to be necessary to trigger the upregulation of genes in *Salmonella* that are essential to orchestrating formation of the replicative vacuole [17]. As such, TLR signaling is necessary to enhance *Salmonella* replication and to promote the establishment of the pathogen's intracellular niche. Conversely, complete loss of TLR signaling in mice lacking TLR2, TLR4, and UNC93B1, a trafficking protein required for proper localization of TLR3, TLR7 and TLR9 [18], results in susceptibility to systemic *Salmonella* infection because the lack of inflammation allows the pathogen to replicate in an extracellular niche [19]. Thus, the TLR response is helpful in terms of limiting the systemic extracellular growth of *Salmonella*, but harmful in terms of facilitating the pathogen's intracellular growth.

NLRC4 and NLRP3 are both activated during systemic *Salmonella* infection and are associated with host-protection. Ligand recognition by these NLRs leads to caspase-1 activation and the subsequent secretion of the pro-inflammatory cytokines IL-1β and IL-18 [20–22], resulting in a form of cell death known as pyroptosis [23]. Whereas flagellin activates NLRC4, the ligand for NLRP3 activation is unknown. It is possible that the production of reactive oxygen species (ROS) during infection could trigger the NLRP3 inflammasome, because the presence of ROS is accompanied by K^+ efflux, which is a known signal for NLRP3 activation [24]. Mice deficient in NLRC4 and NLRP3 exhibit higher bacterial loads in the mesenteric lymph nodes (MLNs), spleen and liver [22]. In fact, *Salmonella* attempts to evade activation of the NLRC4 inflammasome by downregulating expression of flagellin, and of the NLRP3 inflammasome by using two enzymes of the bacterial tricarboxylic acid cycle, aconitase and isocitrate dehydrogenase, to reduce ROS production [25, 26].

In contrast to NLRC4 and NLRP3, activation of NLRP6 and NLRP12 are associated with higher susceptibility to *Salmonella* infection. Mice deficient in either of these NLRs exhibit lower *Salmonella* burden in the liver and spleen, accompanied by the enhanced recruitment

of monocytes and neutrophils, as well as by an increase in phosphorylation of IκB, indicating an increase in the activation of NF-κB signaling [27, 28]. These observations demonstrate that the activation of NLRP6 and NLRP12 during *Salmonella* infection negatively regulates the inflammatory response, thus promoting *Salmonella* dissemination and colonization of systemic organs [27, 28]. Of note, the mechanisms by which *Salmonella* activates NLRP6 and NLRP12 remain to be determined.

Salmonella benefits from host cell death

A consequence of bacterial infection is the death of host cells. Although this process is an effective host strategy to inhibit intracellular replication of bacteria, cell death can also be exploited by *Salmonella* to further its dissemination (Figure 1).

Different mechanisms of cell death occur during *Salmonella* infection, including pyroptosis and necroptosis. Pyroptosis, or inflammatory cell death, is characterized by the activation of caspase-1 or caspase-11, and by the secretion of IL-1β and IL-18 [23]. One of the mechanisms by which *Salmonella* triggers pyroptosis is through secretion of flagellin into the host cell's cytosol via the type-three secretion system encoded in *Salmonella* pathogenicity island 1, which in turn actives the NLRC4 inflammasome [29]. Other mechanisms include activation of the NLRP3 inflammasome by an unknown ligand, and activation of caspase-11 by cytosolic LPS via unknown receptors [30–32]. Although pyroptosis can benefit the host by limiting systemic growth of *Salmonella* within macrophages, it can also contribute to the inflammatory milieu *Salmonella* needs to replicate efficiently in the gastrointestinal lumen, as discussed below. Moreover, pyroptosis induced by *Salmonella* in intestinal epithelial cells is associated with the extrusion of cytosolic *Salmonella* out of the epithelial monolayer, thereby enhancing systemic dissemination of the pathogen [33].

Salmonella also exploits the induction of necroptosis, a form of cell death controlled by the receptor-interacting protein (RIP) family of serine-threonine kinases [34]. Activation of necroptosis in macrophages is dependent on the induction of type I interferon (IFN) signaling by *Salmonella*, which in turn drives the activation of the RIP1 and RIP3 kinases [35]. Whereas wild-type mice succumb to infection and exhibit extensive necrotic cell death of infected macrophages, mice deficient in IFN receptor 1 (IFNAR1) show prolonged survival and the absence of these necrotic cells [35]. *Salmonella* thus exploits type I IFN signaling to induce necroptosis in macrophages, thereby enhancing its proliferation within the host.

Altogether, *Salmonella* can replicate and disseminate better within the host by modulating pyroptosis and necroptosis. An outstanding question is how *Salmonella* only induces pyroptosis or necroptosis in a subset of macrophages, and whether the two processes are occurring simultaneously. Additionally, the mechanisms by which *Salmonella* triggers necroptosis, including whether its induction requires specific virulence factors, remain to be determined.

Establishment of chronic infection

Not all macrophages that engulf *Salmonella* die as a result of infection, and such cells constitute a niche that enhances *Salmonella* persistence during chronic infection. These observations have since prompted several investigations aimed at determining whether the infected macrophages in a persistent infection have a unique phenotype (Figure 2). To this end, *Salmonella* was recently found to preferentially survive in macrophages with an anti-inflammatory M2 phenotype [36, 37]. A subset of these macrophages in the spleen is characterized by having engulfed leukocytes, including B and T cells, and are known as hemophagocytic macrophages [37, 38]. Hemophagocytic macrophages are defective in limiting the replication of *Salmonella*, likely because they secrete very low levels of pro-inflammatory cytokines [37]. Thus, survival within hemophagocytes is one of the mechanisms by which *Salmonella* can persist during chronic infection and avoid clearance by the host immune system.

An essential factor that promotes *Salmonella* replication within M2 macrophages is the altered metabolism of these phagocytes. The acquisition and maintenance of the M2 phenotype requires the action of metabolic regulators such as the peroxisome proliferator-activated-receptors (PPARs) PPARγ and PPARδ [39]. PPARs are cytosolic receptors that sense fatty acids and modulate fatty acid metabolism, insulin sensitivity, and glucose homeostasis. Upon activation by binding fatty acid metabolites, PPARs translocate to the nucleus where they activate or repress the transcription of target genes [40]. During chronic *Salmonella* infection, the upregulation of PPARδ in M2 macrophages results in the increase of glucose availability to the pathogen, thereby enhancing its replication inside these cells and sustaining its long-term survival within the host [36]. Indeed, this phenotype is of such critical importance to the maintenance of *Salmonella* in the host that the pathogen fails to persist in *Ppar*δ-deficient mice [36]. Collectively, these studies demonstrate that *Salmonella* exploits M2 macrophages to establish and maintain a chronic infection in the host, taking advantage of these cells' diminished pro-inflammatory cytokine secretion as well as their relative abundance of a high-energy carbon source.

Another recently discovered means by which *Salmonella* establishes chronic infection in the liver is by direct inhibition of CD4+ and CD8+ T cell proliferation, accompanied by downmodulation of T cell receptor β expression and by suppression of T cell blastogenesis [41]. The mechanism by which *Salmonella* triggers T cell inhibition during chronic infection was shown to be dependent on the secretion of L-Asparaginase II, a *Salmonella* virulence factor that catalyzes the hydrolysis of L-asparagine to aspartic acid and ammonia [41]. Future studies are needed to determine the mechanisms by which L-Asparaginase II impacts T cell function, as well as whether the depletion of L-asparagine impairs T cell metabolism.

Altogether, the aforementioned studies indicate that *Salmonella* manipulates many aspects of the host immune response to establish a systemic infection and to persist in the host's lymphoid tissue, including: The activation of TLR signaling to replicate in an intracellular niche; the downmodulation of NLR ligands to evade pyroptosis; the induction of type I IFN signaling to induce necroptosis; the exploitation of M2 macrophages for intracellular survival; and the direct inhibition of T cell proliferation. Nevertheless, the mechanisms by

which *Salmonella* activates and modulates some of these responses, as well as the interplay between these responses, largely remain uncharacterized. Moreover, it will be important to investigate the contribution of these pathways to *Salmonella* pathogenesis during localized gastrointestinal infection, in which a large fraction of *Salmonella* is found to be extracellular in the intestinal lumen.

Although manipulating and exploiting the innate immune system to dampen and evade an inflammatory response is of the utmost importance for the replication and persistence of *Salmonella* in systemic sites, activation of innate immunity is necessary to enhance *Salmonella* replication in the gastrointestinal tract. On this note, several aspects of the immune response to *Salmonella* infection in the intestine are discussed in the section below.

Salmonella infection in the intestine

Non-typhoidal *Salmonella* triggers a massive inflammatory response, characterized by the secretion of pro-inflammatory cytokines including IL-18 (e.g. by epithelial cells and macrophages undergoing pyroptosis) and IL-23 (by dendritic cells and other mononuclear cells) (Figure 3) [42]. Both of these cytokines rapidly stimulate immune cells (specified below) to secrete pro-inflammatory cytokines during *Salmonella* infection, thereby amplifying the immune response to the pathogen [42–45]. Whereas IL-18 was recently shown to stimulate Th1 cells to release interferon-gamma (IFN-γ) [43], IL-23 induces the production of the pro-inflammatory cytokines IL-17 and IL-22 from a variety of cells of innate and adaptive origin, including: innate lymphoid cells [46], neutrophils [47], gamma delta T cells [48], innate Th17 cells (iTh17; αβ T cells residing in the lamina propria) [49], and Th17 cells [50]. Further contributing to IL-17 and IL-22 production during *Salmonella* infection is the downregulation of PPARγ in epithelial cells by an unknown mechanism independent of TLR4 signaling, which results in maximal induction of IL-6, an activator of Th17 responses [36, 51].

An important consequence of the activation of a Th17 response by *Salmonella* is the induction of CXC chemokine expression, including CXCL1, CXCL2 and CXCL5, which are responsible for the recruitment of neutrophils to the site of infection [52]. Neutrophils follow the chemokine gradient to the gut and extravasate into the gut mucosa, where they encounter and eventually kill *Salmonella* by mechanisms that are not yet fully elucidated. What is known is that disruption of the mucosal barrier – for instance as in mice deficient for the IL-17 receptor – results in reduced neutrophil recruitment to the gut and in the increased dissemination of *Salmonella* to the reticuloendothelial system [44]. The Th17-mediated inflammatory response is thus critical to keeping the infection localized to the gut and protects the host from the potentially dangerous systemic dissemination of *Salmonella*.

Nevertheless, the beneficial effects of Th17 cytokine secretion come at a price: *Salmonella* has evolved numerous means by which to evade and exploit these host responses. Below, we discuss some recent findings on the mechanisms by which *Salmonella* benefits from mucosal immunity to thrive in the inflamed gut.

Salmonella exploits intestinal antimicrobial responses

A major function of IL-17 and IL-22 signaling is to induce the expression of a variety of antimicrobial proteins in the gut, including the metal chelating proteins lipocalin-2 and calprotectin [9, 10, 44] (Figure 3). By releasing these two proteins, the host limits the availability metal ions in the gut in an attempt to starve pathogenic microbes of these essential micronutrients.

Lipocalin-2 is produced by epithelial cells and neutrophils [44, 53], and its secretion serves to reduce bacterial access to iron by sequestering small molecules, known as siderophores, which are released by bacteria to scavenge iron from the surrounding environment [54]. Similarly, calprotectin, a heterodimer of the subunits S100A8 and S100A9, is also produced by epithelial cells and neutrophils [9, 55, 56], and it exerts its antimicrobial activity by sequestering zinc and manganese [55]. In the context of *Salmonella* gastroenteritis, both lipocalin-2 and calprotectin are produced in abundance by neutrophils and colonic epithelial cells, primarily in response to IL-22 signaling [9, 10, 12]. Although secretion of these host antimicrobial proteins should reduce bacterial growth by limiting access to metal ions essential for numerous metabolic functions, quite the opposite effect is achieved with *Salmonella*.

First, *Salmonella* expresses a modified siderophore that is not bound by lipocalin-2, thus enabling the pathogen to acquire iron even when this antimicrobial protein is produced [10, 12]. Secondly, *Salmonella* possesses a high affinity zinc transporter that allows the pathogen to acquire zinc in the presence of calprotectin [9, 10]. These virulence mechanisms are crucial to *Salmonella*, as they provide the pathogen a means by which to evade the detrimental effects of antimicrobial responses produced during intestinal inflammation. Furthermore, they enhance the competitive advantage of *Salmonella* over resident intestinal bacteria (i.e., the microbiota), which are susceptible to the changes in the environmental milieu mediated by these proteins. Indeed, the importance of these host antimicrobial proteins for *Salmonella* to compete with the gut microbiota was recently illustrated in *Il-22* deficient mice [10]: Absence of IL-22 resulted in reduced levels of lipocalin-2 and calprotectin, leading to the loss of *Salmonella*'s competitive advantage over susceptible, closely-related commensal microbes such as *Escherichia coli*. In fact, commensal *E. coli* successfully outcompeted *Salmonella* in the gut lumen of IL-22 deficient mice, leading to a lower *Salmonella* burden in the colon content of these mice [10]. *Salmonella* thus exploits IL-22-mediated induction of antimicrobial proteins – a major component of mucosal innate immunity – to successfully compete with the microbiota and thrive in the inflamed gut.

Salmonella benefits from ROS and RNS stress

Another arm of the mucosal immune response to non-typhoidal *Salmonella* infection consists of generating reactive oxygen and nitrogen species (Figure 3). Inducible nitric oxide synthase (iNOS) is one enzyme involved in this process, and is highly expressed in response to *Salmonella* infection. iNOS is produced by a variety of cells, including intestinal epithelial cells, macrophages and neutrophils [57], and its function is to convert L-arginine to nitric oxide (NO), a reactive nitrogen species (RNS) that inhibits several enzymes central to bacterial metabolism. The expression of iNOS is primarily induced by IFN-γ, but can also

be induced by IL-17 and IL-22 [44, 58]. IL-17 and IL-22 recruit neutrophils to the site of infection, which then, among their many antimicrobial functions, serve to generate reactive oxygen species (ROS). Upon neutrophil activation, NADPH oxidase complexes assemble and produce superoxide radicals by transferring electrons from NADPH to O_2. These radicals can then undergo further reactions to generate various forms of ROS or to form peroxynitrite ($OONO^-$), an RNS and potent cytotoxic oxidant, by reacting with NO [59, 60].

The crucial role of host-derived ROS in defense against *Salmonella* infection can be observed in patients diagnosed with chronic granulomatous disease: These patients possess a non-functional NADPH oxidase and suffer from frequent infections with fungi and bacteria, including *Salmonella* [61]. Indeed, the importance of NADPH oxidase in controlling intestinal *Salmonella* growth was recently shown in the mouse model of infection, where an avirulent strain of *Salmonella* replicated to high numbers and elicited intestinal inflammation in NADPH oxidase deficient mice ($Cybb^{-/-}$) [62]. Similar to ROS, the activity of iNOS is important to restrict the growth of *Salmonella*, as iNOS-deficient mice exhibit increased *Salmonella* colonization in the Peyer's patches and in the underlying gut mucosa [63, 64]. Nevertheless, as with many of the immune responses considered thus far, although RNS and ROS production are of critical importance to the long term health and survival of the host, not all downstream effects of oxidative stress are detrimental to *Salmonella*.

The release of RNS and ROS during intestinal non-typhoidal *Salmonella* infection creates a highly oxidative environment which is not permissive for the growth of Clostridiales and Bacteroidetes, strictly anaerobic bacterial phyla that constitute more than 90% of the healthy gut microbiota [6, 7, 10]. Consequently, the number of obligate anaerobes in the gut dramatically declines during *Salmonella* infection. In contrast, *Salmonella* is, to a certain extent, resistant to RNS and ROS, and can survive and replicate to high numbers in these oxidative conditions [6, 7, 10, 11, 65]. For example, one of the mechanisms by which *Salmonella* is resistant to ROS involves a recently discovered efflux pump, which contributes to *Salmonella* replication in the intestine and in macrophages [66]. Similar to ROS, *Salmonella* is also susceptible to high concentrations of NO, but possesses mechanisms to defend against moderate levels of nitrosative stress [67–69].

In addition to reducing competition by decreasing numbers among the gut microbiota, the highly oxidative environment generates anaerobic electron acceptors for the pathogen, allowing non-typhoidal *Salmonella* to utilize additional carbon sources and replicate to high numbers (Figure 3). Ethanolamine is a non-fermentable compound present in the intestine of mammals, and is generated from phosphatidylethanolamine, the most abundant phospholipid in membranes of enterocytes [70]. Ample quantities of this compound exist in the gut because enterocytes turn over so rapidly in the intestine, living for only a few days before reaching the top of the villus and being released from the epithelium [70]. Nonetheless, ethanolamine cannot be fermented and thus is not utilized by most of the microbiota. In contrast, *Salmonella* is able to anaerobically respire novel electron acceptors generated during inflammation, thus enabling catabolism of ethanolamine and enhancing the competitive advantage of *Salmonella* over the microbiota.

Tetrathionate and nitrate are anaerobic electron receptors which are generated during intestinal inflammation and are exploited by *Salmonella* [11, 71]. Tetrathionate is generated from hydrogen sulfide, a toxic compound produced in large quantities by the gut microbiota, which is rapidly detoxified through oxidation to thiosulfate by intestinal epithelial cells, then converted to tetrathionate by ROS present during gastroenteritis [11] (Figure 3). The innate immune response thus provides *Salmonella* with an electron acceptor to enable anaerobic respiration of ethanolamine and gain an edge over competing microbes [70]. Nitrate is also generated during inflammation, and is an isomerization product of the previously mentioned RNS peroxynitrite. Similar to tetrathionate, host-derived nitrate promotes the growth of non-typhoidal *Salmonella* in the inflamed gut [71], and *Salmonella* has since been described to utilize energy taxis to migrate towards niches where these favorable electron acceptors are present [72]. Taken together, these studies demonstrate that *Salmonella* exploits the highly oxidative environment generated by the host during gastroenteritis to proliferate and outcompete the microbiota.

Concluding remarks

A generally accepted concept is that the immune response is beneficial to the host because it limits the replication and the dissemination of pathogenic organisms. Nevertheless, to replicate in the host and to cause disease, pathogens must have evolved mechanisms to, at least temporarily, evade the immune system. Moreover, an increasing number of studies have demonstrated that pathogens not only evade the immune system, but actively exploit the host response to compete with the microbiota and to carve out a niche in the host. In this sense, the typhoidal and non-typhoidal serovars of *Salmonella* are prime examples of how host immunity is a double-edged sword: On one edge, aspects of the host innate immune response are of critical importance as they limit *Salmonella* replication and systemic dissemination; on the other edge, *Salmonella* can evade, manipulate and exploit aspects of host immunity to replicate, establish a persistent infection, and outcompete the microbiota.

In light of these realities, questions arise as to how we can impact the progression and outcome of *Salmonella* infection. For instance, can aspects of the inflammatory response beneficial to *Salmonella* be dampened or tweaked appropriately to blunt the pathogen's growth without compromising the mucosal barrier? In contrast, multiple aspects of the immune response remain effective against this pathogen. Even so, these host-beneficial responses often come at a price; for instance, Th17-mediated recruitment of neutrophils helps to control dissemination of *Salmonella*, but also results in considerable tissue damage. A challenge for future research is to dissect the overlap among these often simultaneously beneficial and detrimental immune responses, determining how to boost beneficial mechanisms of defense while lessening the impact of their detrimental tradeoffs.

Acknowledgments

References

1. Majowicz SE, et al. The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis. 2010; 50:882–889. [PubMed: 20158401]

2. Crump JA, et al. The global burden of typhoid fever. Bulletin of the World Health Organization. 2004; 82:346–353. [PubMed: 15298225]

3. Nuccio SP, Bäumler AJ. Comparative analysis of Salmonella genomes identifies a metabolic network for escalating growth in the inflamed gut. MBio. 2014; 5:e00929–e00914. [PubMed: 24643865]

4. Gal-Mor O, et al. Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ. Front Microbiol. 2014; 5:391. [PubMed: 25136336]

5. Haraga A, et al. Salmonellae interplay with host cells. Nat Rev Microbiol. 2008; 6:53–66. [PubMed: 18026123]

6. Lupp C, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of *Enterobacteriaceae*. Cell Host Microbe. 2007; 2:204. [PubMed: 18030708]

7. Stecher B, et al. *Salmonella enterica* serovar typhimurium exploits inflammation to compete with the intestinal microbiota. PLoS Biol. 2007; 5:2177–2189. [PubMed: 17760501]

8. Lawley TD, et al. Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota. Infection and immunity. 2008; 76:403–416. [PubMed: 17967858]

9. Liu JZ, et al. Zinc sequestration by the neutrophil protein calprotectin enhances *Salmonella* growth in the inflamed gut. Cell Host Microbe. 2012; 11:227–239. [PubMed: 22423963]

10. Behnsen J, et al. The cytokine IL-22 promotes pathogen colonization by suppressing related commensal bacteria. Immunity. 2014; 40:262–273. [PubMed: 24508234]

11. Winter SE, et al. Gut inflammation provides a respiratory electron acceptor for *Salmonella*. Nature. 2010; 467:426–429. [PubMed: 20864996]

12. Raffatellu M, et al. Lipocalin-2 resistance confers an advantage to *Salmonella enterica serotype* Typhimurium for growth and survival in the inflamed intestine. Cell Host Microbe. 2009; 5:476–486. [PubMed: 19454351]

13. Nuccio, SP., et al. Typhoid. In: Rosenberg, E., et al., editors. The Prokaryotes. Berlin Heidelberg: Springer; 2013. p. 375-399.

14. Benoit M, et al. Macrophage polarization in bacterial infections. J Immunol. 2008; 181:3733–3739. [PubMed: 18768823]

15. Weiss DS, et al. Toll-like receptors are temporally involved in host defense. J Immunol. 2004; 172:4463–4469. [PubMed: 15034062]

16. Wong CE, et al. *Salmonella enterica* serovar typhimurium exploits Toll-like receptor signaling during the host-pathogen interaction. Infection and immunity. 2009; 77:4750–4760. [PubMed: 19720755]

17. Arpaia N, et al. TLR signaling is required for *Salmonella* typhimurium virulence. Cell. 2011; 144:675–688. [PubMed: 21376231]

18. Tabeta K, et al. The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9. Nat Immunol. 2006; 7:156–164. [PubMed: 16415873]

19. Sivick KE, et al. Toll-like receptor-deficient mice reveal how innate immune signaling influences *Salmonella* virulence strategies. Cell Host Microbe. 2014; 15:203–213. [PubMed: 24528866]

20. Franchi L, et al. Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in *Salmonella*-infected macrophages. Nat Immunol. 2006; 7:576–582. [PubMed: 16648852]

21. Miao EA, et al. Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. Nat Immunol. 2006; 7:569–575. [PubMed: 16648853]

22. Broz P, et al. Redundant roles for inflammasome receptors NLRP3 and NLRC4 in host defense against *Salmonella*. J Exp Med. 2010; 207:1745–1755. [PubMed: 20603313]

23. Bergsbaken T, et al. Pyroptosis: host cell death and inflammation. Nat Rev Microbiol. 2009; 7:99–109. [PubMed: 19148178]

24. Munoz-Planillo R, et al. K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. Immunity. 2013; 38:1142–1153. [PubMed: 23809161]

25. Cummings LA, et al. FliC-specific CD4+ T cell responses are restricted by bacterial regulation of antigen expression. J Immunol. 2005; 174:7929–7938. [PubMed: 15944299]

26. Wynosky-Dolfi MA, et al. Oxidative metabolism enables *Salmonella* evasion of the NLRP3 inflammasome. J Exp Med. 2014; 211:653–668. [PubMed: 24638169]

27. Zaki MH, et al. *Salmonella* exploits NLRP12-dependent innate immune signaling to suppress host defenses during infection. Proc Natl Acad Sci U S A. 2013; 111:385–390. [PubMed: 24347638]

28. Anand PK, et al. NLRP6 negatively regulates innate immunity and host defence against bacterial pathogens. Nature. 2012; 488:389–393. [PubMed: 22763455]

29. Miao EA, et al. Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. Nat Immunol. 2010; 11:1136–1142. [PubMed: 21057511]

30. Broz P, et al. Caspase-11 increases susceptibility to *Salmonella* infection in the absence of caspase-1. Nature. 2012; 490:288–291. [PubMed: 22895188]

31. Kayagaki N, et al. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. Science. 2013; 341:1246–1249. [PubMed: 23887873]

32. Hagar JA, et al. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. Science. 2013; 341:1250–1253. [PubMed: 24031018]

33. Knodler LA, et al. Dissemination of invasive *Salmonella* via bacterial-induced extrusion of mucosal epithelia. Proc Natl Acad Sci U S A. 2010; 107:17733–17738. [PubMed: 20876119]

34. Humphries F, et al. RIP kinases: key decision makers in cell death and innate immunity. Cell death and differentiation. 2014

35. Robinson N, et al. Type I interferon induces necroptosis in macrophages during infection with *Salmonella enterica* serovar Typhimurium. Nat Immunol. 2012; 13:954–962. [PubMed: 22922364]

36. Eisele NA, et al. *Salmonella* require the fatty acid regulator PPARdelta for the establishment of a metabolic environment essential for long-term persistence. Cell Host Microbe. 2013; 14:171–182. [PubMed: 23954156]

37. McCoy MW, et al. Hemophagocytic macrophages in murine typhoid fever have an anti-inflammatory phenotype. Infection and immunity. 2012; 80:3642–3649. [PubMed: 22868497]

38. Nix RN, et al. Hemophagocytic macrophages harbor *Salmonella enterica* during persistent infection. PLoS Pathog. 2007; 3:e193. [PubMed: 18085823]

39. Grygiel-Gorniak B. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications--a review. Nutrition journal. 2014; 13:17. [PubMed: 24524207]

40. Berger J, Moller DE. The mechanisms of action of PPARs. Annu Rev Med. 2002; 53:409–435. [PubMed: 11818483]

41. Kullas AL, et al. L-asparaginase II produced by *Salmonella* typhimurium inhibits T cell responses and mediates virulence. Cell Host Microbe. 2012; 12:791–798. [PubMed: 23245323]

42. Godinez I, et al. Interleukin-23 orchestrates mucosal responses to *Salmonella enterica* serotype Typhimurium in the intestine. Infection and immunity. 2009; 77:387–398. [PubMed: 18955477]

43. O'Donnell H, et al. Toll-like receptor and inflammasome signals converge to amplify the innate bactericidal capacity of T helper 1 cells. Immunity. 2014; 40:213–224. [PubMed: 24508233]

44. Raffatellu M, et al. Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes Salmonella dissemination from the gut. Nat Med. 2008; 14:421–428. [PubMed: 18376406]

45. Godinez I, et al. T cells help to amplify inflammatory responses induced by *Salmonella enterica* serotype Typhimurium in the intestinal mucosa. Infection and immunity. 2008; 76:2008–2017. [PubMed: 18347048]

46. Takatori H, et al. Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. J Exp Med. 2009; 206:35–41. [PubMed: 19114665]

47. Zindl CL, et al. IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. Proc Natl Acad Sci U S A. 2013; 110:12768–12773. [PubMed: 23781104]

48. Sutton CE, et al. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. Immunity. 2009; 31:331–341. [PubMed: 19682929]

49. Geddes K, et al. Identification of an innate T helper type 17 response to intestinal bacterial pathogens. Nat Med. 2011; 17:837–844. [PubMed: 21666695]

50. Ouyang W, et al. The biological functions of T helper 17 cell effector cytokines in inflammation. Immunity. 2008; 28:454–467. [PubMed: 18400188]

51. Kundu P, et al. Absence of intestinal PPARgamma aggravates acute infectious colitis in mice through a lipocalin-2-dependent pathway. PLoS Pathog. 2014; 10:e1003887. [PubMed: 24465207]

52. Liu JZ, et al. Th17 cytokines and host-pathogen interactions at the mucosa: dichotomies of help and harm. Cytokine. 2009; 48:156–160. [PubMed: 19665391]

53. Goetz DH, et al. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell. 2002; 10:1033–1043. [PubMed: 12453412]

54. Flo TH, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004; 432:917–921. [PubMed: 15531878]

55. Corbin BD, et al. Metal chelation and inhibition of bacterial growth in tissue abscesses. Science. 2008; 319:962–965. [PubMed: 18276893]

56. Urban CF, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. PLoS Pathog. 2009; 5:e1000639. [PubMed: 19876394]

57. Kobayashi Y. The regulatory role of nitric oxide in proinflammatory cytokine expression during the induction and resolution of inflammation. Journal of leukocyte biology. 2010; 88:1157–1162. [PubMed: 20807706]

58. Zhang Y, et al. IL-17A synergizes with IFN-gamma to upregulate iNOS and NO production and inhibit chlamydial growth. PLoS One. 2012; 7:e39214. [PubMed: 22745717]

59. Fang FC. Antimicrobial actions of reactive oxygen species. MBio. 2011; 2

60. Henard CA, Vazquez-Torres A. Nitric oxide and salmonella pathogenesis. Front Microbiol. 2011; 2:84. [PubMed: 21833325]

61. Holland SM. Chronic granulomatous disease. Clinical reviews in allergy & immunology. 2010; 38:3–10. [PubMed: 19504359]

62. Felmy B, et al. NADPH oxidase deficient mice develop colitis and bacteremia upon infection with normally avirulent, TTSS-1- and TTSS-2-deficient Salmonella Typhimurium. PLoS One. 2013; 8:e77204. [PubMed: 24143212]

63. Alam MS, et al. Nitric oxide produced in Peyer's patches exhibits antiapoptotic activity contributing to an antimicrobial effect in murine salmonellosis. Microbiol Immunol. 2008; 52:197–208. [PubMed: 18426394]

64. Ackermann M, et al. Self-destructive cooperation mediated by phenotypic noise. Nature. 2008; 454:987–990. [PubMed: 18719588]

65. Burton NA, et al. Disparate impact of oxidative host defenses determines the fate of *Salmonella* during systemic infection in mice. Cell Host Microbe. 2014; 15:72–83. [PubMed: 24439899]

66. Bogomolnaya LM, et al. The ABC-type efflux pump MacAB protects *Salmonella enterica* serovar typhimurium from oxidative stress. MBio. 2013; 4 e00630-00613.

67. De Groote MA, et al. Periplasmic superoxide dismutase protects Salmonella from products of phagocyte NADPH-oxidase and nitric oxide synthase. Proc Natl Acad Sci U S A. 1997; 94:13997–14001. [PubMed: 9391141]

68. Henard CA, Vazquez-Torres A. DksA-dependent resistance of *Salmonella enterica* serovar Typhimurium against the antimicrobial activity of inducible nitric oxide synthase. Infection and immunity. 2012; 80:1373–1380. [PubMed: 22311927]

69. Husain M, et al. Ferric uptake regulator-dependent antinitrosative defenses in *Salmonella enterica* serovar Typhimurium pathogenesis. Infection and immunity. 2014; 82:333–340. [PubMed: 24166960]

70. Thiennimitr P, et al. Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota. Proc Natl Acad Sci U S A. 2011; 108:17480–17485. [PubMed: 21969563]

71. Lopez CA, et al. Phage-mediated acquisition of a type III secreted effector protein boosts growth of *Salmonella* by nitrate respiration. MBio. 2012; 3

72. Rivera-Chavez F, et al. *Salmonella* uses energy taxis to benefit from intestinal inflammation. PLoS Pathog. 2013; 9:e1003267. [PubMed: 23637594]

73. Spees AM, et al. Colonization resistance: battle of the bugs or Menage a Trois with the host? PLoS Pathog. 2013; 9:e1003730. [PubMed: 24278012]

74. Lawhon SD, et al. Intestinal short-chain fatty acids alter *Salmonella* typhimurium invasion gene expression and virulence through BarA/SirA. Mol Microbiol. 2002; 46:1451–1464. [PubMed: 12453229]

75. Deriu E, et al. Probiotic bacteria reduce *Salmonella typhimurium* intestinal colonization by competing for iron. Cell Host Microbe. 2013; 14:26–37. [PubMed: 23870311]

76. Lamichhane-Khadka R, et al. The Hyb hydrogenase permits hydrogen-dependent respiratory growth of *Salmonella enterica* serovar Typhimurium. MBio. 2010; 1

77. Maier L, et al. Microbiota-derived hydrogen fuels *Salmonella* typhimurium invasion of the gut ecosystem. Cell Host Microbe. 2013; 14:641–651. [PubMed: 24331462]

78. Ng KM, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature. 2013; 502:96–99. [PubMed: 23995682]

79. Stentz R, et al. Cephalosporinases associated with outer membrane vesicles released by Bacteroides spp. protect gut pathogens and commensals against beta-lactam antibiotics. J Antimicrob Chemother. 2014

80. Ganesh BP, et al. Commensal Akkermansia muciniphila exacerbates gut inflammation in Salmonella Typhimurium-infected gnotobiotic mice. PLoS One. 2013; 8:e74963. [PubMed: 24040367]

Box 1 *Salmonella* exploits the microbiota

A stable, healthy gut microbiota provides "colonization resistance" to pathogens like *Salmonella* by competing with them for nutrients and space (reviewed in [73]). Moreover, intestinal anaerobic bacteria ferment dietary fiber to short chain fatty acids, which in turn downregulate the expression of *Salmonella* invasion genes [74]. Additionally, closely related bacteria such as the probiotic *E. coli* Nissle 1917 can outcompete *Salmonella* by scavenging for iron more effectively in the already iron-scarce environment of the inflamed gut, thus limiting the pathogen's access to this vital micro-nutrient and reducing *Salmonella* colonization [75].

Although the microbiota plays a vital part in limiting pathogen colonization, some commensal microbes and their metabolic byproducts are exploited by *Salmonella* to colonize the gut. As discussed in the main text, the microbiota produces hydrogen sulfide, which is eventually converted into tetrathionate, an anaerobic electron acceptor for *Salmonella* in the inflamed gut [11]. Additionally, fermentation of complex sugars by the microbiota results in the formation of molecular hydrogen (H_2); *Salmonella* expresses three hydrogenases, which in turn enable the pathogen to utilize H_2 [76]. As such, hydrogenases are important virulence factors for *Salmonella*, especially during the early stages of infection when the microbiota is not yet perturbed and when energy sources are relatively limited [76, 77].

Some commensals also directly help *Salmonella* to access carbon sources. One example is *Bacteroides thetaiotaomicron*, a commensal bacterium that encodes a sialidase which cleaves and releases sialic acid from mucin. Although *B. thetaiotaomicron* itself lacks the pathways to catabolize sialic acid, it presumably cleaves it to gain access to underlying carbohydrates. *Salmonella*, however, can catabolize this liberated monosaccharide, and access to it supports the pathogen's proliferation in the gut [78]. *B. thetaiotaomicron* also produces a beta-lactamase that protects the bacteria from beta-lactam antibiotics, including *Salmonella* [79]. Another microbe that helps *Salmonella* to degrade mucin is *Akkermansia muciniphila*, which exacerbates *Salmonella*-induced intestinal inflammation by disturbing host mucus homeostasis [80].

Taken together, these studies suggest that the microbiota, much like the inflammatory immune response, is a double-edged sword during *Salmonella* infection: While some commensals limit *Salmonella*'s ability to colonize the intestine, others promote it.

Highlights

1. Inflammation is a double-edged sword: it benefits but eventually kills *Salmonella*

2. *Salmonella* exploits TLR/NLR signaling and cell death to establish infection

3. *Salmonella* persists in M2 macrophages during chronic infection

4. *Salmonella* benefits from host-derived ROS, RNS and antimicrobial proteins
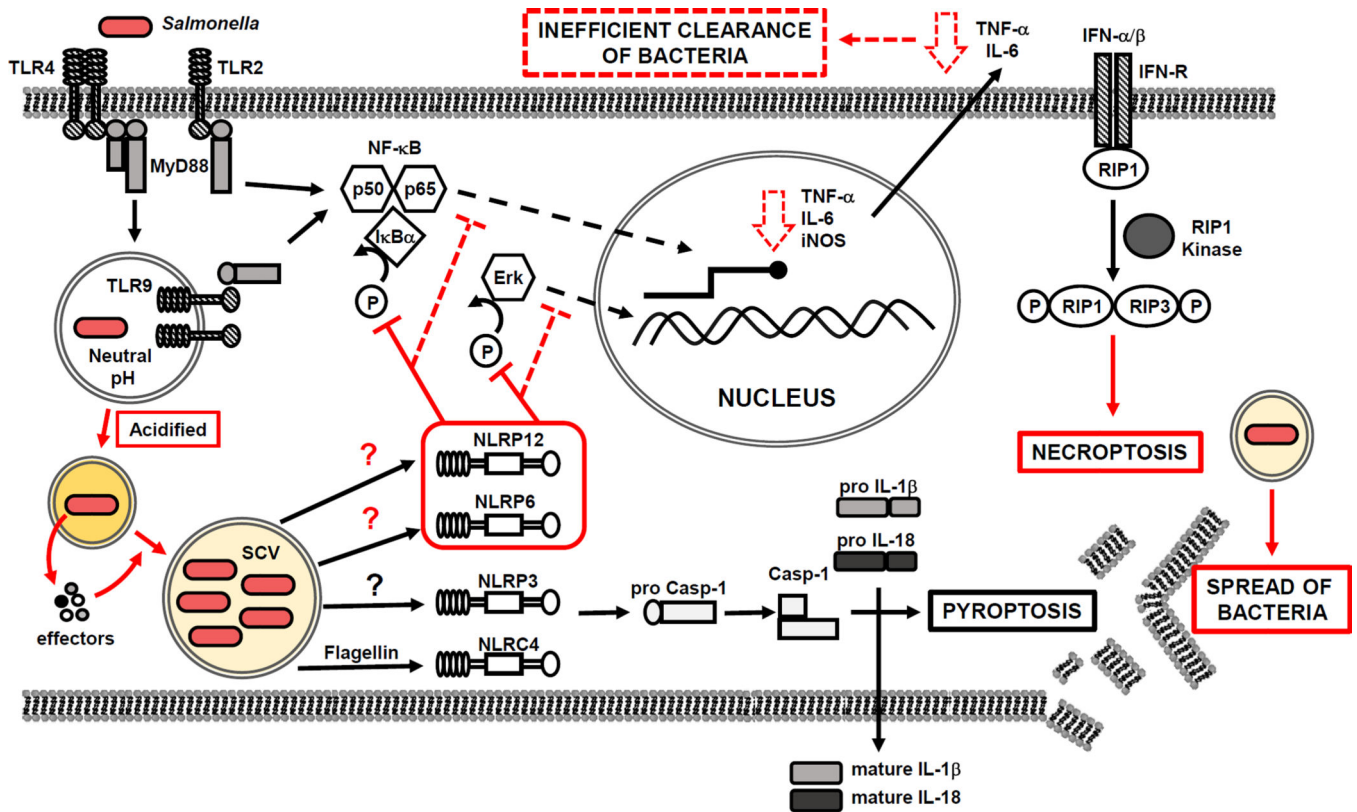
Figure 1. *Salmonella* **takes advantage of innate immune responses inside the macrophage**
PAMPs from *Salmonella* are recognized by TLRs, signaling from which induces an array of
responses, including acidification of the phagosome. This drop in pH triggers *Salmonella* to
secrete effectors, which in turn modify the phagosome to generate a replicative compartment
known as the *Salmonella*-Containing Vacuole (SCV). *Salmonella* can also activate NLRs,
including NLRC4 (activated by flagellin) and NLRP3 (unknown ligand). Activation of
NLRC4 and NLRP3 lead to processing of pro- Caspase-1 into its active form, followed by
cleavage of pro-IL-1β and pro-IL-18 into their active forms and by the induction of
pyroptosis. In addition to NLRC4 and NLRP3, NLRP6 and NLRP12 are also activated by
Salmonella, albeit trough unknown ligands. These latter NLRs inhibit phosphorylation of
IκBα and ERK, thus preventing nuclear translocation of NF-κB and ERK, respectively. This
subsequently diminishes the production of proinflammatory mediators and results in the
inefficient clearance of phagocytized bacteria. During *Salmonella* infection, type I IFN
signaling is activated through the production of type I IFNs, leading to the association
between IFNAR and RIP1. This in turn induces the formation of the RIP1–RIP3 complex,
resulting in necroptosis of macrophages. The reduction in proinflammatory mediators and
induction of macrophage death by necroptosis diminishes the immune system's ability to
control the replication and spread of *Salmonella* within the host. Activities promoting the
growth or survival of *Salmonella* are depicted with red lines and boxes.
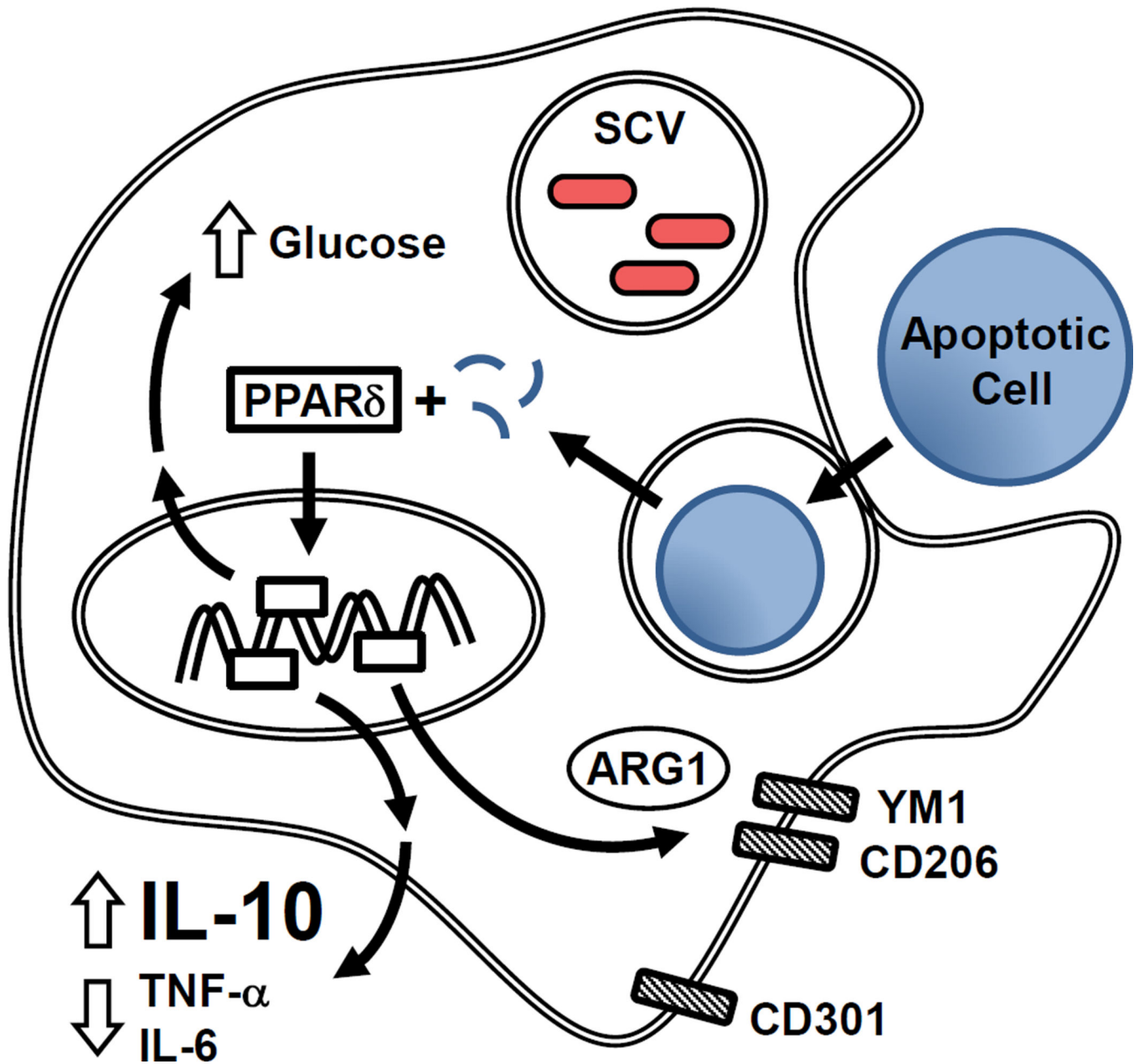
Figure 2. *Salmonella* uses M2 macrophages to establish a chronic infection
PPARδ is upregulated in CD301^{+} macrophages during *Salmonella* infection. Following
activation by fatty acids present in engulfed apoptotic cells, PPAR receptors translocate to
the nucleus where they induce the M2 macrophage phenotype (ARG1^{+}, YM1^{+}, CD206^{+}).
M2 macrophages produce high levels of anti-inflammatory cytokines such as IL-10, and low
levels of proinflammatory cytokines such as TNFα and IL-6. In addition, activation of
PPARδ increases intracellular glucose availability and enhances *Salmonella* replication in
macrophages and in mice, whereas this pathogen fails to persist in *Pparδ* null mice. The
anti-inflammatory environment and metabolic state present in M2 macrophages allow

Salmonella to establish a chronic infection. Abbreviations: SCV, *Salmonella* containing vacuole.
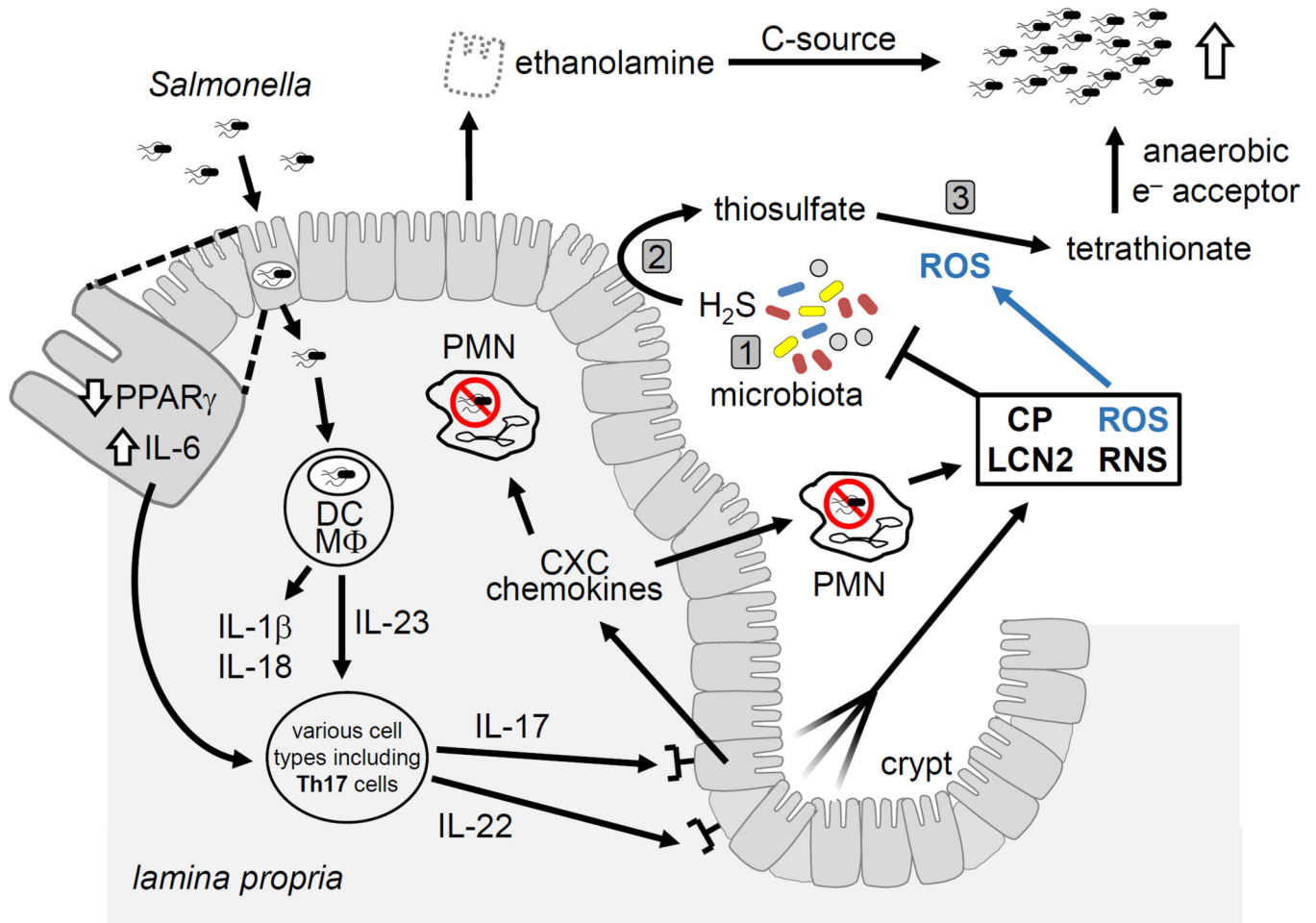
Figure 3. *Salmonella* exploits intestinal antimicrobial responses

During colonization of the gastrointestinal tract, *Salmonella* invades and escapes from epithelial cells, gaining access to the lamina propria. There, *Salmonella* invades/is taken up by macrophages (MΦ) and dendritic cells (DC), and resides inside the *Salmonella* containing vacuole (SCV). Once infected, these phagocytes produce IL-1β, IL-18 (see Fig. 1) and IL-23. IL-23 signals to various cells types (e.g. Th17 cells) to produce IL-17 and IL-22. Production of these cytokines is further increased by IL-6, which is released in greater abundance by epithelial cells following *Salmonella*-mediated PPARγ downregulation. Epithelial cells also express receptors for IL-17 and IL-22, and ligand binding induces production of antimicrobial proteins such as Lipocalin-2 (LCN2; iron starvation) and calprotectin (CP; zinc and manganese starvation). Contrary to the majority of the resident microbiota, *Salmonella* has evolved to evade the detrimental effects of these antimicrobial proteins, producing an additional iron-scavenging siderophore that cannot be bound by LCN2, and expressing a high affinity zinc transporter to overcome zinc sequestration by CP. Epithelial cells also produce CXC chemokines following IL-17 and IL-22 stimulation, leading to the recruitment of polymorphonuclear cells (PMN; primarily neutrophils) to the site of infection, which also produce the aforementioned antimicrobial proteins. PMNs phagocytose *Salmonella* and kill it with the help of reactive oxygen (ROS) and nitrogen species (RNS), among other mechanisms. IL-17 and IL-22 together with IFN-γ

also induce iNOS in epithelial cells, an enzyme involved in the production of NO (an RNS). Whereas *Salmonella* is resistant to moderate levels of ROS and RNS, these responses transform the gut into an oxidative, inhospitable environment for many members of the anaerobic microbiota. Moreover, this oxidative environment provides *Salmonella* with additional electron acceptors, for example: (1) Hydrogen sulfide (H_2S), produced by the microbiota, is converted into (2) thiosulfate by epithelial cells; ROS oxidize thiosulfate to (3) tetrathionate. *Salmonella*'s ability to anaerobically respire tetrathionate allows it to utilize non-fermentable carbon sources (C-source) such as ethanolamine, which is generated from the membranes of dead enterocytes. Together, these resistance mechanisms and metabolic properties give *Salmonella* a competitive advantage over the gut microbiota, allowing it to exploit host inflammation and grow to high numbers in the gastrointestinal lumen.