Review Article

Journal of Innate Immunity

J Innate Immun 2019;11:289–299
DOI: 10.1159/000490953

Received: April 3, 2018
Accepted after revision: June 10, 2018
Published online: July 24, 2018

Hoodwinking the Big-Eater to Prosper: The *Salmonella*-Macrophage Paradigm

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Keywords
Macrophage · TLR · NLR · ROS · Pyroptosis · Metal starvation · AMP · Exosome

Abstract
Salmonella is a major cause of morbidity and mortality in the developing and underdeveloped nations. Being a food-borne disease, *Salmonella* infection is primarily contracted through the ingestion of contaminated food or water, or due to close contact with infected/carrier individuals. It is an intracellular pathogen, which can survive and replicate in various cells including macrophages, dendritic cells, epithelial cells, and other white blood cells. Once *Salmonella* crosses the intestinal barrier, it disseminates to various systemic sites by circulation via immune cells. One of the major cell types which are involved in *Salmonella* infection are host macrophages. They are the niche for intracellular survival and proliferation of *Salmonella* and a mode of dissemination to distal systemic sites. These cells are very crucial as they mediate the mounting of an appropriate innate and adaptive anti-*Salmonella* immune response. In this review, we have tried to concise the current knowledge of complex interactions that occur between *Salmonella* and macrophages.

Introduction

Salmonella is a Gram-negative pathogen which causes a variety of diseases ranging from life-threatening systemic infection like typhoid to mild gastroenteritis in humans. Global estimates indicate 21 million typhoid cases and 5 million cases of paratyphoid fever along with 215,000 fatalities each year [1]. There is an estimated 93 million non-typhoidal salmonellosis with 155,000 fatalities every year [2]. These estimates make *Salmonella* a major cause of morbidity and mortality in developing and underdeveloped nations. Annually, 175–388 cases per 100,000 children and 2,000–7,500 cases per 100,000 HIV-infected adults are approximated in sub-Saharan Africa [3]. In 95% of the cases, nontyphoidal serovars cause gastroenteritis; however, 5% of the cases result in bacteremia and systemic infection called invasive nontyphoidal salmonellosis (iNTS). The incidence of iNTS is prevalent in African countries, particularly among children. 20–25% of iNTS tends to be fatal [4]. Apart from the human host, *Salmonella enterica* subspecies have been reported to infect other warm-blooded animals of economic importance such as poultry, cattle, etc. The vast diversity showed by these serovars with respect to host range adaptation and virulence strategy makes *Salmonella* a daunting pathogen.

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Salmonella infection is primarily contracted through ingestion of contaminated food or water or due to close contact with infected/carrier individuals. Once ingested, *Salmonella* crosses the host intestinal barrier by entering through "M cells" (microfold cells) and dendritic cells present in Peyer's patches. Additionally, it can induce bacterial uptake by intestinal epithelial cells via injection of virulence factors into the host cell. These virulence factors are encoded by various horizontally acquired pathogenicity islands present in the *Salmonella* genome, called the *Salmonella* pathogenicity islands (SPIs), which are critical for the entry and intracellular survival [5]. After colonization, the bacteria disseminate through the reticuloendothelial system and reside in host macrophages, dendritic cells, polymorphonuclear cells, and hepatic cells. During its systemic phase, the pathogen spreads from the intestine to the mesenteric lymph node (MLN), spleen, liver, gallbladder, and even into bone marrow [6].

Macrophages play an essential role in anti-*Salmonella* response as it is involved in mediating both innate and adaptive immune responses. Its importance in *Salmonella* pathology is further emphasized by the fact that the mutants which are incapable of intracellular life in macrophages are in general defective in causing a systemic infection [7]. As a part of innate immune response, macrophages sense the presence of *Salmonella*-derived pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs) like Toll-like receptors (TLRs) and NOD-like receptors (NLRs). This subsequently leads to an antibacterial response which comprises reactive oxygen species (ROS), reactive nitrogen species (RNS), acidic environment, metal starvation, and antimicrobial peptides (AMPs). As a part of adaptive immune response, it is essential to mount a Th1 response against *Salmonella* to avoid a chronic infection. During *Salmonella* infection, macrophage polarization to M1 or M2 phenotype plays a determinant role in dictating the disease outcome. In this review, we have tried to bring together the intricacies of macrophage-*Salmonella* interaction.

Intracellular Life of *Salmonella* in Macrophage

In the host phagocytic cells, *Salmonella* resides in a modified endosome known as *Salmonella*-containing vacuole (SCV). The membrane properties of SCV are dynamic and the nascent bacteria containing endosome matures into a modified late endosome. *Salmonella* avoids SCV lysosome fusion, providing it a safe intracellular niche for bacterial survival and replication [8, 9]. Early SCV contains markers like EEA1 and Rab4 and 5 and transferrin receptors. During the late maturation stage, about 20–40 min after internalization, the late endosomal markers like LAMPs, Rab7, 11, and vATPase replace the early endosome markers [10]. For replication of bacteria within the cell, perinuclear localization of SCV is obligatory. During its intracellular life, SPI-2 effectors are instrumental for both replication and SCV positioning [11]. In case of host epithelial cells, SCVs form filamentous structures called *Salmonella*-induced filaments (Sifs) [9, 12]. MYD88- and TRIF-mediated TLR signalling is essential for initial SCV acidification [13]. Within an hour of infection, the SCV attains the pH of 4–5. In response to the acidic environment of SCV, the cytoplasm of the bacteria also undergoes acidification. This acidification process is EnvZ sensor kinase and OmpR mediated. EnvZ senses the osmotic stress and in response to it OmpR regulates expression of various pH regulatory genes, including CadC/BA operon and ATP synthase gene of the bacteria [14]. The low pH and low Mg^{2+} environment of SCV activates the PhoP/Q two-component system. This leads to the upregulation of the Mg^{2+} transporter (mgtCBR operon), lipopolysaccharide (LPS) modification, and SPI-2 expression [15]. During the process of internalization as well as inside the host macrophage, *Salmonella* activates signalling of the host PRRs. This process activates various antibacterial responses in macrophages.

TLR and NLR Signalling

TLRs and NLRs are the PRRs responsible for sensing most of the PAMPs. Early detection of *Salmonella* is dependent on both surface and endosomal TLRs. TLR2 and TLR4 are present on the macrophages and recognize the *Salmonella* PAMPs. Mice lacking these TLRs exhibit higher extracellular bacterial burden, thus implicating their role in host innate immune responses [16]. Conversely, the absence of TLR signalling in macrophages impairs its acidification, which in turn compromises intracellular replication [13]. Collectively, TLR signalling helps the intracellular replication of bacteria, but inhibits the extracellular growth of the same. As compared to TLR2/4 and TLR4/9 double knockout mice, mice lacking all the three TLRs (i.e., TLR2, 4, and 9) are less susceptible to *Salmonella*-mediated lethality. Further, complete ablation of TLR (TLR2, 4, 3, 7, 9) signalling results in reduced inflammatory response which in turn enhances susceptibility to *Salmonella* infection [17].

Apart from the TLRs, NLRs also detect intracellular PAMPs. *Salmonella* infection activates NLRC4 and NLRP3 signalling, resulting in inflammasome formation [18]. This further leads to activation of caspase-1 and secretion of IL-1β and IL-18 and pyroptosis. NLRC4 recognizes *Salmonella* flagellin and in order to avoid activation of NLRC4, *Salmonella* downregulates flagellin production during its intracellular life [19]. The *Salmonella* PAMP responsible for NLRP3 activation is yet unknown. However, NLRP3 signalling might be linked to ROS production and ROS production is often accompanied by K^+ efflux which is a known signal for NLRP3 activation [20]. A deficiency in either of NLRC4 and NLRP3 results in a higher systemic bacterial burden, emphasizing its role in antibacterial response against *Salmonella* [21]. In contrast, NLRP6 and NLRP12 deletion reduces the bacterial burden and enhances neutrophil and macrophage recruitment to the site of infection [22, 23]. Pathogen sensing by host PRRs induce oxidative burst as an antibacterial response.

ROS and RNS

Recognition of *Salmonella* PAMPs induce proinflammatory cytokine production by various immune cells. These cytokines further activate macrophages and dendritic cells to produce antibacterial ROS and RNS response. In response to *Salmonella* infection in macrophages, the initial bactericidal respiratory burst is driven by phagocyte oxidase (phox) also known as NADPH oxidase [24]. This is followed by a long bacteriostatic nitrosative stress driven by inducible nitric oxide synthase (iNOS) [25]. The regulatory effect of cytokines on ROS is proven by the fact that in vivo administration of IFNγ results in increase in ROS, which in turn inhibits systemic spread of *Salmonella* in rats. The importance of ROS is demonstrated by the fact that mice lacking gp91phox, an essential subunit of NADPH oxidase, succumb to *Salmonella* infection much faster than wild-type mice. This is also accompanied by a 1,000-fold increase in bacterial burden in the liver of these knockout mice [26]. In addition, patients suffering from chronic granulomatous disease, a condition compromising ROS production, suffer from recurring *Salmonella* and other bacterial infections [27]. Studies on murine peritoneal macrophages reveal that clearance of up to 99% of intracellular bacteria within the first 6 h of infection is predominantly ROS dependent [28]. However, ROS production reduces after 6 h of infection. *Salmonella* mutants, which are deficient is ROS and RNS detoxification enzymes such as superoxide dismutase (SodC) [29] and oxidative burst scavenger glutathione, are unable to lead a successful intracellular life in macrophages [30]. Thus, the bacteria are compromised in causing a systemic infection. The SPI-2 is essential for successful evasion of intracellular ROS assault. This is demonstrated by the fact that PhoPQ mutants, responsible for SPI-2 expression in the SCV, are unequipped to handle intra-macrophage ROS [31]. The integrity of the SCV is also crucial to evade ROS. Cytosolic population of *Salmonella* has been reported to face enhanced ROS levels in both human and murine macrophages [32].

Once the ROS production diminishes, a prolonged iNOS-mediated nitrosative stress follows. iNOS converts L-arginine to nitric oxide (NO) and citrulline, which show a bacteriostatic effect at a later stage of infection [33]. Being a free radicle, NO can react with various intracellular molecules to generate more destructive reactive nitrogen intermediates. The reaction of ROS and NO produces peroxynitrite, which damages DNA and hence results in mutagenesis, further accompanied by damage to proteins as well as lipids. Although, IFN-γ is the main inducer of iNOS [34] even IL-17, IL-22, IL-1, and TNFα can induce its upregulation [33, 35]. Genetically deficient iNOS mice can control the bacterial burden at the initial stage of infection, but are unable to do so at a later stage and show enhanced mortality [36]. This indicates the role of iNOS in controlling *Salmonella* infection at a later stage. *Salmonella* also evades RNS by limiting substrate for iNOS. *Salmonella* infection of murine macrophages results in upregulating of iNOS but simultaneously it enhances arginase II production, which converts L-arginine to ornithine and urea, thus limiting iNOS substrate [37]. PhoPQ plays a crucial role in evasion of nitrosative stress as its deletion results in hyper-susceptibility to NO. Recent reports suggest that PhoPQ-mediated Mg^{2+} transport is crucial for surviving RNS [38]. The hyper-susceptibility of this mutant to RNS is due to nitrotyrosine formation, DNA damage, and oxidation of iron sulfur cluster. Apart from its antibacterial effect, NO also has immune-modulatory effects as it can suppress T-cell proliferation [38].

Apart from ROS and RNS response, macrophages control bacterial replication by limiting the availability of metal ions.

Metal Starvation/Toxicity

Intracellular availability of trace elements is essential for the survival of an intracellular pathogen. One of the innate immune responses against pathogens is metal star-

vation. Macrophages and neutrophils restrict intracellular bacterial replication by limiting iron and zinc. Additionally, macrophages utilize iron as a co-factor for the generation of ROS and RNS, indicating that the right equilibrium of metal ions is essential for successful pathogen clearance.

Iron is most commonly available in bound form with proteins like transferrin, ferritin, lactoferrin, and lipocalin-2. Transferrin receptor present on the surface of the macrophage binds to plasma ferric ion-bound transferrin which results in receptor-mediated endocytosis. During the endosomal maturation, iron dissociates from transferrin protein due to the acidic pH of the vacuole and empty transferrin receptors are recycled to the cell surface for the next cycle [39].

The importance of iron availability is demonstrated by the fact that individuals suffering from iron overload such as hemochromatosis are more susceptible to *Salmonella* infection. Mice lacking HEF allele which is associated with hemochromatosis show better survival during *Salmonella* infection [40, 41]. Furthermore, iron supplementation increases intracellular survival of *Salmonella* [42]. During *Salmonella* infection, macrophages increase iron efflux by upregulating an iron export protein ferroprotein-1, thereby limiting the bioavailability of iron to the pathogen [43]. In addition, *Salmonella* further decreases the intracellular iron pool by enhancing the level of both haem oxygenase, a haem-degrading enzyme, and lipocalin-2, an iron siderophore [39]. All these changes in macrophage iron homeostasis are reported to be IFNγ mediated [44]. Apart from being an iron siderophore, lipocalin-2 also represses IL-10 production and enhances proinflammatory response by upregulation TNFα, IL-6, and NOS2 levels [45]. Macrophage iron-regulatory protein (IRB) 1 and 2 deficiency results in increased mortality in response to *Salmonella* infection, emphasizing the role of iron homeostasis in disease severity [46]. Live *Salmonella* also induce the transformation of macrophages to hemophagocytes, these are phagocytes which have engulfed erythrocyte or leukocyte. In these cells, intracellular bacterial survival is greatly enhanced within hemophagocytes, due to a surplus of iron [47, 48].

The importance of divalent transition metal ions like iron and manganese in host defense is further supported by the fact that the presence of natural resistance-associated protein 1 (Nramp1) [49] in mice results in survival of acute infection, whereas those which lack them are susceptible and succumb to the infection. In some individuals, conversion of one glycine residue to asparagine due to a single nucleotide polymorphism results in a nonfunctional Nramp1 protein [50]. This results in uncontrolled replication of *Salmonella* in the SCV. Nramp1 is expressed in phagocytic cells and is localized to late endosome and lysosome. It is LPS inducible and exports manganese ions from the phagosome in a pH-dependent manner [51]. Further, Mn^{2+} is reported to inversely regulate NO production and IFNγ response [52].

Zinc is crucial for both the structural aspect and gene expression of various proteins and its sequestration is one of the host defense mechanism against microbial assault. As a response to infection, macrophages upregulate zinc scavengers, metallothioneins 1 and 2 [53]. Their ablation results in a reduction in ROS and RNS levels and an increase in free zinc levels. Collectively, this leads to increased bacterial intracellular survival of *Salmonella*. The increase in intracellular free zinc level inhibits NFκB and hence proinflammatory responses [54].

Metal ion availability can act as a double-edged sword: on the one hand, it is essential for bacterial survival in trace amounts, and on the other hand, at a higher concentration they can be antibacterial in nature. The best example for this is zinc and copper toxicity. Both human and murine macrophages show increased uptake of copper in response to TLR4 activation. In the intracellular vacuoles, *Salmonella* is subjected to an increased copper concentration. In murine bone marrow-derived macrophages, treatment with copper chelator enhances intracellular survival of *Salmonella* [55]. The antibacterial role of copper is further validated by reduced organ load in CueO, a copper oxidase mutant as compared to wild-type *Salmonella* [56]. *Salmonella* also express CopA, a copper efflux pump to avoid copper toxicity.

In human monocyte-derived macrophages, although *Salmonella* induces the formation of zinc-containing vesicles, simultaneously it also upregulates the expression of a zinc efflux pump ZntA [57]. Further, prolonged stimulation of NOD-2 results in accumulation of intracellular zinc which in turn induces autophagy and hence clearance of *Salmonella* [58]. In addition to starving the intracellular bacteria with the unavailability of metal ions, macrophages also mount an AMP response.

AMP Response

AMPs are generally 10- to 50-amino-acid-long cationic peptides which show direct bactericidal effects. As the name suggests, it contains positively charged basic amino acids along with hydrophobic amino acid residues

Table 1. The miRNAs altered in macrophages upon *Salmonella* infection

miRNA	Modulation by *Salmonella* infection	Molecules/pathways altered	Authors [Ref.], year
let-7a	Downregulation	IL-6 and IL-10/ pro- and anti-inflammatory responses, respectively	Das et al. [64], 2016
miR-155	Downregulation	Inflammatory mediators, cytokines	Das et al. [64], 2016
miR-146a	Upregulation	IRAK4 and TRAF6/important molecules of TLR signalling	Heale et al. [72], 2010
miR-125b	Downregulation	Alteration in TNFα levels	Heale et al. [72], 2010

that can interact with the LPS and bacterial membrane resulting in pore formation. Cathelicidins and defensins are the two major classes of cationic AMPs. Most of the AMP studies regarding *Salmonella* have been carried out in epithelial cells as they are the major producers of AMPs in vertebrates. The status of macrophage-derived AMPs during *Salmonella* infection remains largely unexplored. However, the existing limited literature suggests a role of cathelicidin in macrophages [59]. In macrophages, cathelicidin resides in the lysosome. It is composed of a C-terminal antimicrobial domain which is masked by a conserved N-terminal domain. Removal of the N-terminal domain by a cellular serine protease is crucial for its antimicrobial activity. Humans express cathelicidin hCAP-18 gene, whereas mouse expresses cathelicidin-related antimicrobial peptide (CRAMP). *Salmonella* infection of macrophages results in the upregulation of CRAMP [60]. It has been reported to show an antibacterial effect both in vitro and in vivo. *Salmonella*-mediated upregulation of CRAMP is both ROS and serine protease activity dependent. *Salmonella* infection of antioxidant pretreated macrophages were unable to show upregulation of CRAMP, confirming that the role of ROS is cathelicidin function. PhoP mutants are more sensitive to CRAMP which might be due to the absence of PhoP-mediated LPS modification [61]. In case of human macrophages, hCAP-18 production is TLR dependent. Stimulation of TLR2, 4, and 9 results in the upregulation of hCAP expression in alveolar macrophages. Furthermore, TLR regulation of hCAP-18 is vitamin D dependent [60, 62]. In case of human monocyte-derived macrophages, *Salmonella* does not induce cathelicidin hCAP-18 [61]. The effects of other macrophage-derived AMPs in anti-*Salmonella* response requires further investigation. Another area which remains hugely unexplored is the miRNA regulation in macrophages upon *Salmonella* infection.

miRNAs in *Salmonella* Infection

miRNAs belong to the class of noncoding RNAs of about 18–22 nucleotides in length. They play major roles in regulating the expression of protein-coding genes by either controlling at the transcriptional level or by repression of translation [63]. Since the host-pathogen interaction is a complex network, many of the host gene expression levels are, or should be, altered by the pathogen to tend to its own needs. Thus miRNA regulation also has a role to play during *Salmonella* infection of macrophages (Table 1).

Salmonella is known to modulate the levels of certain miRNAs in vitro in both macrophages and epithelial cells. In murine macrophage-like RAW 264.7 cells, miRNAs like miR-21, miR-146, and miR-155 were found to be upregulated [64]. Interestingly, None of these miRNAs have known roles associated with invasion or intracellular replication of *Salmonella*. Another miRNA, let-7a, was found to be downregulated in both RAW 264.7 cells and HeLa cells. This downregulation was found to be a response to the TLR4 stimulation by the LPS. Target genes of this let-7a miRNA are IL-6 and IL-10 whose repression is relieved upon its downregulation. These cytokines mediate the pro- and anti-inflammatory responses, respectively [65, 66].

The mammalian adenosine deaminases, ADAR1 and ADAR2, have been found to deaminate the adenosines in dsRNAs leading to the A to I conversions. ADAR1 is an ubiquitously expressed protein [67] ADAR2 has high levels of expression in the brain [68]. ADAR1 knockout mice die at the embryogenesis stages [69], whereas ADAR2 knockout mice die by postnatal day 20 with epileptic seizures [70]. Defective editing by these enzymes has been implicated in several diseases like amyotrophic lateral sclerosis, cancers, and metabolic disorders like type 2 diabetes mellitus [71]. These A to I conversions in miRNAs lead to the reduction of the mature miRNA levels. This is either due to the failure of the processing enzymes to bind

to the pri-miRNAs after editing, or due to the inability of the miRNA to bind to their targets as a result of the loss of complementarity after editing. It has been found that the levels of ADAR1 increase dramatically upon *Salmonella* infection. LPS stimulation of macrophages is found to alter the levels of miR-146a, miR-155, and miR125b [72]. miR-146a has roles in regulating the levels of IRAK1 and TRAF6; two important members of TLR signalling. miR-146a also regulates the levels of TNFα which is one of the major cytokines produced via TLR signalling that mediates proinflammatory responses.

Exosomes

Many intracellular pathogens induce exosome secretion which has immunomodulatory properties [73]. Exosomes are small vesicles secreted by various cells of the dimension of 50–100 nm. The role of exosomes during *Salmonella* infection is largely unexplored. *Salmonella* stimulates exosome secretion in infected THP-1 macrophages [74]. These exosomes are $CD63^+$ and $CD9^+$ positive. They contain LPS and can activate a TLR4-dependent pathway in naive macrophages. They can induce TNFα production in naive macrophages in a Myd-88-dependent manner. In addition to LPS, *Salmonella*-induced exosomes have also been reported to contain OUT deubiquitinase1 which does not contain any secretory motif. The secretion of exosomes is mediated via multivesicular bodies [74, 75]. Furthermore, *Salmonella* Typhi infection of both epithelial cells and macrophages also results in the secretion of outer membrane vesicles containing cytolethal distending toxins (CDTs) [76]. This process requires the microenvironment of SCV or its mimic. Infected cells release CDT-containing exosomes via actin and microtubule-dependent anterograde transport. Internalization of these OMVs by bystander cells requires active endocytosis and retrograde transport, ultimately resulting in DNA damage. *Salmonella* infection also results in various types of host cell death [77].

Cell Death

One of the many strategies employed by various pathogens to manipulate host is via host cell death. *Salmonella* causes host cell death by both apoptosis, pyroptosis, necroptosis, and autophagy.

Pyroptosis is an inflammatory cell death. It is a caspase-1-dependent programmed cell death which results in the lysis of the infected cell. It is characterized by the secretion of proinflammatory cytokines IL-1β, IL-18, nuclease-mediated cleavage of DNA in the nucleus and membrane lysis, and LDH release [78]. *Salmonella* stimulates pyroptosis in infected macrophages in a SPI-1-dependent manner. Translocation of flagellin to the cytosol by the type three secretion system (T3SS) results in activation of cytosolic NLRC4-mediated activation of inflammasome which ultimately results in caspase-1-mediated cell death. NLRP3-mediated activation of inflammasome also results in pyroptosis of *Salmonella*-infected macrophages. Inflammasome formation, Ipaf and adaptor protein ASC interaction is crucial for caspase-1 activation [79]. Further, SPI-1 mutants cannot secrete flagellin and hence cannot stimulate pyroptosis in macrophages. Caspase-1 activation results in IL-1β, IL-18, and LDH release [80]. IL-1β and IL-18 secretion affect the recruitment of T cells and NK cells. Being a well-known pyrogen, IL-1β also affects the extent of fever during salmonellosis. Flagellin-mediated cell death is the most prominent in the early phase of infection but not during the systemic phase. However, prolonged *Salmonella* infection also results in a caspase-1-dependent delayed cell death in a SPI-2-dependent manner. SPI-2 effector SpvB is crucial for this phenotype. Caspase-1-deficient mice are 1,000-fold more susceptible to *Salmonella* infection and display a higher organ burden in Peyer's patches, MLN, and spleen when the infection is through the oral route [81]. Peritoneal infection of these mice does not exhibit any difference when compared to wild type, highlighting its role in intestinal invasion and dissemination. Release of proinflammatory cytokines by macrophages undergoing pyroptosis can recruit more immune cells which can be infected by the bacteria and result in dissemination. Deficiency of caspase-1 in resistant mouse strain (Nramp1 positive) also enhances lethality to *Salmonella* [82]. Among other signalling cascades, Raf-1 kinase is also known to mediate inflammatory signals upon LPS stimulation in macrophages. Raf-1-deficient macrophages are hypersensitive towards pathogen-induced caspase-1 activation and cell death [78].

Infected macrophages also undergo SPI-2-dependent apoptosis. SpvB, a SPI-2 cytotoxin, depolymerizes actin cytoskeleton in human macrophages and causes apoptosis. This process requires TLR4 signalling and bacterial LPS. *Salmonella*-mediated activation of PKR kinase results in phosphorylation of eIF2α which in turn inhibits protein synthesis. This ultimately leads to delayed apoptosis of infected cells. NFκB and MAPK activity negatively regulates *Salmonella*-mediated apoptosis of macro-

phages [83]. *Salmonella* effector AvrA shows MAPKK acetyl transferase activity which results in inhibition of JNK and NFκB. PhoP has also been reported to play a role in *Salmonella*-mediated cell death of human macrophages. This kind of apoptosis of macrophages is induced by only invasive *Salmonella* strains irrespective of their intracellular replication ability. During *Salmonella*-mediated apoptosis of macrophages, infected cells show membrane blebbing, DNA fragmentation, and activation of effector caspase-3 [78, 80].

Apart from apoptosis and pyroptosis, *Salmonella* infection has been reported to induce necroptosis in macrophages. *Salmonella* infection of macrophages stimulates type I interferon signalling which in turn activates RIP1 and 3 kinases resulting in necroptosis of infected cells. Mice deficient in IFN receptor or RIP3 show prolonged survival as compared to wild-type mice. This is attributed to the absence of macrophage necroptosis [84].

Salmonella has also been reported to cause autophagy-mediated cell death in macrophages deficient in caspase-1. This process is SipB dependent. SipB disrupts host mitochondria in infected cells which results in autophagy induction and cell death [85].

Metabolism

In human and murine macrophages, glycolysis is the major source of energy and glucose is the main carbon source. The tri-carboxylic acid (TCA) cycle is partially required for amino acid synthesis; however, complete TCA cycle is not essential for intracellular life in macrophages.

In contrast to epithelial cells, *Salmonella* present in the macrophages does not require a functional electron transport chain and ATP synthase. Instead, it depends on substrate level phosphorylation for ATP generation rather than proton gradient [86]. *Salmonella* has been reported to use host amino acids for its survival. For example, *Salmonella* upregulates arginine uptake by the host macrophage by increasing the cationic amino acid transporter mCAT expression [87]. Localization of mCAT to SCV results in transport of arginine from cytosol to the vacuole. From the vacuolar pool, bacteria uptake arginine in an ArgT-dependent manner [87]. *Salmonella* also requires a functional purine biosynthesis pathway for successful survival inside macrophages. Macrophage ROS response results in DNA damage in the bacteria and to repair this damage it is crucial to have functional purine biosynthesis. This pathway also regulates Sif formation and SPI-2 T3SS formation [86].

Macrophage Polarization during *Salmonella* Infection

The initial inflammatory response of the host, upon *Salmonella* infection may prime the differentiation of the macrophages into two major types, the classically activated macrophages (CAMs or M1 type) or the alternatively activated macrophages (AAMs or M2 type).

The polarization into M1 or M2 phenotypes is majorly dictated by the cytokines the macrophages encounter [88]. Cytokines like IFNγ or stimulation of TLRs like TLR4 by LPS primes the macrophage to develop into an M1 phenotype which then secretes proinflammatory cytokines and helps in the activation of Th1 arm of the adaptive immune system. This activation of macrophages into CAMs is very tightly regulated as they can also induce tissue damage. Stimulation by cytokines like IL-4 results in the differentiation of macrophages into M2 phenotype, which secrete anti-inflammatory cytokines. These macrophages have critical roles in resolving the inflammation and wound healing. They have reduced microbicidal activity and aid the Th2 arm of the adaptive immune system.

Studies have found that *Salmonella* prefer inhabiting the AAM during the establishment of chronic infections [89]. There have been rigorous studies performed to elucidate the reason for which the pathogen tends to "choose" one phenotype of the macrophage over another. This can happen because either the bacteria can survive or replicate better in these phagocytes or the bacteria actively guide the polarization to be shifted towards M2 phenotype. The latter seems to be the case with *Salmonella*.

The nuclear peroxisome proliferator-activated receptors, PPARγ and PPARδ, via their signal transduction are pivotal in dictating the gene regulation patterns of the AAMs [90]. Drugs that target the activity of these PPARs have been known to be effective in a variety of diseases including diabetes, cardiac diseases, and also inflammatory bowel disease [91]. Infection of cultured macrophages with *Salmonella* induces the expression of genes encoding PPARγ and PPARδ [89]. The same study showed that modulation of PPARγ levels in AAMs directly correlated with the ability of *Salmonella* to replicate inside these macrophages, whereas they did not show any effect on the proliferation of bacteria such as *Mycobacterium*, *Francisella*, and *Listeria*. This suggests that the pathogen plays an active role in driving the macrophage polarization into the AAM or M2 phenotype.

Protein kinase C (PKC) isotypes belong to the family of serine/threonine protein kinases and play a vital role through signal transduction in the regulation of a variety

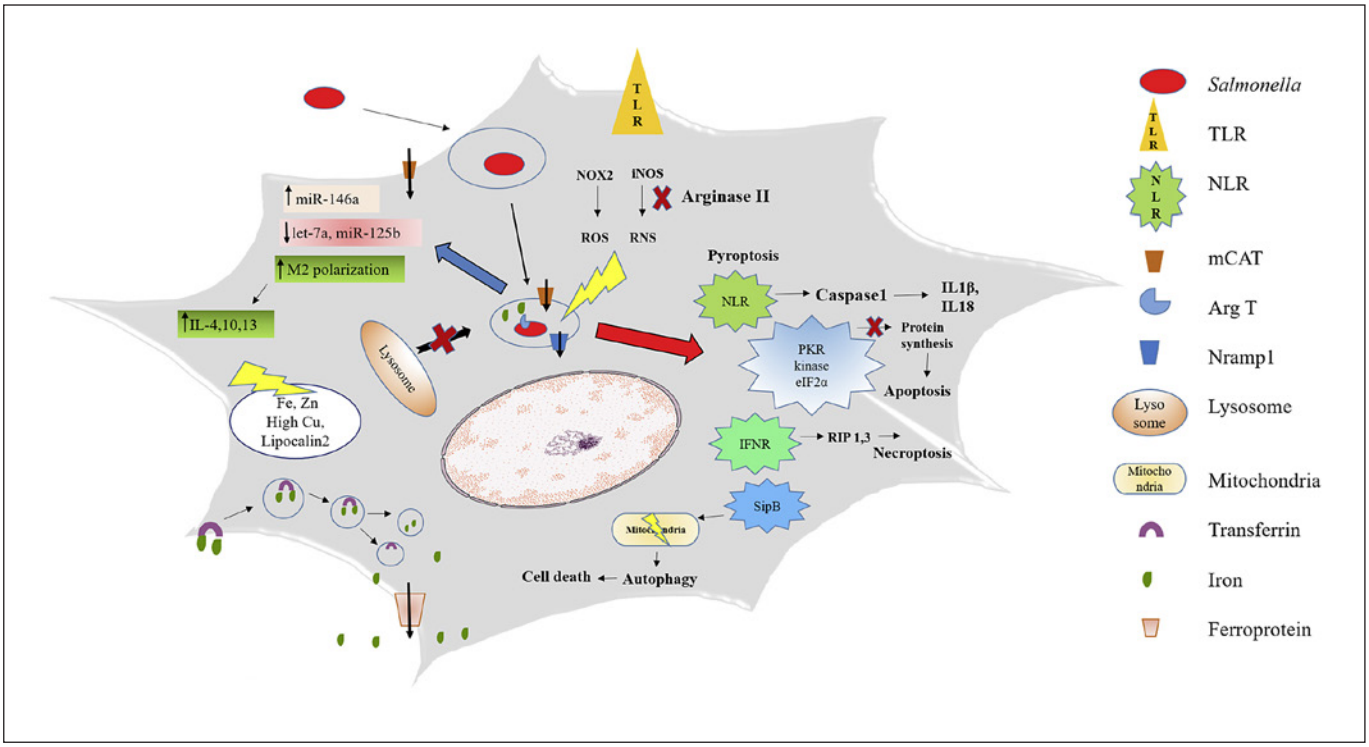

Fig. 1. Summary of the *Salmonella*-macrophage interactions. TLR stimulation by the *Salmonella* PAMPs trigger the burst of ROS and RNS. *Salmonella* tries to avoid the ill effects of RNS by inducing the simultaneous enhancement in the production of arginase II, which limits the substrate for iNOS activity. Arginase is also recruited into the SCVs. *Salmonella* actively avoids the SCV fusion with the lysosomes. Macrophages try to establish the balance in the level of metal ions in order to limit the comforts extended to the pathogen. *Salmonella* effectors trigger various kinds of cell death, like (a) caspase-1-mediated pyroptosis, (b) activation of PKR kinase which leads to the phosphorylation of eIF2α, hampering protein synthesis, leading to apoptosis, (c) activation of IFNR, leading to necroptosis, and (d) SipB mediating the autophagy. *Salmonella* infection also leads to the alteration of the levels of certain miRNAs. *Salmonella* prefers the alternatively activated macrophages over the classically activated macrophages for its intracellular life.

of cellular functions. One of the isotypes, PKCθ, is known to be predominantly expressed in T cells. Recent reports suggest a role for PKCθ in cholesterol metabolism in human macrophages [92]. Other isotypes like PKCα, PKCβ, PKCδ, and PKCζ have been shown to have critical roles in antimicrobial immune responses of the macrophages. In a recent study, it was shown that in mice lacking PKCθ, the disease progression and lethality of *Salmonella* Typhimurium infection was exacerbated [93]. It was found that the mRNA levels of PKCθ were strongly upregulated in CAMs but not in AAMs. The results were similar both in murine macrophages and human monocytes. It is suggested that PKCθ might have a subset selective role as signalling intermediate of proinflammatory macrophages. This is speculated to be happening by IL-10 repression which affects the microbicidal activity of the macrophages and also by the regulation or influencing the transcription of certain miRNA clusters [94]. This might be another reason for the pathogen to not choose CAMs.

Since AAMs have reduced microbicidal activity, *Salmonella* choosing them as a better vehicle than the CAMs whose responses are more robust seems logical. However, direct evidence of this being the reason has not yet been established. It was elegantly suggested by Roop II et al. [95] that this bias could be not because of the reduced microbicidal activity, but something more fundamental. The shift in the cellular metabolism is one of the consequences of macrophage polarization. CAMs rely on glycolysis for their energy and end up consuming a lot of glucose, whereas AAMs degrade fatty acids for acquiring energy. Since glucose is more readily available in the AAMs, as beautifully suggested, the bacteria might have found a "sweet spot" in the AAMs.

Conclusion

Significant advances have been made in understanding the *Salmonella* pathogenesis in both human and murine models. In this review, we have tried to summarize the complex interaction between *Salmonella* and macrophage (Fig. 1). Among all cells types that serve as a niche for *Salmonella*, epithelial cells and macrophages are most studied. However, large gaps still remain to be filled in our understanding of the pathogenesis. Specially, the role of AMPs, metal availability, and *Salmonella* metabolism in macrophage with respect to acute versus chronic infection require further attention. We are just beginning to understand the role of exosomes, microRNA, and tissue resident macrophages in *Salmonella* pathogenesis and they might play a crucial role in determining the disease outcome. Further studies are needed to explore these unchartered territories to have a better understanding of the disease. This can help design a new therapeutic strategy against *Salmonella* infection.

Disclosure Statement

The authors declare no conflicts of interest.

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