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Salmonella enterica serovars Typhimurium and Typhi as model organisms

Revealing paradigm of host-pathogen interactions

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The lifestyle of intracellular pathogens has always questioned the skill of a microbiologist in the context of finding the permanent cure to the diseases caused by them. The best tool utilized by these pathogens is their ability to reside inside the host cell, which enables them to easily bypass the humoral immunity of the host, such as the complement system. They further escape from the intracellular immunity, such as lysosome and inflammasome, mostly by forming a protective vacuole-bound niche derived from the host itself. Some of the most dreadful diseases are caused by these vacuolar pathogens, for example, tuberculosis by Mycobacterium or typhoid fever by Salmonella. To deal with such successful pathogens therapeutically, the knowledge of a host-pathogen interaction system becomes primarily essential, which further depends on the use of a model system. A well characterized pathogen, namely Salmonella, suits the role of a model for this purpose, which can infect a wide array of hosts causing a variety of diseases. This review focuses on various such aspects of research on Salmonella which are useful for studying the pathogenesis of other intracellular pathogens.

Salmonella as a Model Intracellular Pathogen

Salmonella represents a group of Gram-negative facultative anaerobic pathogenic bacteria which costs millions of lives across the world every year. At present, the genus Salmonella is categorized into two species *S. bongori* and *S. enterica*, based on the high (96–99%) sequence similarity of the genome. There is only one subspecies under *S. bongori* namely subspecies V, whereas *S. enterica* comprises the remaining seven subspecies I, II, IIIa, IIIb, IV, VI and VII.[1] Where subspecies I is specific to warm-blooded animals like mammals, others can infect only cold-blooded animals including reptiles. Further division into serovars increases the number of variants to more than 2,500. Out of these, *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Typhi have been discussed here, as they have previously served as tools to study host-pathogen interactions.

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While *S.* Typhi infection is strictly limited to humans and higher primates, *S.* Typhimurium has a wide range of host such as rodents, cattle and mammals.

Intracellular pathogens can either survive in a self-constructed niche in the form of a vacuole or they may choose to live in the cytoplasm of the host cell. Salmonella chooses the most commonly preferred option of forming an intracellular vacuole termed as Salmonella containing vacuole (SCV). SCV arrests the host endosomal pathway at the late endosome stage. It does acquire the late endosome markers, such as vATPases and LAMP1, but loses some of them like mannose-6-phosphate receptor which differentiates it from the late endosome.[2] Later the SCV gets juxtaposed to the nucleus by utilizing the microtubule meshwork of the host cell and derives nutrition from the Golgi apparatus.[3] Few intracellular pathogens follow an alternate less preferred strategy to survive inside host cells, as they do not form a niche but develop strategies to survive inside the cytoplasm,[4] including the examples of Shigella and Listeria.

Other best studied vacuolar pathogens also hijack the endophagocytic pathway of the host at various stages bearing the surface markers of that specific stage. Mycobacterium infection involves formation of Mycobacteria pathogen vacuole (MPV) that does not mature after the early endosome stage while being associated with the corresponding markers like EEA1 and Rab5.[2] This arrest at early endosome stage prevents the fusion of the MPV with the phagolysosome and hence the clearance of the pathogen. Another example, Brucella containing vacuole (BCV), displays early endosome related markers like EEA1, Rab5, etc. and eventually takes an unconventional route of becoming endoplasmic reticulum (ER) derived autophagosome maturing into ER.[2] In case of Legionella infection, Legionella containing vacuole (LCV) bears autophagosome associated markers like Atg7 and Atg8 and further matures into rough ER like organelle.[2] Chlamydia form *Chlamydia trachomatis* inclusion (INC) which moves to the microtubule organizing center (MTOC) like Salmonella.[2] Notably, the vacuolar structure INC is segregated from the typical endomembrane pathway unlike other pathogens. Toxoplasma forms a host plasma membrane derived parasitophorous vacuole (PV), which is completely independent of vesicular trafficking of the host cell. The membrane of PV gets incorporated with LDL cholesterol with the help of post-lysosomal vesicles.[2]

Although a substantial amount of work about the mechanism of establishment of these intracellular structures have been done, there are yet many unanswered questions about the changes induced upon host by the pathogen to maintain the integrity of these vacuoles. Hence understanding the system of a model pathogen will address such questions to great extent.

A faster rate of growth and feasibility of modification of the genome by using recombinant DNA technology makes Salmonella an ideal pathogen to study host-pathogen interaction. Availability of mouse model for typhoid fever as well as gastroenteritis[5] and *C. elegans* for innate immune response during Salmonella infection[6] makes it a preferential model pathogen to study. Further, *S.* Typhimurium alone can be used as a model for two modes of infection, local gastroenteritis as well as systemic typhoid fever. Taking these points in consideration we intend to explain the pathogenic features that render Salmonella eligible to be used as a model intracellular pathogen.

Keys to Success

The key factors behind the success of Salmonella as an intracellular pathogen are described in subsequent sections which cover features specific to Salmonella, making it interesting to venture into the details of Salmonella pathogenesis.

Multiple targets. Salmonella possesses extremely versatile strategies to infect different target host cells (**Table 1**). Interestingly it prefers to proliferate in the usually non-permissive environment of immune cells such as macrophages instead of the much permissive epithelial cells. The mode of entry as well as the strategy followed to survive inside the target cell varies according to the type of cell and depends on the temporal expression of particular genes by Salmonella such as the type three secretion systems. Salmonella is controversially shown to be able to survive and replicate most preferentially inside the microbicidal neutrophils instead of macrophages according to the conventional paradigm.[7] Other target cells that encounter Salmonella in different locations include B cells, T cells, monocytes, dendritic cells, granulocytes and gut epithelial cells (**Fig. 2**). Salmonella displays variety in mechanisms of not only entry and survival but also cytotoxicity, which relies on its virulence factors (**Table 1**).

Horizontally acquired pathogenicity islands. The astonishing variation in pathogenesis of the serovars of the same species is accountable to the acquisition of genes laterally from external sources over the course of million years. One-fourth of the Salmonella genome is estimated to be acquired horizontally. The divergence of Salmonella from *E. coli* in the process of evolution involved the horizontal transfer of various genes that turned Salmonella into a successful pathogen compared with *E. coli*. These genes are collectively termed as pathogenicity islands, which are further categorized based on their function. The serovars Typhi and Typhimurium have 11% difference in their genome, which are otherwise 99% similar in their sequences of housekeeping genes. Also the same serovar has variations in their genome within the strains. The genes acquired play roles in pathogenesis (SPI1, SPI2, SPI3, etc.) as well as metabolism (*aroA*), resistance against antibiotics and many such important functions.[8] The horizontal gene transfer could occur by various modes like phage infection, conjugative plasmids, transposition or transformation[1] and most commonly by inserting genes within tRNA genes. There are 12 pathogenicity islands known at present and the continuous process of evolution may add up more genes to the list. Apart from gaining external genes, Salmonella may also tend to lose certain genes to maintain virulence, like loss of *lac* operon during evolution has enhanced the fitness and virulence of Salmonella.[9]

Two-component systems. The ability of Salmonella to sense the extracellular cues in the surrounding micro-environment and accordingly regulate the expression of genes is dependent majorly upon few two-component systems. The *phoP* system encodes the sensor PhoQ and response regulator PhoP, whose expression is induced by Mg^{2+} starvation and low pH, regulates acid tolerance and major virulence genes, such as genes required for invasion,[10] intracellular survival[11] and resistance to antimicrobial peptides. Another two-component system, *ompR*, responds to change in osmolarity and regulates invasion[12] as well as intracellular survival.[13,14] The system *pmrAB* mediates resistance specifically against the anti-microbial peptide polymyxin B and is further regulated by other two-component systems such as PhoP/PhoQ and PreA/PreB[15] reflecting the importance of polymyxin B in Salmonella pathogenesis. Nevertheless, there are many other two-component systems to mediate virulence and adaptation to environmental stresses, like SPI1 induction as well as biofilm formation by SirA/BarA[16,17] and SPI2 expression regulation by SsrA/SsrB.[18] Some two component systems regulate nutrition uptake too, as seen in the case of *ttrRS* system which helps in utilization of tetrathionate to produce an alternate electron donor thiosulphate.[19] Interestingly these two-component systems are inter-dependent generating a complex network of their activity and regulation.

Global regulators. The success of a pathogen in establishing infection is predicted by the appropriate expression of virulence associated genes tuned by a set of regulators. These regulators control the expression of various genes in favor of the cell in response to certain environmental cues. Global regulators are called so due to their ability to regulate multiple genes simultaneously. To quote some examples, SirA regulates the genes required

Table 1. Multiple cellular targets

Cell type	Cell culture model	Mode of internalization	Outcome
M cells	Mixed culture of CaCo-2 and Raji B cells	Caveolae mediated endocytosis	Cell lysis
Dendritic cells	Primary cells	Phagocytosis	Apoptosis, cell lysis
Macrophages	RAW 264.7, J774, etc., primary cells	Phagocytosis	Apoptosis
Epithelial cells	HeLa, CaCo-2, HT-29, Intestine 407, etc. and primary cells	Macropinocytosis	Apoptosis, cell lysis

for gastroenteritis[20] as well as biofilm formation.[16] Another global regulator HilA, which is itself regulated by SirA, acts as a transcriptional regulator for SPI1, SPI4 and SPI5 that together mediate invasion of the host cells.[20] Similarly CsrA is a very well-known global regulator which controls multiple functions including invasion, flagella synthesis, chemotaxis, biofilm formation, vitamin B12 synthesis and maltose operon.[16,21] Interestingly, two or more global regulators can dictate the expression of similar genes; for example, Fnr regulates flagellar synthesis and chemotaxis along with CsrA. On the other hand, Fnr is assigned to regulate several important genes including genes for aerobic metabolism, $NO^{\cdot}$ detoxification and anaerobic carbon utilization.[22]

Sentinels of Salmonella: Virulence Factors

Type three secretion systems. Type III secretion systems (T3SS) are present on the cell wall and possess a needle like structure. The major T3SS of Salmonella are encoded by two pathogenicity islands, SPI1 and SPI2. The assembly and functions of these T3SS are coordinated spatially and temporally. The T3SS are dedicated to secrete certain proteins which bring about specific effects in the microenvironment of the cell.

Salmonella pathogenicity island 1 (SPI1). SPI1 plays pivotal role in both forms of diseases caused by Salmonella, i.e., gastroenteritis as well as systemic infection.[23] It carries out multiple functions, which include cytotoxicity of macrophages,[24] invasion of epithelial cells,[25] inflammation and fluid secretion in ileum[26] and cytokine secretion.[27,28] SPI1 also induces apoptosis in macrophages[24] and executes the exact opposite function in epithelial cells.[29] A series of modifications are brought about by SPI1 inside the host to facilitate internalization of Salmonella[30] (**Table 2**). For example, some SPI1 encoded proteins like InvG, InvJ, PrgH, PrgI, PrgK and SpaO assemble the needle complex, whereas others, including SipB, SipC and SipD, translocate effector proteins through this needle.[28] Effector proteins may or may not be encoded by SPI1.

Salmonella pathogenicity island 2 (SPI2). Genes within this pathogenicity island are not essential for gastroenteritis but are indispensable for systemic infection as they support the intracellular survival of Salmonella inside host cells. The formation and maintenance of the SCV involves a number of events controlled by this pathogenicity island[30] (**Table 2**). SPI2 confers protection against reactive oxygen species (ROS)[31] as well as reactive nitrogen intermediates (RNI)[32] inside macrophages. SPI2 encoded tetrathionate reductase acts on tetrathionate to generate thiosulphate which acts as an alternate electron donor for Salmonella in tetrathionate containing environments like human gut, soil, decomposing carcasses.[19,33] An additional T3SS, Spi/Ssa, is encoded by SPI2 during intracellular life of Salmonella. It is regulated by PhoP/PhoQ system and serves as the portal for the exchange of materials within the SCV and host cytoplasm.[11]

On account of their divergent roles, the simultaneous expression of these two type three secretion systems is not expected naturally. But in actual scenario, the spatiotemporal expression of these two pathogenicity islands cannot be demarcated clearly as there is evidence of their overlapping expression. This includes

Table 2. Functions of the SPI encoded proteins

Major event	Proteins encoded
SPI1	
Assembly of needle and secretion of effector proteins	SpaO, InvJ, InvG, PrgI, PrgJ, PrgK, SipB, SipC
Actin cytoskeletal rearrangement via Rho GTPases and tight junction disruption	SopE, SopE2, SopB (or SigD)
Actin polymerization by decrease in critical concentration	SipA
Modulation of actin cytoskeleton by actin nucleation	SipC (or SspC)
Regaining of cytoskeleton by reversing action of SopE, SopE2 and SopB	SptP
Fluid accumulation in intestine	SopA, SopD, SopB
Modulation of chloride channel to induce diarrhea	SopB, SopE
Inhibition of NFkappaB activity and IL-8 secretion	AvrA, SspH1
Transmigration of polymorphonuclear leukocytes	SipA, SopA
Activation of caspase 1 and autophagy in macrophages	SipB (or SspB)
SPI2	
Needle assembly	SpiB, SpiC, SpiD etc. (also known as Ssa genes)
Effector protein translocation	SseB, SseC, SseD
Interference with endosome trafficking	SpiC
Maintenance of SCV integrity	SifA
Salmonella induced filament (Sif) formation and microtubule bundling	SifA, SseF, Sseg, SopD2, PipB2
Inhibition of actin polymerization	SspH2
Downregulation of Sif formation	SpvB
Host cell dissemination	SseI
Anaerobic respiration by reducing tetrathionate	TtrABC, TtrRS

pre-emptive expression of SPI2 in gut lumen before invasion of epithelial cells in order to prepare Salmonella for traversing across basal side of epithelium into lamina propria[34] as well as for the upcoming intracellular stress[35] and residual SPI1 expression after internalization by macrophage to counteract host immune response by suppressing cytokine expression.[36]

Other pathogenicity islands. SPI3 encoded *mgtC* enables Salmonella to survive in Mg^{2+} starvation conditions, partly controlled by *phoP/Q* system, and is required for survival within macrophages as well as systemic infection in mouse model.[37] SPI4 encodes a type I secretion system and mediates adhesion, whereas SPI5 encodes SopB. SPI7 is exclusively present in the host specific serovar *S.* Typhi and absent in Typhimurium. The main functions of this pathogenicity island include synthesis as well as the export of Typhi specific Vi antigen. Additionally, genes encoding SPI1 effector SopE and type IVB pilus lie within SPI7.[38] Very little information is available about other pathogenicity islands, like Typhi specific SPI6[39] and SPI10[40] encode chaperon-usher fimbrial operon. Similarly SPI8 encodes a pseudo bacteriocin and degenerate integrase, whereas SPI9 codes for a type I secretion system like SPI4.[40]

Adhesins. The mere attachment of the bacterium to the target cell consists of many steps mediated by various adhesins encoded either by fimbrial genes like type 1 fimbriae (fim),[41] plasmid encoded fimbriae (pef),[42] long polar fimbriae (lpf)[43] and thin aggregative fimbriae (Agf)[44] or non-fimbrial genes such as the autotransporters MisL[45] and ShdA[46] or SPI4 member SiiE.[47] Each adhesin belonging to this pool is assigned to mediate adhesion to particular kind of cells due to specificity for the receptors present on the surface of these cells,[48] for example, SPI4 is responsible for adhering to polarized cells[47] whereas type 1 fimbriae *fimH* mediates attachment to dendritic cells.[49] The same kind of cell can be bound by different adhesins with the progression of adhesion, as described recently in the form of irreversible docking by SPI1 that enhances adhesion mediated by type 1 fimbriae.[48] Nevertheless, the flagellum is required to reach the target cell as well as to aid in adhesion in accordance with fimbriae.[50]

Plasmid encoded virulence genes. Salmonella possesses extrachromosomal genes which are equally important for infection. For example, *Spv* works in a SPI2 dependent manner and is essential for virulence of *S.* Typhimurium[51] but not for Typhi. Similarly, *Pef* is a plasmid encoded adhesin to mediate adhesion for infecting gut epithelial cells.[42]

Counteracting the Worst

Like any other pathogen, Salmonella has its share of risks while entering the host system. After ingestion along with the contaminated food, Salmonella needs to withstand the highly acidic pH of the stomach. Once it reaches the intestine it establishes the infection in two modes. The invasive mode involves breaching of M cells leading to uptake by phagocytes, whereas the non-invasive mode refers to direct phagocytosis by dendritic cells. The various stresses induced upon Salmonella by the host act as environmental cues to be sensed by response regulators present within Salmonella for the expression of particular set of proteins to sustain the stress.

Acidic stress. The gastric pH acts as the first line of defense against Salmonella infection. The passage through the highly acidic environment of stomach generates the acid tolerance response (ATR) which ensures the escapade of Salmonella from acidic stress (**Fig. 1**). During ATR, the response regulator PhoP and alternate sigma factor RpoS protect from inorganic acid encountered inside stomach.[52] On the other hand RpoS and Fur facilitate the survival in presence of weak organic acids like lactic acid in the intestine.[53] Acidic pH also induces expression of certain virulence associated genes. For example, acidity induced STM1485 enables better intracellular replication of Salmonella.[54]

Physical barrier. To begin an intracellular lifestyle Salmonella must cross the gut epithelia (**Fig. 2**). While M cells allow easy entrance, epithelial cells do not favor passive entry. Salmonella induces membrane ruffling in epithelial cells by modifying actin cytoskeleton, exerted by SPI1, ultimately resulting in macropinocytosis of Salmonella as described in previous sections in this review. Also SPI2 mediated apoptosis helps Salmonella to cross the epithelial lining.[55] The alternate path of breaching epithelial barrier includes uptake by $CD18^{+}$ phagocytes traversing the gap between epithelial cells.[56]

Evasion of host defense. Immune responses generated by host constantly try to eliminate the pathogen (**Fig. 3**). Anti-microbial peptides produced by Paneth cells of gut epithelia and macrophages can kill extracellular and intracellular Salmonella respectively. To avoid this, Salmonella may undergo changes in the lipidA composition on the cell surface to prevent interactions of the cationic peptides or synthesize proteins like that coded by operons *yejABEF*[57] and *sap*[58] assigned to export these peptides outside cells. The smartness of Salmonella in deviating host defense is reflected in the strategy of retaining one bacterium per SCV to reduce the count of lysosome per SCV.[59] Oxidative and nitrosative stresses are two most prominent immune strategies of host which are countered by Salmonella by various mechansims.[60] The detrimental nitric oxide generation from arginine by host is counteracted by arginase production by Salmonella that competes with iNOS for arginine.[61] Most interestingly, arginine is being transported inside SCV by recruiting host mCAT1 and mCAT2B to the SCV. From the SCV, arginine transporter encoded by ArgT operon is used to transport arginine inside Salmonella.[62]

Nutritional stress. Starvation for nutrients inside the host is very common which leads to starvation stress response that enable Salmonella to withstand the stress as well as to counteract other environmental stresses.[63] An excellent example of Salmonella induced host manipulation to meet its own nutritional requirements is presented by tetrathionate production induced inflammation during Salmonella infection.[33] The excess of tetrathionate aids in competing with gut micro-flora for electron source and hence better survival.

Availability of Tools

Cell-culture model/in vitro model. *M cells.* M cells, present in Peyer's patches, serve as the gateway for Salmonella to enter host reticuloendothelial system. As M cells lack glycocalyx, Salmonella can conveniently enter these cells to be further taken up by the

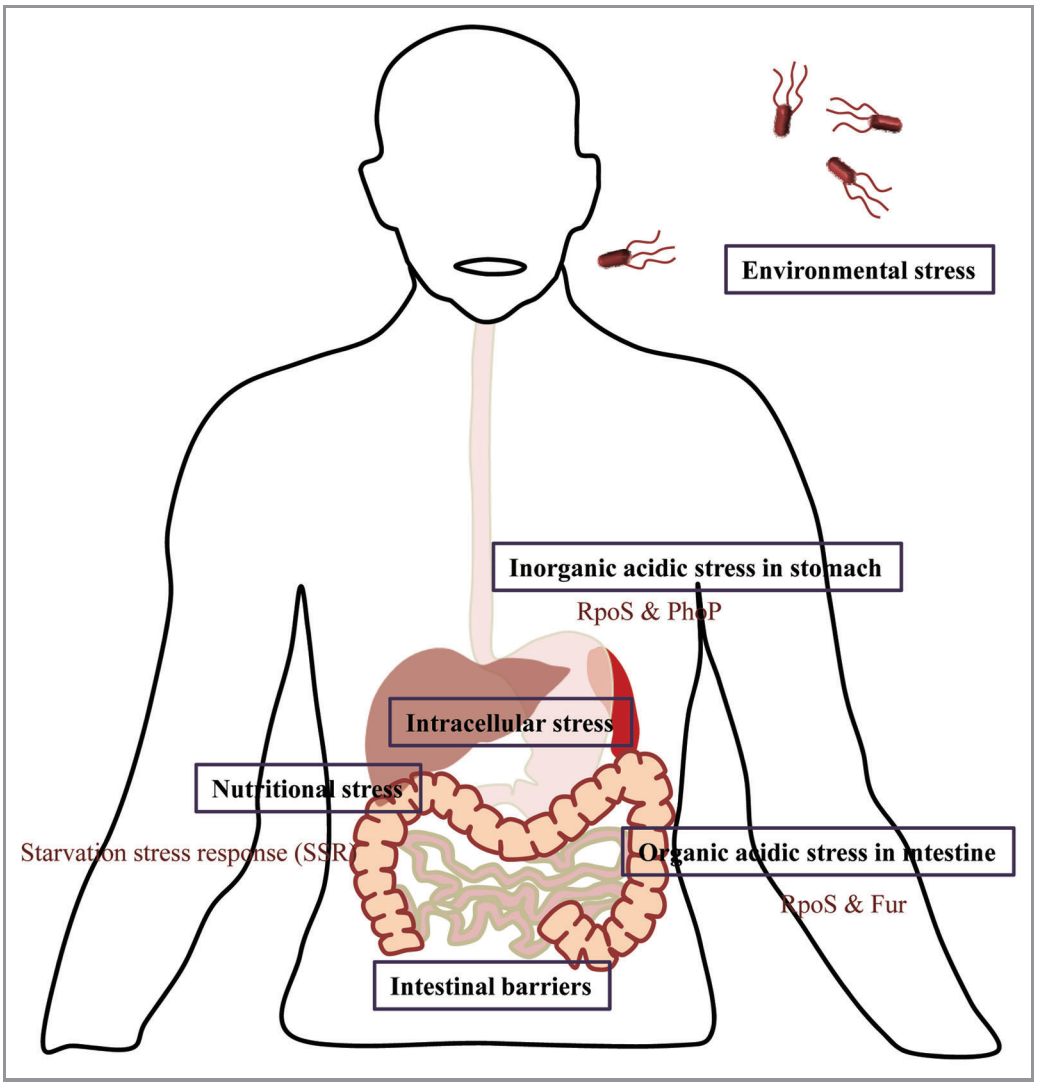

Figure 1. Challenges encountered by Salmonella. The text boxes represent the various stresses encountered by Salmonella during its life cycle and the open text describes the factors and signals generated by Salmonella in order to combat these stress conditions.

underlying macrophages. The adhesion of Salmonella to M cells is believed to be mediated by the fimbrial assembly chaperone and the invasion is receptor mediated[64] independently of SPI1 and SPI2.[65] Although the cytotoxicity of M cells by Salmonella is not clearly understood, the regulator SlyA seems to play a role in damaging M cells and strains defective in invasion are attenuated in killing M cells.[64] The caveolae mediated entry in M cells, was deduced by using a co-culture of Caco-2 cells and Raji B cells.[66,67]

Epithelial cells. Epithelial cells beside M cells or within an organ like gall bladder engulf Salmonella by macropinocytosis, in SPI1 dependent manner. In cell-culture model, after internalization the monolayer epithelial cells are directed toward caspase 3 mediated apoptosis depending upon the effector proteins encoded by SPI2 and spv loci,[55] whereas polarized enterocytes are lysed due to lipid peroxidation by Salmonella induced ROS generation.[68] To name few, HeLa, CaCo-2, HT-29, etc. serve as very good model cell-lines for studying invasion of epithelial cell by Salmonella and henceforth its proliferation.

Dendritic cells. Salmonella can breach gut epithelia by an alternate mechanism by being engulfed by dendritic cells (DCs). These DCs are also major antigen presenting cells like macrophages which phagocytose Salmonella and present antigen to the specific $CD4^+$T and $CD8^+$T cells. Although, they do not provide hospitable environment for the survival of the pathogen, they act as steady carrier of Salmonella for its passive dissemination to systemic sites. Also Salmonella induces caspase-1 mediated cytotoxicity in DCs depending upon SPI1 needle assembly and the expression of SPI1 effector protein SipB.[30] The killing is possibly mediated by stimulation of $P2X_7$ receptor or pore forming property of SPI1 which leads to leakage of cytoplasmic matter.[69] Primary cells isolated from bone marrow for animal models or healthy humans are used as in-vitro model for dendritic cells.

Macrophages. They act as reservoir for Salmonella and play the most vital role in the dissemination as well as the antigen presentation of Salmonella. The macrophages present in the gut associated lymphoid tissue phagocytose Salmonella as soon as the

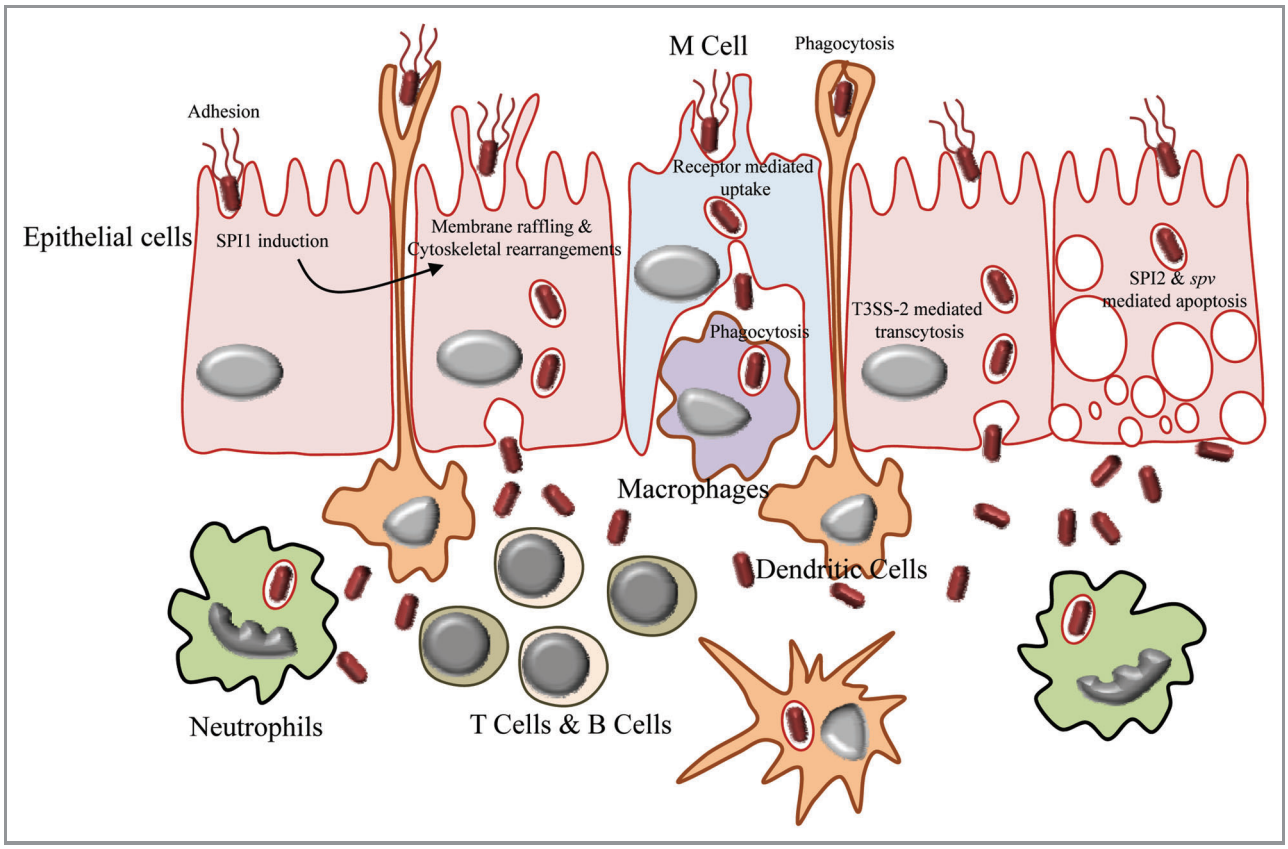

Figure 2. Breaching of gut epithelia by Salmonella. The mode of entry of Salmonella in gut lumen varies according to type of cell encountered on the gut epithelium. The M cells take up the bacteria by means of receptor mediated endocytosis, whereas dendritic cells engulf them by phagocytosis. The membrane of epithelial cells is modified by the action of SPI1 to facilitate the entry of bacteria. Once inside the gut lumen, Salmonella is being taken up by macrophages, T cells, B cells, neutrophils, etc.

intestinal epithelium is breached and harbor them until they undergo apoptosis induced by SPI1.[24] The route of macrophages through reticuloendothelial system, while carrying Salmonella, is believed to be one major reason behind systemic site infections. A murine macrophage like cell line RAW 264.7 is the most useful model cell line to study intracellular survival of Salmonella within macrophages. Other cell lines like murine macrophages J774-A.1, most preferred by *S.* Typhimurium, can also be used.

Monocytes and granulocytes. The idea of dendritic cells being the major antigen presenting cells vanished when it was discovered that transport and antigen presentation of Salmonella in lymph is mainly performed by monocytes and granulocytes instead of dendritic cells.[70] The example of survival within immature granulocytes in association with malaria[71] presents a special situation of survival of Salmonella in unconventional targets. The model used for such non-DC myeloid cells are primary cells isolated from animal models. The human monocyte cell line THP-1 can provide for the in-vitro model for monocytes.

Recently it was discovered that pathogenesis of Salmonella varies based on the polarization status of the cells. Polarized cells allowed easier internalization of Salmonella than non-polarized cells and phagocytes and intracellular survival in polarized cells was found to be independent of SPI2, which is otherwise essential for surviving inside other cells types.[72]

Animal models/in vivo model. Salmonella infects a wide range of animal hosts and the causative agent of human infection usually comes from livestock in the form of meat, eggs and similar products. Hence animal models are essential to improvise understanding of pathogenesis as it helps to extrapolate the results to humans. Animal models are used for the two major forms of diseases that occur in humans namely enteritis and systemic typhoid. There are suitable models for each kind of infection. The susceptible mouse strain BALB/c, lacking Nramp1 protein, is used most commonly for *Salmonella* Typhimurium infections, as the manifestation of disease in this model resembles closely to that of humans. C57BL/6 is another common strain of mouse used as a model system. In comparison to mouse, bovine model is considerably more suitable to study enteritis.[5] Rhesus monkeys are also used for enteritis.[5] Unfortunately, there is no ideal animal model available for *S.* Typhi infection. The mouse model for typhoid varies from that of human typhoid fever. For example, in case of Typhimurium infection of mice, few genes, such as *spv* operon, are essential that are not required by Typhi to infect humans. Hence it remains a challenge to study the pathogenesis of Typhi infection at physiological level. Although there is provision of artificial systems like iron-treated mice for Typhi infection,[73] these are not preferred over the natural mouse model for Typhimurium infection. Introduction of humanized mice has

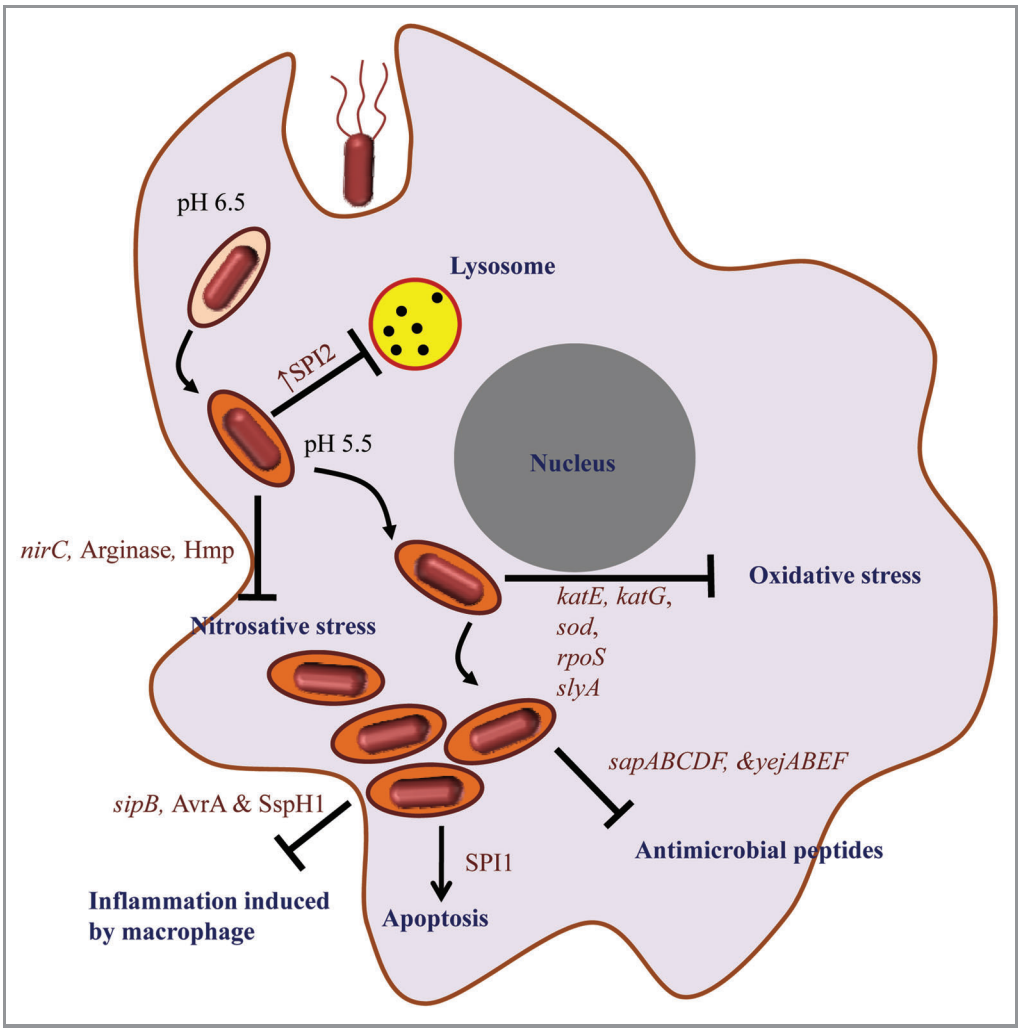

Figure 3. Immune evasion strategies of Salmonella. The intracellular life-cycle of Salmonella includes the entry of the bacterium in the host cell, SCV formation (whose pH changes from 6.5 to 5.5 depicted by change in the color of SCV compartment), evasion of host immune response and ultimately host cell death by apoptosis. The text in dark blue shows the immune responses and processes within the host cell that take place during Salmonella infection and text in dark red depicts the factors that help Salmonella to evade these immune responses.

generated some hope. One such example, namely the humanized immune system (HIS) mouse model, has the incorporation of human immune cells into the reticuloendothelial system of mouse.[74] Typhoid can also be induced in chimpanzees by oral infection.[5] Also, *C. elegans* can serve as a potential animal model for studying Salmonella pathogenicity.[75]

Different Outcomes of Infections

The peculiarity of Salmonella infection is depicted by its ability to bring about different outcomes in different targets during the course of infection. As described previously, each cell type targeted by Salmonella meets a different fate. Similar effects are implied in organs harboring these cells. In spleen, the highest population of cells containing Salmonella are found to be monocytes and neutrophils, whereas liver shows accumulation of macrophages, both resulting in splenomegaly and hepatomegaly respectively.[5,7] The gall bladder harbors Salmonella within the favorable environment of the epithelial cells.[76] Mesenteric lymph node (MLN) prohibits dissemination by restricting the trafficking of Salmonella containing DCs.[77] The fact that B cells carry Salmonella to bone marrow, gives a possible explanation for Salmonella induced osteomyelitis.[7] On the other hand, some regions like gall stones serve as a platform for biofilm formation.[78] Occasionally Salmonella crosses the blood brain barrier and causes meningitis, mainly in infants.[79] The ultimate stage of infection is reached in the form of bacteremia which describes the presence of bacteria in blood circulation.

Other Facets

Salmonella and cancer. It was observed more than 100 y ago that bacterial infections can reduce tumor growth and since then several studies have been conducted to use bacteria as a vector for cancer therapy including *Shigella flexneri*, *Listeria monocytogenes*, *Lactococcus lactis*, *E. coli*, etc.[80] Researchers showed specific interest

in Salmonella because of their preferential colonization in solid tumors and the retardation of the tumor growth.[81,82] Avirulent strains of Salmonella can reduce the tumor size directly[83] or through the expressed therapeutic proteins[84] in mouse models. Different attenuated strains of Salmonella have been used for cancer therapy[85-90] and some of them made it to phase I clinical trials[9,52,53,57] as in case of metastatic melanoma in human patients, attenuated Salmonella strain VNP20009 was used. Although it showed only moderate tumor targeting.[91] Type 3 secretion system of Salmonella (T3SS) has been exploited for cancer therapy as well as cancer vaccination.[92] The siRNA against *MDR1* gene that codes for P-glycoprotein (ATP binding cassette transporters), has been delivered through attenuated *Salmonella* Typhi to revert multidrug-resistant tumor cells. Salmonella could retard the tumor growth and the tumor cells have responded to the chemotherapy after siRNA delivery through Salmonella.[82] Obligate anaerobic bacteria are restricted to anaerobic region of tumor and hence cannot target the vascular system, whereas Salmonella, which is a facultative anaerobe, can target the vascular system as well as cause vessel destruction and tumor retardation.[93]

There are few reports addressing the specificity of Salmonella toward tumor.[94,95] High-throughput screening of Salmonella mutants for their preferential growth in tumors showed that *STM3120* is even more efficient than *aroA* for the tumor colonization and targeting.[95] Twelve elements which are expressed only in tumor but not in normal tissues have been predicted using promoter trap library in *Salmonella* Typhimurium. These elements are very specific and can be used to express therapeutic proteins only in the tumor cells.[96] From the phase I human trials, it was observed that the bacterial strain could not localize in the tumor cells in human, whereas the ability and colonization in the tumor was very efficient in murine model.[91] These studies clearly show that there are specific host pathogen interactions which are not well understood.

Salmonella and vaccine delivery. Apart from the tumor therapy, Salmonella has also been known as a vector for vaccination because of the ability to induce immune response.[97] Salmonella T3SS is widely exploited to deliver the antigen and elicit immune response against cancer.[92,98-100] Apart from cancer Salmonella was also tested as a vaccine candidate for pneumonia by delivering pneumococcal PspA antigen[101] and against *Helicobacter pylori* by delivering A and B subunits of *H. pylori* urease.[102] Although, attenuated Salmonella induces cell mediated immune response (Th1 cells and IgG2a class switching) in cancer immunity,[92,99] mixed Th1 and Th2 responses have also been reported in case of Salmonella mediated vaccination against *H. pylori*[102] and *Streptococcus pneumonia*.[101]

Mathematical Models of Salmonella Infection

A number of mathematical models are developed in the field of infection biology that help researchers to look into the infectious diseases virtually. It is an interdisciplinary approach where biological experiments are translated into equations used to analyze and interpret the data easily.

Mathematical models for intracellular distribution and population dynamics at whole cell level of *Salmonella enterica* have been developed from experimental data. Wild-type isogenic tagged strains were used to infect the same animal simultaneously to check the spread of the bacteria in vivo and based on that the model has been developed.[103,104] The heterogeneous behavior of Salmonella infection (early rapid replication of bacteria and local spread of bacteria in later stage) was used in this model to understand Salmonella spread in vivo.[103] Mathematical model for Salmonella infection of macrophages at single cell level explains the possibility of two populations of macrophages. The variation in infection at single cell level may be due to the heterogeneity in the cell population.[105] Apart from infection models, a mathematical model for Salmonella T3SS (SPI1) regulation by SirA through HilA and HilC was developed and this model can be used to predict the virulence and intermediate components in the regulation in accordance with the experimental results.[106]

Mathematical models can provide some clue for antiviral therapy against HIV by understanding the basic mechanism of viral spread and immunity.[107,108] Few models are only available for bacterial infection and diseases and a number of models have been developed for Salmonella dynamics.[109-112] In future more accurate mathematical models can be used for a better prediction and treatment of the diseases.

Model for Other Intracellular Pathogens

Despite having stark differences in pathogenesis, many intracellular pathogens resemble Salmonella in various aspects of infection and strategies for survival within the host. Additionally, it is more feasible to perform genetic engineering in Salmonella as compared with other pathogenic bacteria. This virtue lets one to exploit Salmonella to generate information for the comparatively fastidious pathogens. For example, the gene *noxR3* was shown to be required by Mycobacterium for combating oxidative and nitrosative stress by expressing *noxR3* in *S.* Typhimurium.[113] The process of vacuole formation in case of intravacuolar pathogens is similar to SCV formation up to certain stage, for instance, Mycobacterium, Brucella, Legionella and Chlamydia exploit endosomal pathway and avoid fusion with lysosome by various strategies, one of them being the accumulation of cholesterol in vacuolar membrane, as seen in Salmonella.[2] Recently it was shown that Salmonella effector protein SipC interacts with host protein syntaxin6 to recruit LAMP1 on SCV in order to stabilize the vacuole and prevent LAMP1 recruitment on lysosome,[114] which may hint at similar mechanism adapted by other intracellular pathogens too, to maintain their intracellular niche. To quote example of extrapolation of information obtained from work on Salmonella to other pathogens, the characterization of arylamine *N*-acetyltransferases (NATs) in Mycobacterium in inactivating the antitubercular drug isoniazid was done based on the knowledge of NATs in Salmonella.[115] Thus it is plausible to utilize the information obtained from the studies on SCV formation and integrity for studying intracellular life of other intravacuolar pathogens, hence fulfilling the purpose of Salmonella being a model pathogen.

Challenges

Although Salmonella represents the group of well-studied intracellular pathogens, certain challenges do exist in the research dealing with pathogenesis of Salmonella. The non-availability of an animal model for *S.* Typhi presents the best example. On the other hand, there is no mouse epithelial cell-line available for studying intracellular life of *S.* Typhimurium within epithelial cells. Hence extrapolation of results of *S.* Typhimurium to *S.* Typhi is not possible in all cases. Moreover, the ability of Salmonella to survive in various extreme conditions within cells like macrophages, neutrophils and dendritic cells with the help of numerous virulence proteins generates a complex network of functioning of all these effector proteins. As a result, deciphering the underlying mechanisms becomes quite challenging. Further, complete understanding of the mechanism of evasion of lysosomal fusion to SCV remains as one of the biggest challenges, pointing at the requirement of better detection of intracellular Salmonella by means of appropriate markers as well as better imaging techniques. The development of such tools would certainly lead us to the answers of many questions related to Salmonella pathogenesis.

Conclusion

Salmonella displays most elegant mechanisms of manipulation of the host. The diversity in the modes of evasion of Salmonella from host immune system gives an overall view of major strategies followed by most of the intracellular pathogens makes it a model pathogen. Difference in pathogenesis of different serovars, Typhi and Typhimurium, demonstrate the complicated lifestyle of a pathogen that can be tuned according to the type of host. Adaptation of such variable lifestyles is attributable to acquisition of numerous virulence associated genes over millions of years. The orchestrated action of these virulence proteins result in two major modes of Salmonella infection, local gastroenteritis or systemic typhoid. The latter imparts minimal damage to host providing optimal conditions for survival of pathogen. Recent contradictions of many established facts, such as spatiotemporal overlapping expression of SPI1 and SPI2, survival within hostile environment of neutrophils and dendritic cell mediated dissemination, describe the challenges lying in future of Salmonella related studies. Apart from being a successful pathogen, Salmonella has served to be a useful system for various therapeutic and biotechnological applications. This further paves the path for extensive research to dissect the unknown aspects of Salmonella infection.

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