Immunological Reviews

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Cellular aspects of immunity to intracellular *Salmonella enterica*

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Acknowledgements
This work was supported by grants of the Deutsche Forschungsgemeinschaft to J. J. and M. H. We thank Roopa Rajashekar, Cédric Cheminay, and Serkan Halici for cartoons and micrographs displayed in the figures. The authors declare no conflicts of interest.

Immunological Reviews 2011
Vol. 240: 185–195

Immunological Reviews
0105-2896

Summary: *Salmonella enterica* is a frequent gastrointestinal pathogen with ability to cause diseases ranging from local gastrointestinal inflammation and diarrhea to life-threatening typhoid fever. *Salmonella* is an invasive, facultative intracellular pathogen that infects various cell types of the host and can survive and proliferate in different populations of immune cells. During pathogenesis, *Salmonella* is confronted with various lines of immune defense. To successfully colonize host organisms, the pathogen deploys a set of sophisticated mechanisms of immune evasion and direct manipulation of immune cell functions. In addition to resistance against innate immune mechanisms, including the ability to avoid killing by macrophages and dendritic cells (DCs), *Salmonella* interferes with antigen presentation by DCs and the formation of an efficient adaptive immune response. In this review, we describe the current understanding of *Salmonella* virulence factors during intracellular life and focus on the recent advances in the understanding of interference of intracellular *Salmonella* with cellular functions of immune cells.

Keywords: *intracellular pathogen, innate immune response, Salmonella-containing vacuole*

Introduction

Salmonella enterica is a frequent agent of food-borne infections with the ability to cause a spectrum of diseases ranging from self-limiting gastroenteritis to the life-threatening systemic disease, typhoid fever (1). The disease outcome is mainly dependent on the serotype of *S. enterica* encountered. *S. enterica* serovar Typhi and, to a lesser extent, *S. enterica* serovar Paratyphi cause systemic infections that are major health issues in developing countries and among human immunodeficiency virus (HIV)-infected individuals. Gastrointestinal infections by *Salmonella* are a global problem and primarily caused by serovars such as Enteritidis and Typhimurium. The various serovars of *S. enterica* also show remarkable differences in their host range and specificity, with serovar Typhi being specific for humans and primates and serovars Enteritidis or Typhimurium infecting humans, livestock animals, and various wild animals. Further *Salmonella* serovars are specific to defined animal hosts and cause persistent infections, for example in swine or chickens. *Salmonella* infections can also result in a carrier state

with patients shedding *Salmonella* with feces for prolonged periods of time after asymptomatic encounter or acute disease.

The study of *Salmonella* virulence factors contributing to systemic disease and of host immunity against *Salmonella* is mainly based on experimental systems using *S. enterica* serovar Typhimurium for infection of mice. In susceptible mouse strains, this serovar induces a systemic disease with characteristics of human typhoid fever. Only recently, a novel model has been reported that allows analyses of the pathogenesis of *S. enterica* serovar Typhi in a humanized non-obese diabetic (NOD) severe combined immunodeficient (SCID) mouse model (2).

The molecular analysis of the gastroenteritis by non-typhoidal *Salmonella* serovars has been hampered by the lack of suitable small animal models. However, reduction of the intestinal commensal flora by streptomycin pretreatment renders mice susceptible to *Salmonella* infection. Several hallmarks of human salmonellosis can be observed in this model (reviewed in 3).

Salmonella as a facultative intracellular pathogen

Salmonella can proliferate in epithelial cells and non-activated macrophages. The bacteria are thought to primarily replicate in macrophages, as they are found in the lymphatic tissues and organs during systemic infection. Mutant strains of *Salmonella* defective in macrophage replication are avirulent in murine models of infection, which underscores the importance of bacterial survival and replication in macrophages for disease outcome (1). The survival in activated macrophages and persistence in dendritic cells have been observed, while the situation in fibroblasts appears to be more diverse – here, a restriction of replication was seen (4).

The current models of intracellular replication of *Salmonella* are mainly based on observations in cell culture models. However, recent analyses of the growth dynamics of *Salmonella* during systemic pathogenesis indicate that intracellular replication may be much more restricted *in vivo* (reviewed in 5).

A model for the different interactions of *Salmonella* with host cells during systemic infection is shown in Fig. 1. The pathogenesis of diseases by *Salmonella* depends on the coordinated function of various sets of virulence proteins encoded by genes clusters on the virulence plasmid or by specific chromosomal loci, referred to as *Salmonella* pathogenicity islands (SPI). During the course of evolution, *Salmonella* obtained numerous pathogenicity islands from related species by repeated events of horizontal gene transfer (6). While SPI1 is required for the invasion of non-phagocytic host cells and elicitation of diarrheal disease, SPI2 is essential for the intracellular survival and

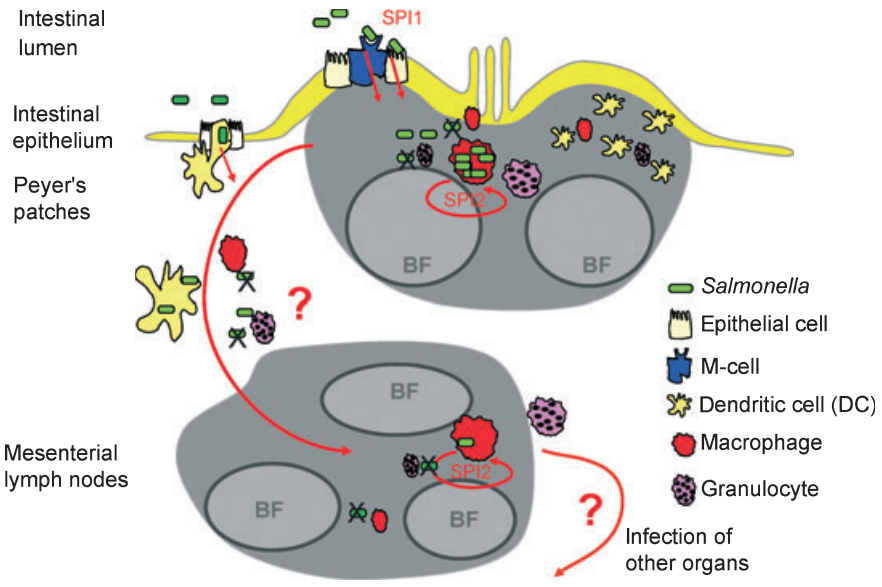

Fig. 1. Routes of infection by *Salmonella enterica*, host barriers, and immune defense mechanisms in various tissues. After oral uptake, *Salmonella* can enter the host by SPI1-T3SS-mediated invasion of non-phagocytic cells, by uptake via M cells, or by phagocytosis by dendritic cells (DCs) that sample intestinal luminal content. Within Peyer's patches, the bacteria were predominantly found within host cells and extracellular bacteria are controlled by granulocytes and macrophages. The subsequent spread from the intestinal lymphatic tissue to other lymphatic organs might involve the transport by immune cells. DCs that internalize but fail to kill *Salmonella* are considered as 'Trojan horses' for the systemic spread of the pathogen. Within other lymphatic tissues such as mesenteric lymph nodes, *Salmonella* is found intracellular and the survival and replication inside host cells is dependent on the function of SPI2. The mechanisms and potential cellular vehicles for the spread to other organs of the infected host are not fully understood and need further investigation. BF, B-cell folicles.

replication of the bacteria. *Salmonella* is able to infect a variety of host cells and can invade non-phagocytic cells (reviewed in 7). Entry into host cells can either occur via bacteria-mediated invasion or by phagocytosis. Invasion is mediated by effector proteins of the SPI1-encoded type III secretion system (SPI1-T3SS). The effector proteins are toxin-like virulence factors that induce the reorganization of the host cell actin cytoskeleton, leading to macropinocytosis. The translocation of SPI1-T3SS effectors is also linked to the inflammatory response of the epithelium and the triggering of apoptosis of host cells. Following SPI1-induced macropinocytosis, a few SPI1-T3SS effectors, such as SipA, SopB, SopD, and SopE2, persist within the cell and have recently been implicated in contributing to the intracellular stages of the infection process (8–10).

The ways of entry into the cell probably affects the initial phase of maturation of the compartment containing the bacterium. However, *Salmonella* internalized by either invasion or phagocytosis induces the formation of a specialized pathogen-inhabited compartment.

The *Salmonella*-containing vacuole: the intracellular habitat of *Salmonella*

Intracellular *Salmonella* are able to actively modify the biogenesis of a novel membrane-bound compartment in which the bacteria can survive and efficiently replicate. In the following section, we describe the features of this compartment, termed '*Salmonella*-containing vacuole' (SCV). Whether *Salmonella* has specific mechanisms to egress infected cells or, more specifically, for egress from the SCV has only been partially studied (11).

Although the SCV has some features in common with late endosomes, such as the presence of lysosomal glycoproteins and the acidic luminal pH, other properties are unique and may be the result of a sophisticated manipulation of normal host cell functions. Within the SCV, the bacteria can persist intracellular for hours to days, making it a unique compartment with respect to the normal progression of phagolysosomal maturation and recycling. Though there has been some controversy within the field, several reports have shown that *Salmonella* can survive within macrophages in which the lysosomal compartments have fused with the SCV (12, 13). Consequently, the avoidance of phagolysosomal fusion is unlikely to be a major pathogenic strategy of *Salmonella*. Studies in various cell types also demonstrated that the vacuole acidifies; however, depending on the mechanism of host cell entry, vacuolar acidification may be delayed in both macrophages and epithelial cells (14, 15). The SCV interacts transiently with the early endocytic pathway and quickly recruits and loses early endocytic markers, such as EEA1 (early endosomal antigen 1), TfR (transferrin receptor), and the early endocytic trafficking guanosine triphosphatases (GTPases) Rab5 and Rab11. Several late endosomal markers are commonly associated with the SCV at later time points, including the GTPase Rab7, LAMP1 (lysosomal associated membrane protein 1), LAMP2, LAMP3, and the vacuolar adenosine triphosphatase (ATPase) (16, 17). Other markers such as M6PR (mannose 6-phosphate receptor), LBPA (*lyso*-bisphosphatidic acid), and the lysosomal hydrolase cathepsin D appear transiently associated with the SCV. Furthermore, cholesterol has been reported to accumulate in the membrane of the SCV (18). The ability of *Salmonella* to survive exposure to lysosomal contents is mediated by its resistance to anti-microbial peptides, nitric oxide, and oxidative killing – important features for its survival within macrophages and to virulence (19, 20). This is supported by the observation that *Salmonella* spp. mutants that are sensitive to these compounds are attenuated in virulence in the murine model, whereas knockout mice deficient for the production of these compounds have increased susceptibility to *Salmonella* spp. (19, 20).

The virulence gene clusters for intracellular life

For establishing a successful life within the SCV, *Salmonella* deploys a second T3SS, encoded by SPI2, and injects at least 21 effector proteins across the phagosomal membrane into host cell cytoplasm (reviewed in 7, 21). Briefly, these proteins, together with the target host proteins, alter the host endosomal trafficking and contribute to bacterial replication. Furthermore, the secreted effector proteins also play an important role in excluding or evading the effects of anti-microbial compounds such as reactive oxygen and nitrogen intermediates (ROI and RNI, respectively). After juxtanuclear positioning of the SCV by the balanced activities of the kinesin and dynein motor proteins and a lag phase of about 3–4 h, the bacteria start to replicate and at the same time, the SCV are found associated with an extended tubular network. This unique *Salmonella*-induced phenotype is termed '*Salmonella*-induced filaments' or SIF. Though initial microscopic studies suggested that SIF formation results from continuous fusion of endosomal vesicles, the biogenesis of SIF still remains enigmatic.

In addition to SPI2-encoded proteins (SpiC, SseF, and SseG), the SPI2-T3SS also translocates a large number of effector proteins encoded by genes that are located elsewhere on the chromosome (SifA, SifB, SlrP, SopD2, PipB, etc.) (7, 21). SifA is

required for the maintenance of the SCV integrity during intracellular proliferation of *Salmonella*, as well as for the induction of SIF (22, 23). The host proteins interacting with SifA, SKIP and PIKfyve, a PtdIns(5) kinase, that mediates acidification of SCV, are known to be crucial for the SIF formation. Despite numerous studies, the role of majority of the SPI2 effectors is not known, probably because of redundancy of the effectors, and requires further investigation. More recently, SopB, a SPI1-T3SS effector acting as bacterial phosphoinositide phosphatase, was found to manipulate the SCV surface charge resulting in the inhibition of SCV and lysosome fusion (24). In addition to lysosomal glycoproteins and vATPase, like those present on SCV membrane, SIFs are also decorated with several SPI2 effector proteins including SseF, SseG, SifA, SifB, and SopD2. Though the biological role of SIF induction remains to be resolved, their appearance is closely linked to the intracellular survival and replication of *Salmonella*. In contrast to unrestricted growth in epithelial cells, the restricted bacterial replication in activated macrophages, dendritic cells (DCs), and fibroblasts suggests that the fate of bacterial multiplication is cell type-specific. Recently, our group and others have identified that SIF are highly dynamic structures that extend, retract and branch, which in a way are thought to depend on the microtubules and the host cell motor proteins (25, 26). In contrast to earlier belief that SIF are only induced in epithelial cells and fibroblast cell lines (27), we observed SIF formation as well in macrophage cell lines, primary peritoneal macrophages, and bone marrow-derived dendritic cells (BMDCs) (25). Though short tubular endosomes can be frequently observed in living phagocytic cells, the formation of extensive tubular endosomes is dependent on the function of the SPI2-T3SS of intracellular *Salmonella* (Fig. 2). The visualization of tubular endosomes was enabled by stimulation by interferon-gamma (IFNγ) that led to a strongly adherent, flattened morphology of the phagocytic cells. Compared to epithelial cells, the SIF formation is found to be delayed in macrophages, while SIF dynamics is relatively similar in different cell types.

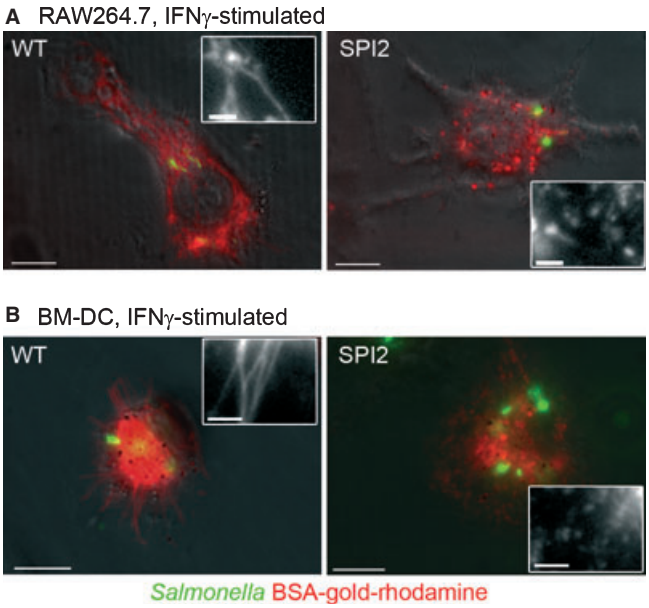

Fig. 2. Modification of the endosomal system in *Salmonella*-infected macrophages and dendritic cells. Infection by *Salmonella enterica* leads to induction of extensively tubular, highly dynamic endosomal compartments. RAW264.7 macrophages (A) or murine bone marrow-derived dendritic cells (B) were stimulated with interferon-gamma (IFNγ). Subsequently, the cells were infected with *Salmonella* wildtype (WT) or SPI2-deficient strains (SPI2) constitutively expressing GFP (green). Infected cells were pulsed with the fluid phase marker BSA-gold-rhodamine (red) to trace endosomal uptake. Living cells were imaged. Further details are described in (25). Scale bars, 10 μm and 2 μm in overview and insert, respectively.

Regulation of virulence factors for intracellular life

High salt and low oxygen conditions, prevailing in the intestinal milieu, activate the expression of *SPI1* genes and enable invasion. Once intracellular, the intra-phagosomal environment, including acidic pH and limiting concentrations of Ca^{2+}, Mg^{2+}, and inorganic phosphate, inhibits the *SPI1* gene expression and activates *SPI2* gene expression (28, 29). The *SPI2* genes encode a second T3SS, used to inject a second set of effector proteins into the host cell cytoplasm, and modify the SCV as a favorable place for bacterial replication (21). Though colonization of intestinal Peyer's patches was not affected, mutants for various *SPI2* genes had reduced systemic spread in mice infections (30). While HilA is the major transcriptional activator of *SPI1* gene expression, most of the *SPI2* genes are subjected to regulation by a two-component regulatory system, SsrAB (29).

Salmonella sense the acidic environment of the SCV, resulting in the induction of various regulatory systems that promote intracellular survival, for example, by surface remodeling of the protein, carbohydrate, and phospholipid components of the bacterial envelope (31). Such regulatory systems include OmpR/EnvZ, PhoP/PhoQ, RpoS/RpoE, PmrA/PmrB, Cya/Cyp, and cyclic diGMP, all of which confer resistance to anti-microbial peptides and oxidative stress (32). The phagosomal environment is acidic, with a pH range of 5.5 to <5, has a concentration of magnesium and calcium in the 1 mM range, and contains anti-microbial peptides ROI and RNI that can damage the bacterial cell (33). Various studies indicate that both pH and anti-microbial peptides are important signatures of the phagosomal environment and such conditions activate many of the regulators that are implicated in *Salmonella* viru-

lence (reviewed in 7). It is likely that several sensory systems respond to the conditions in the phagosome and cooperate to orchestrate the complex cascade of events that are necessary to alter the bacterial surface and promote intracellular survival (32). The best characterized of these is the PhoQ sensor, which promotes resistance to anti-microbial peptides (34). PhoQ contains a novel acidic domain that is bridged to the bacterial inner membrane by interactions with metal ions and binds to and initiates responses to anti-microbial peptides (35). It also responds to pH by structural changes that are determined by regions of the protein separate from the metal ion bridges involved in anti-microbial peptide sensing (36, 37). After phagocytosis, *Salmonella* undergoes extensive bacterial surface remodeling, as has been shown for the lipid A component of lipopolysaccharide (LPS) during growth within macrophages (35). Bacterial molecules that the host can recognize as indicators of infection, such as the SPI1-T3SS and flagellin, are repressed and the LPS structure is altered (38). Some of the specific surface modifications include a decrease in length of the O-antigen, which is the repeating carbohydrate polymer of the LPS, alterations to the number of acyl chains in the structure of the lipid A component of LPS, and changes in the protein content of the outer membrane, the inner membrane, and the peptidoglycan layer. Synthesis of enzymes that allow the bacteria to cope with oxidative and nitrogenous radicals also occurs. Microarray studies have shown that up to 919 *S. enterica* serovar Typhimurium genes are differentially regulated in response to the phagosomal environment, demonstrating that dramatic transcriptional and post-translational changes probably occur when *Salmonella* makes the transition from a nutrient-rich extracellular environment to the intracellular environment (39).

Avoidance of innate immunity by intracellular *Salmonella*

Oxygen and nitrogen radicals and multiple defense mechanisms

There is a great body of evidence that ROI and RNI participate in killing of bacterial species (reviewed in 20, 40). Regarding *Salmonella*, it has been shown that ROI mediate rapid clearance of the pathogen. This may not exclusively be explained by a direct ROI-dependent action on intracellular *Salmonella*, but may also involve an ROI-dependent induction of anti-microbial peptides such as CRAMP (41). Furthermore, an active NADPH-oxidase is linked to early killing of *Salmonella* mediated by the constitutive acid sphingomyelinase in macrophages (42). At later time points after orogastric infection with *Salmonella*, NO modulates the ability to combat *Salmonella* (43–45). Accordingly, application of inhibitors of the inducible nitric oxide synthase (iNOS) increased the bacterial burden in macrophages and consequently in liver and spleen of infected mice (46–48). Furthermore, after infection with an avirulent strain of *S. enterica* serovar Typhimurium iNOS-deficient mice succumbed while wildtype (WT) mice survived (49). Chakravortty *et al.* (50) demonstrated that SPI2 is able to prevent nitrite accumulation at the SCV in non-activated macrophages, thereby enabling *Salmonella* to grow within macrophages. This observation is mirrored by the fact that SPI2 is able to prevent ROI production in the vicinity of the SCV (51). However in case, macrophages are activated with IFN-γ, SPI2 as well as PhoPQ activity is impeded by high order RNI resulting in SCV maturation and bacterial containment (52, 53). Interestingly, upon NO challenge, *Salmonella* induce NO detoxifying pathways (54–56). However, after macrophage activation with IFN-γ, the resulting high amounts of RNI cannot be sufficiently detoxified by *Salmonella* (57).

Iron limitation as defense against *Salmonella*

Production of ROI may be influenced by subcellular availability and distribution of iron, as ferrous iron is necessary to reduce hydrogen peroxide by the Haber–Weiss and Fenton reaction (58). However, there are several lines of evidence strongly suggesting that *Salmonella*-infected macrophages restrict their intracellular iron pool. Slc11A1 [formerly termed natural resistance-associated macrophage protein 1 (NRAMP1)] has been associated with export of iron from phagosomes. This protein was originally identified as a protein important in innate resistance to intracellular, intra-phagosomal pathogens (59). It has been clear for some years that this protein also plays a role in intracellular iron homeostasis within cells (60). It has been demonstrated that functional NRAMP1 expression increases iNOS expression and activity in macrophages (61). Furthermore, iron chelation as well as functional NRAMP expression is a robust inductor of SPI2 virulence genes (62). However, the host's efforts to restrict iron abundance in macrophages prevail in case NRAMP is functional or the infected macrophages are stimulated with IFN-γ. Nairz *et al.* (63–65) demonstrate that *Salmonella*-infected NRAMP1 positive or IFN-γ activated macrophages reduce their intracellular iron pool by increasing the iron efflux and reducing the uptake of iron and thereby contribute to the containment of intracellular *Salmonella*.

While establishing the infection in the gut, *Salmonella* outcompetes the host's efforts to restrict iron. Under steady-state

conditions, dietary iron represents an abundant nutrient source for the intestinal microbiota and must be rapidly sequestered to keep the commensals at bay. However, during inflammation and intestinal tissue damage, host-derived iron-containing molecules are released and accumulate in an uncontrolled fashion. To scavenge these iron molecules, granulocytes and epithelial cells produce lipocalin-2 (Lcn-2). Lcn-2 binds and inactivates enterobactin-type siderophores (66). Recent observations indicate that *Salmonella* by virtue of Salmochelin expressed by the enterochelin *iroBCDEiroN* cluster (67–69) are Lcn-2-resistant (70). The expression of a stealth siderophore by *Salmonella* may explain why granulocytes, despite being a prominent source of Lcn-2, do not interfere with the establishment of a systemic disease (71).

Salmonella interference with the establishment of an adaptive immune response

Interference of intracellular *Salmonella* with DC immunobiology

Salmonella penetrates the epithelial lining by preferentially invading M cells (72–74) and by hijacking $CD18^+$ cells and DCs (75, 76). Interestingly, the route of entry decides whether a robust IgA response develops, because only invasion via M cells results in fecal release of IgA (78). After entering the host via M cells, subepithelial DCs are the first cells engulfing the invading pathogen, finally resulting in accumulation of *Salmonella* in large numbers in Peyer's patches (77). These observations suggest that *Salmonella* must be prepared for the specific requirements of the intracellular life of different DC subsets to establish a systemic disease.

In contrast to macrophages, *Salmonella* are not replicating within DCs (79, 80) but rather form a static, non-dividing population (81) whose fate is independent of Slc11A1 (82). Furthermore, SPI2 as well as virulence factors encoded on SPI1, SPI3, SPI4, and SPI5 are dispensable for survival in DCs (80, 81). Neither bacterial nor host cell *de novo* biosynthesis affect the persistence of *Salmonella* in DCs, but the *Salmonella* O-antigen of the LPS determines the intracellular fate of *Salmonella* in DCs (83). Within DCs, *Salmonella* change their gene transcription profile. It has been demonstrated that *Salmonella* dramatically reduce FliC expression (84), thereby rendering the rapid induction of FliC-specific T cells by subepithelial *lamina propria* DCs (LP DCs) inefficient in protecting the host (85). Likewise, the induction of T cells specific for a SPI1 effector SipC may not be helpful in protecting the host (86), because within host cells, *SPI1* gene expression is downregulated. In contrast to FliC and SPI1, SPI2 is induced by *Salmonella* within DCs, and SPI2-encoded virulence proteins are translocated into the cytoplasm of DC (81, 87), indicating that SPI2 exerts functions beyond intracellular replication and that SPI2-T3SS effector protein are targeting specific functions of DCs such as migration and antigen presentation.

Recent evidence indicates that the *Salmonella* SPI2-T3SS effector SseI interferes with DC migration. After intraperitoneal injection of BM DCs harboring *Salmonella* into mice, migration of infected cells was delayed in an SseI-dependent manner (88). However, after orogastric infection, it has been shown that $CD103^+$ CX_3CR1^- subset of LP DCs are capable to transport *Salmonella* to the MLNs (89) and may thereby support spread of *Salmonella* to distant sites, promoting development of systemic disease. The fact that *Salmonella* do not use CX_3CR1^+ LP DCs is surprising, as CX_3CR1-expression is required for allowing DCs to cross with their protrusions the epithelial lining of the gut (90). These CX_3CR1^+ LP DCs may undergo *Salmonella*-induced pyroptosis during host invasion, because it has been shown that PrgJ, a molecule of the SPI1-T3SS needle, is detected by the NLRC4 inflammasome (91) and that DCs are rapidly killed in a SPI1-dependent manner (92). Alternatively, the migration of CX_3CR1^+ LP DCs may be inhibited exclusively by *Salmonella*, while the $CD103^+$ CX_3CR1^- LP DCs migratory capacity is not affected by *Salmonella*. It is intriguing to speculate about the overall consequences of this targeting of LP DC subsets by *Salmonella*, because $CD103^+$ LP DCs have been associated with Treg cell induction and establishment of tolerance to food antigens (93), while CX_3CR1^+ LP DCs are supporting Th17 development (94) and thereby drive the inflammation of the gut. A hallmark of orogastric infection by *Salmonella* is the absence of inflammation of the gut, despite a developing systemic disease. In addition to the commensal flora being able to suppress inflammatory activation (95), it can be hypothesized that $CD103^+$ CX_3CR1^- LP DCs may transport *Salmonella* to the MLN and induce Tregs, resulting in further suppression of intestinal inflammation after encounter with *Salmonella*.

Apart from modulating DC migration and engagement of DC subsets, there is substantial evidence that intracellular *Salmonella* interfere with antigen presentation. After *Salmonella* infection, $CD4^+$ and $CD8^+$ T-cell-mediated immune responses are slow and inefficient *in vivo* (96–100), yet $CD4^+$ T-cell responses are especially necessary to clear the tissues from bacteria (101, 102). Wick *et al.* (103, 104) first reported that *phoP*, a *Salmonella* virulence regulator, negatively influences the capacity of myeloid cells in antigen presentation. In line with these observations, it was demonstrated that antigen presentation and induction of $CD4^+$ and $CD8^+$ T-cell responses are

impaired through SPI2-T3SS function of intracellular *Salmonella* (105, 106). The inhibition of antigen presentation could be partially overcome by opsonization of *Salmonella* and directing *Salmonella* to lysosomes via FcγRIII-mediated phagocytosis (107, 108), indicating that subcellular targeting of *Salmonella* is a critical process contributing to impaired antigen presentation. In line with this hypothesis, it was demonstrated that SPI2 function affects subcellular trafficking in the endocytic pathway (25, 81, 106). However, whether there is vacuolar escape in DCs dependent on SPI2-T3SS effector SifA remains controversial (87, 109). Apart from modulating the subcellular localization of its own compartment, it has been reported that *Salmonella* is able to ubiquitinate and degrade MHC II molecules in human DCs in a SPI2-dependent manner (110). Correspondingly, MHC molecules that avoid the SCV and MHC class II molecules are not loaded properly (113). However, the intracellular fate of *S.* Typhimurium in murine and human DCs is divergent (111). This may explain why *Salmonella* does not alter the expression of MHC molecules or costimulatory molecule surface expression on murine DCs (104, 105, 112). However, it has been reported that SPI2 functions interfere with the loading of antigenic peptides on MHC class II molecules in murine DCs (87, 105, 106). Interestingly, only a subset of SPI2-T3SS effectors is able to suppress antigen presentation (87), and further studies are mandatory to clarify which host cell factors are affected. The identification of these molecules targeted by *Salmonella* in DCs may provide interesting insights into the cell biology of DCs and bacterial evasion strategies. Representative images of the interaction of *Salmonella* with DCs and a model for the interference of intracellular *Salmonella* with antigen presentation can be found in *Fig.* 3.

Salmonella and B cells

Despite the progress in understanding the interaction of *Salmonella* and DCs, there are only scarce data on the intimate interplay between *Salmonella* and B cells. This is surprising because there is evidence that B cells play an important role in the outcome of primary and secondary *Salmonella* infection (114). Interestingly, passive transfer of *Salmonella* immune serum did not rescue B-cell-deficient mice after *Salmonella* infection, indicating that factors beyond antibody production such as antigen presentation may be involved (43). Rosales-Reyes *et al.* (115) described that *Salmonella* resides in B cells in a late endosomal compartment that is divergent from that in macrophages. Activation with IFN-γ does not result in clearance of *Salmonella* within B cells (115). Furthermore, B-cell precursors may serve as a reservoir of *Salmonella* in the bone marrow (116). Whether *Salmonella* virulence genes modulate B-cell functions has not been investigated in detail and requires further studies.

Direct interference with T-cell functions

Apart from modulating the capacity of antigen-presenting cells, *Salmonella* possesses a second contact-dependent but SPI1- and SPI2-independent mechanism to prevent the development of an adaptive immune response by directly inhibiting the proliferation of naive T cells by downmodulating the TCR expression on T cells (112, 117). This mechanism may be helpful to suppress immune responses in case extracellular *Salmonella* have reached secondary lymphatic organs. However, further studies need to clarify whether this inhibitory contact-dependent mechanism may partially be explained by the capacity of bacterial flagellin to inhibit TCR activation by inducing SOCS-1 signaling (118).

Conclusions and outlook

The recent investigations of the molecular mechanisms of *Salmonella* virulence factors provide an increasingly detailed view on the manipulation of eukaryotic cells function by this pathogen. While there is a comprehensive understanding of the mechanism of invasion of non-phagocytic cells and the factors that contribute to the remodeling of the host cell endosomal system. Also, there is an increasing amount of studies that investigate the interactions of *Salmonella* with the various populations of cells encountered during immune defense against *Salmonella* infections. The cellular and molecular understanding of the interaction of *Salmonella* with primary immune cells has emerged and revealed novel pathogenic interferences. A further challenge will be the observation of the intracellular lifestyle of *Salmonella* in an *in vivo* setting, and such studies obviously require improved methods to detect and visualize the relatively low number of bacteria within circulating immune cells or with solid tissues of a living host.

Future studies have to identify the molecular targets of *Salmonella* virulence factors during intracellular life in immune cells and characterize the molecular mechanisms of interference. This should provide novel insight into the cell biology of DCs and other immune cells. Furthermore, understanding of the intracellular life of *Salmonella* should lead to new approaches to generate improved vaccines against *Salmonella* infections, to use *Salmonella* strains as live carriers for recombinant vaccines, and to develop novel forms of treatment that target the function of specific virulence factors.

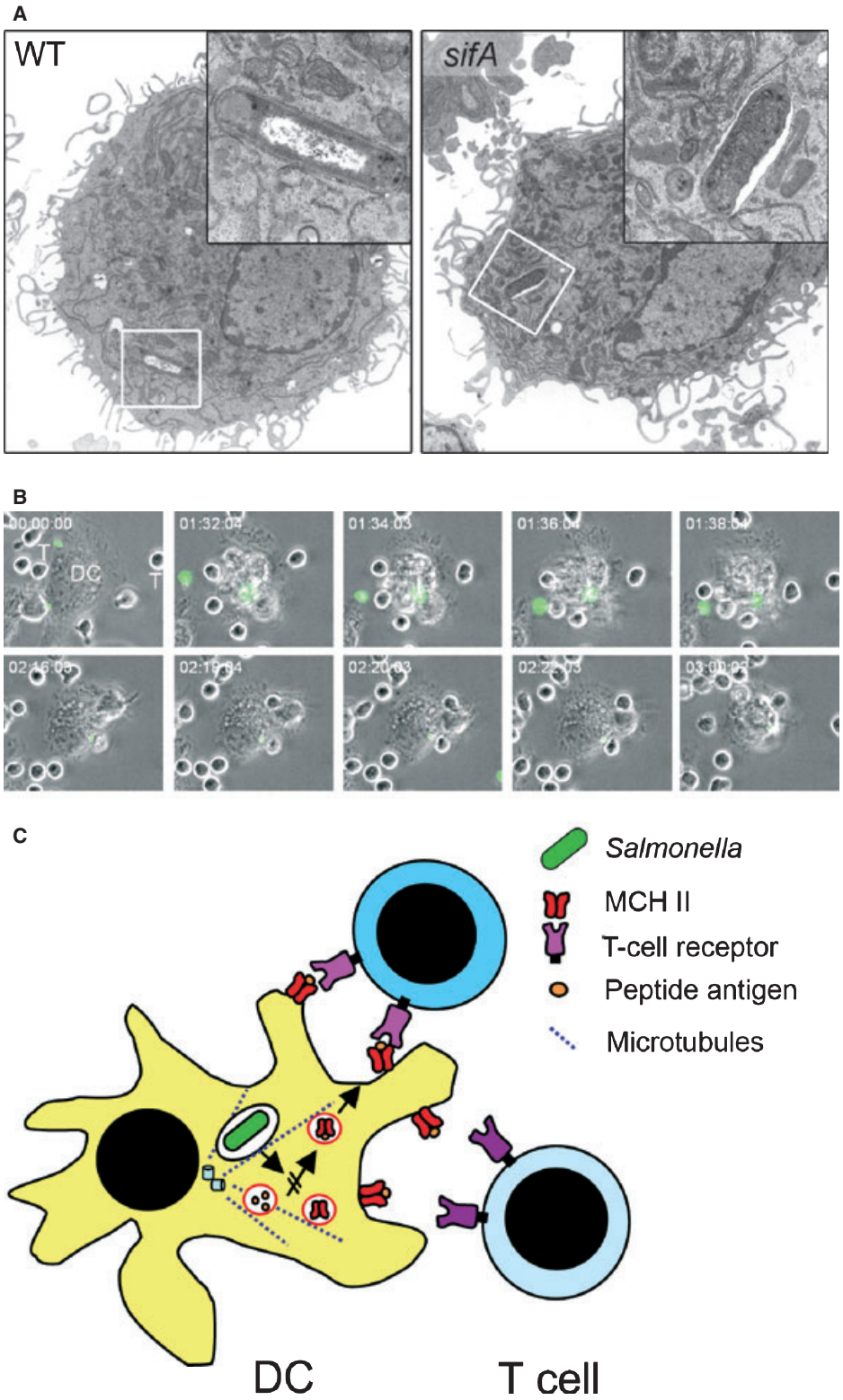

***Fig. 3.* Interactions of intracellular *Salmonella enterica* with dendritic cells.** (A) After phagocytosis by murine bone marrow-derived dendritic cells (BMDCs), *Salmonella* wildtype as well as *sifA*-deficient strains are contained in a membrane-bound compartment. (B) Murine BMDCs (marked DC) were infected with GFP-expressing *Salmonella* (green) and pulsed with the model antigen ovalbumin (OVA). Subsequently, the cells were co-incubated with OVA-specific T cells (marked T), and live cell imaging was performed (time stamp, h:min:sec). Note the high number of DC:T-cell contacts within the period of observation. (C) Model for the interference of intracellular *Salmonella* with the presentation of antigens by DCs (adapted from 87).The activities of intracellular *Salmonella* in DCs interfere with the loading of antigens onto MHC class II complexes. The underlying mechanism has to be revealed, and the SPI2-T3SS-dependent interference with vesicular transport is a candidate molecular mechanism of this phenotype.

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