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Salmonella interactions with host cells: *in vitro* to *in vivo*

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Salmonellosis (diseases caused by *Salmonella* species) have several clinical manifestations, ranging from gastroenteritis (food poisoning) to typhoid (enteric) fever and bacteraemia. *Salmonella* species (especially *Salmonella typhimurium*) also represent organisms that can be readily used to investigate the complex interplay that occurs between a pathogen and its host, both *in vitro* and *in vivo*. The ease with which *S. typhimurium* can be cultivated and genetically manipulated, in combination with the availability of tissue culture models and animal models, has made *S. typhimurium* a desirable organism for such studies. In this review, we focus on *Salmonella* interactions with its host cells, both in tissue culture (*in vitro*) and in relevant animal models (*in vivo*), and compare results obtained using these different models. The recent advent of sophisticated imaging and molecular genetic tools has facilitated studying the events that occur in disease, thereby confirming tissue culture results, yet identifying new questions that need to be addressed in relevant disease settings.

Keywords: pathogenesis; typhoid fever; gastroenteritis; type III secretion

1. *SALMONELLA TYPHIMURIUM*: A MODEL SYSTEM FOR THE STUDY OF INVASIVE PATHOGENS THAT SURVIVE WITHIN A VACUOLAR COMPARTMENT

The pathogenic strategy of *Salmonella* species includes penetration of the mucosal barrier and interaction with cells of the immune system where it functions as an intracellular pathogen. As such, the virulence mechanisms used by these pathogens are necessarily complex, interfacing with diverse host cell types ranging from epitheliod to macrophages, interacting with both their surface and intracellular compartments. Indeed, it is estimated that 4% of the *S. typhimurium* chromosome (about 200 genes) are virulence factors (Bowe *et al*. 1998). These factors include, to date, five pathogenicity islands, numerous smaller pathogenicity 'islets', other virulence factors on the chromosome and at least one virulence plasmid (Groisman & Ochman 1997; Salama & Falkow 1999).

Salmonella species infect a broad range of animals and can cause different diseases in different hosts. For example *Salmonella typhi* causes typhoid fever in humans, an invasive disease that can be fatal. In contrast, *S. typhimurium* usually causes a self-limiting gastroenteritis in humans but induces a systemic disease in mice that is similar to typhoid fever. As such, the study of *S. typhimurium* in mice has provided a valuable animal model for the study of these clinically relevant invasive pathogens that survive within a vacuolar compartment in host cells. However, an often overlooked concept is that this is not a diarrhoea model, but instead a systemic disease model, indicating that different virulence factors may participate in these different diseases. For example, SigD/SopB is not a virulence factor in *S. typhimurium*-infected mice, yet is essential for fluid accumulation in bovine ileal loops infected with *S. dublin* (Galyov *et al*. 1997; Wood *et al*. 1998).

In vitro models of *S. typhimurium* have allowed genetic, cell biological and biochemical analysis of the infection process. By using cultured mammalian cells, investigators have learned much about *Salmonella* interactions with epithelial and macrophage lines. Although these models are consistent, reliable, and relatively easy to use, it is becoming apparent that they also have their limitations and problems. For example, because these cells are studied in isolation from the other cells and factors with which they usually interact (such as cells of the immune system or cytokines), defining the role of the host immune response and other cell responses is not feasible. Cultured cells are usually immortalized, and normal disease processes such as apoptosis in cultured cells are thus affected. Some cell types, such as M cells, which appear to play a major role in *Salmonella* penetration across the intestinal barrier, are difficult if not impossible to grow in tissue culture. Finally, mammalian cells often lose important characteristics upon culturing, such as lack of polarity for most epithelial lines, and lack of an oxidative burst for macrophage lines. However, despite these limitations, these cells have provided enormous amounts of information about how *Salmonella* interacts with them, much of it at the molecular level. A major challenge for the future is to confirm that events studied in tissue culture actually occur during disease. At least for invasion, the events seen with cultured cells look remarkably similar to those described in animal infection models (Jones *et al*. 1994; Takeuchi 1967).

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2. INVASION OF EPITHELIAL CELLS

Studies of *S. typhimurium* infection in mice suggest that M cells are the preferred target of this pathogen, with invasion of these cells occurring at early times (15 min) following inoculation (Jones & Falkow 1996). Binding to M cells is thought to be mediated by the *lpf* fimbrial operon, though the host receptor for this adhesin is unknown (Baumler *et al*. 1996). However, several other adhesins have been described for *S. typhimurium*, and their functions may be redundant. Interestingly, recent evidence suggests that the host receptor for *S. typhi* may be the cystic fibrosis transmembrane conductance regulator, a chloride channel that is non-functional in patients with cystic fibrosis (Pier *et al*. 1998). However, it does not appear to be a receptor for *S. typhimurium*, despite the striking similarities in invasion loci and mechanism (Mills & Finlay 1994). Invasion of M cells rapidly leads to cellular destruction and the dissemination of *S. typhimurium* to deeper tissues. M cell destruction by *S. typhimurium* may be similar to perforation of the intestine witnessed in typhoid patients infected with *S. typhi* (Jones & Falkow 1996). Although *S. typhimurium* triggers apoptosis in cultured macrophage cells (Chen *et al*. 1996; Monack *et al*. 1996), M cell death appears morphologically to be cytoxic or necrotic in nature.

S. typhimurium and other *Salmonella* species can also invade columnar absorptive cells at their apical surface. Since M cell models remain difficult to study *in vitro*, studies of *Salmonella* invasion have largely been performed with cell lines that resemble absorptive enterocytes. The use of polarized epithelial cell lines such as Caco-2 and T84 have facilitated the study of invasion, although the events appear to be the same in polarized and non-polarized cells. However, polarized cells have also been used to study epithelial monolayer penetration, which cannot be examined in non-polarized cells. Additionally, by adding inflammatory cells such as polymorphonuclear leucocytes (PMNs) to the basolateral surface of polarized cell monolayers, transepithelial PMN migration and other events can be reconstructed *in vitro* (Gewirtz *et al*. 1999; McCormick *et al*. 1993, 1995, 1998). As depicted in figure 1, invasion involves denuding of the microvilli and ruffling of the cell surface in both cultured cells and the small bowel. These cell surface rearrangements lead to uptake of the bacterium in large vacuoles that resemble macropinosomes (figure 2).

In contrast to other pathogenic bacteria that use adhesin–receptor interactions for their uptake (i.e. a zipper mechanism), *Salmonella* species use a type III secretion system to cause host epithelial cell ruffling, which drives their internalization (Collazo & Galan 1997). This secretion system, encoded within *Salmonella* pathogenicity island 1 (SPI-1), delivers a variety of effectors into the host cell that mediate uptake of the pathogen (figure 3). These effectors directly interface with host cell signalling systems, independently of receptor–ligand interactions on the host cell surface. As such, invasion by *Salmonella* species could be considered distinct from receptor-mediated phagocytosis (cell eating) and more likened to 'force feeding' of the host cell (Brumell *et al*. 1999). *S. typhimurium* mutants lacking SPI-1 activity are unable to invade and destroy M cells and are somewhat attenuated for systemic virulence when delivered orally to mice. Interestingly, injection of these mutants into the peritoneum of these animals (thereby bypassing the need for invasion of the intestine) reveals that these pathogens have normal virulence for causing systemic disease (Galan & Curtiss 1989). Thus, SPI-1 appears to be specialized for gastrointestinal events during the infection process, enabling the pathogen to penetrate epithelial barriers both *in vitro* and *in vivo*.

Recent progress has revealed some of the mechanisms by which the translocated effectors of SPI-1 mediate invasion by *S. typhimurium* in cultured epithelial cells. Crucial to invasion are rearrangements of the actin cytoskeleton, which is accomplished by the actions of at least four effectors. SopE acts as a guanine nucleotide exchange factor for Cdc42 and Rac, Rho family GTPases that regulate the actin cytoskeleton (Hardt *et al*. 1998*a*). Interestingly, SopE is not encoded within the SPI-1 yet is translocated by this secretion system into the host cell (Hardt *et al*. 1998*b*). Downstream actions of SopE also include the activation of kinase cascades that lead to the production of pro-inflammatory cytokines. SptP has two functional domains, a tyrosine phosphatase domain and a region with homology to both exoenzyme S from *Pseudomonas aeruginosa* and YopE from *Yersinia* spp. Both domains of SptP initiate cytoskeletal rearrangements in the host cell (Fu & Galan 1998). Recent data indicate that it functions as a GTPase-activating protein, which promotes inactivation of Rho family GTPases (Fu & Galan 1999). This effect indicates that invading *Salmonella* have the ability to downregulate the signals that mediate cytoskeletal rearrangements once internalized, and provides a mechanism to explain previous results, which indicated that the cytoskeleton returns to normal following bacterial uptake (Finlay *et al*. 1991). How the tyrosine phosphatase domain of SptP contributes to actin rearrangements in the host cell remains to be determined.

In addition to modulating regulators of the host cell actin cytoskeleton, effectors of *S. typhimurium* can also modulate actin dynamics in a direct manner. For example, SipA is an actin-binding protein that lowers its critical concentration and inhibits depolymerization of actin filaments, thereby stabilizing actin polymers at the site of *S. typhimurium* interaction with the host cell (Zhou *et al*. 1999*b*). SipA also forms a complex with T-plastin, resulting in an increase in the actin-bundling activity of this protein (Zhou *et al*. 1999*a*). Recent data suggest that SipC can also bind to actin and mediates bundling of actin filaments directly with its N-terminal domain, in the absence of T-plastin. In addition, the C-terminus of SipC was found to mediate nucleation of actin polymers, a novel function for a bacterial effector protein. Interestingly, SipC was found to associate with lipid bilayers, suggesting that these activities may be directed from the host cell membrane upon delivery by the SPI-1-encoded type III secretion system (Hayward & Koronakis 1999).

Together, these *in vitro* studies suggest that the invasion of *S. typhimurium* involves a very complex and highly calibrated set of interactions between its translocated effectors and the host's actin cytoskeleton. Despite the appeal of these mechanisms, whether these events occur *in vivo* has not been established (not even actin has been shown to accumulate under bacteria in relevant disease models!).

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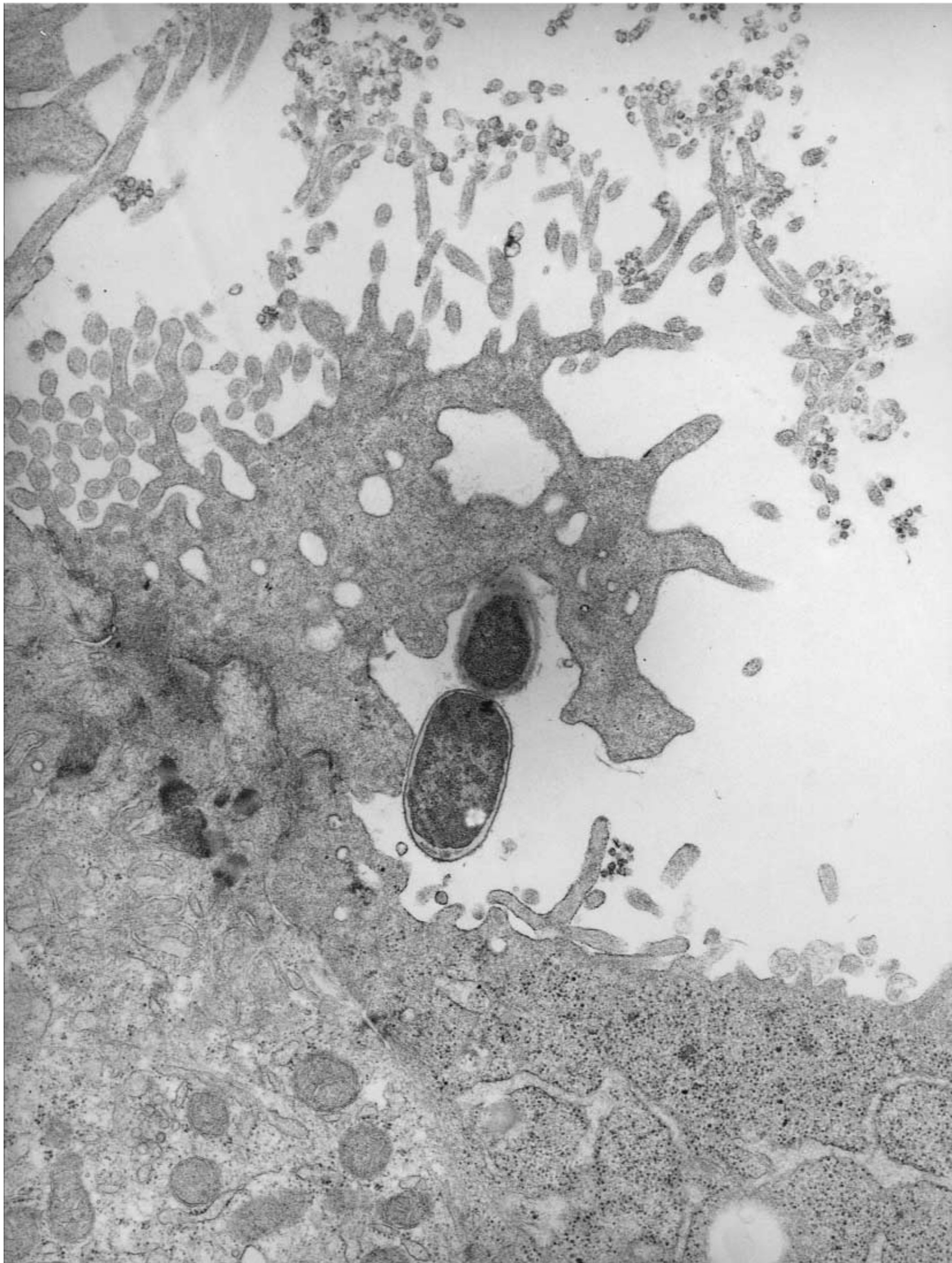

Figure 1. Invasion of epithelial cells *in vitro*. Transmission electron micrograph illustrating cell surface ruffling of Caco-2 epithelial cells induced by *S. typhimurium*. The bacteria induce dramatic ruffling of the host cell surface at the site of contact with the plasma membrane. These ruffles engulf *S. typhimurium* and lead to their internalization.

However, the similarity of morphological changes that occur in intestines upon infection (ruffling, microvilli denuding, cellular protrusions and macropinocytosis) are quite similar to the tissue culture events, suggesting these molecules function in a similar manner during disease.

SopB from *S. dublin* is a phosphatase, capable of acting on polyphosphoinositides and inositol phosphates (Norris *et al*. 1998). Its phosphatase activity in host cells is thought to activate Ca^{2+}-dependent chloride channels and thereby contribute to fluid secretion induced by *S. dublin* in bovine calf ileal loop models of infection. Though delivered by the SPI-1-encoded type III secretion system, SopB is encoded within a separate pathogenicity island, SPI-5, which appears to be required for enteropathogenicity (Wood *et al*. 1998). The SopB homologue in *S. typhimurium*, SigD, was identified as an invasion gene in SPI-1 mutants (Hong & Miller 1998). This suggests that SopB/SigD may also play a role in invasion. Interestingly, mutations in *sigD* have little if any effect on virulence in the murine typhoid model (Wood *et al*. 1998), yet SopB seems to be required for fluid secretion in the diarrhoea model (Galyov *et al*. 1997). However, *sigD* is induced inside host cells (C. Pfeifer, unpublished data), indicating it may play another, perhaps redundant, role in *S. typhimurium* infections.

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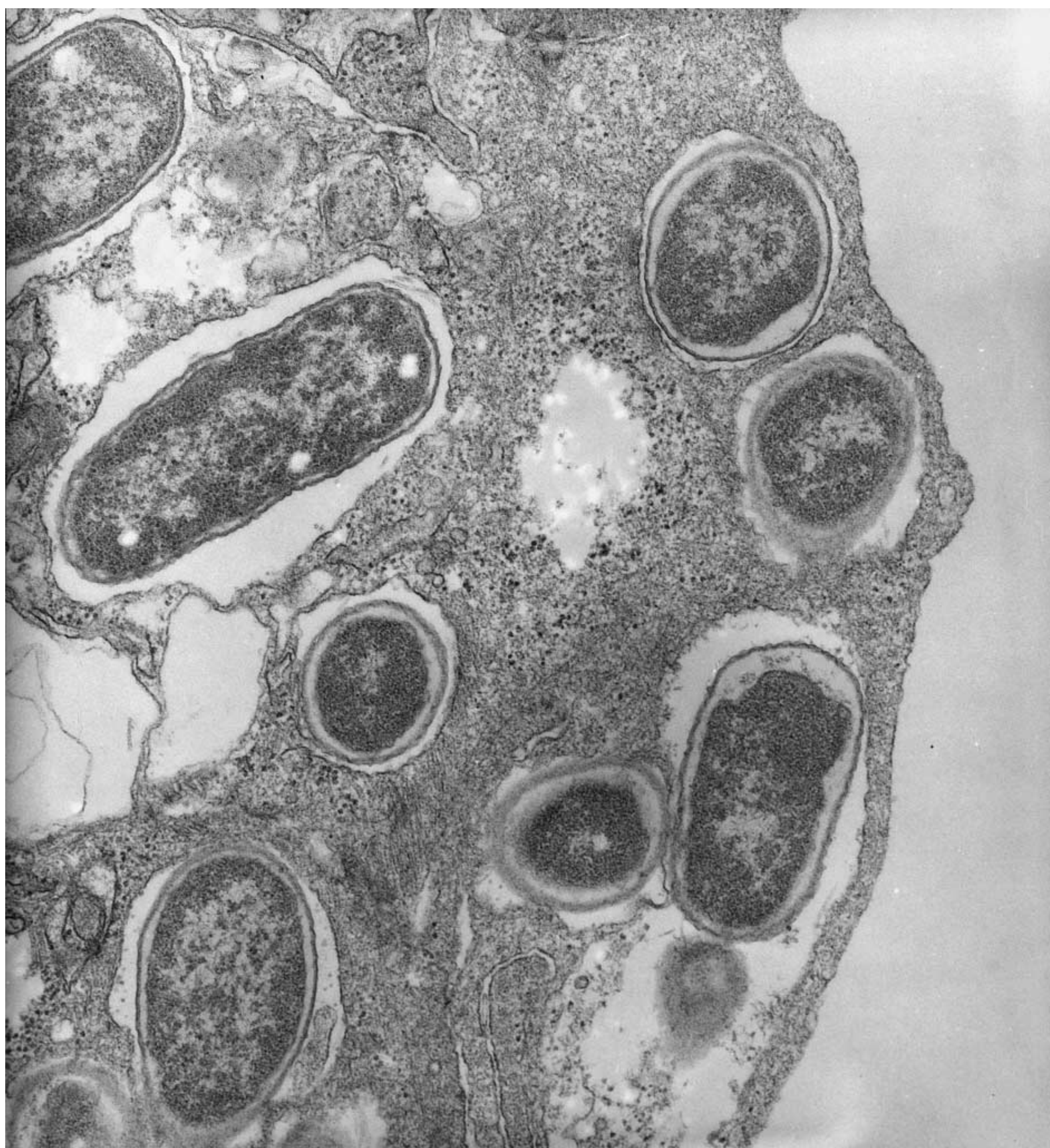

Figure 2. Invasion of epithelial cells *in vitro*. Shortly following invasion, *S. typhimurium* are present in large membrane-bound vacuoles, morphologically similar to macropinosomes. Depicted are *S. typhimurium* 60 min after invasion of Caco-2 epithelial cells *in vitro*.

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Invasion of macrophages by *S. typhimurium* using its SPI-1 secretion system (as opposed to phagocytosis) has been shown to induce rapid apoptosis of the infected cell (Chen *et al*. 1996; Monack *et al*. 1996). Recently, it was shown that SipB, a translocated effector encoded on SPI-1 that also helps to form a pore in the host plasma membrane during secretion, is a key mediator of this effect. SipB binds to and activates caspase-1, a pro-apoptotic protease that converts the precursor form of interleukin-1β to its mature form (Hersh *et al*. 1999). The cytotoxic effect of invasive *S. typhimurium* may be relevant to infection at Peyer's patches, where macrophages are found to underlie target M cells. Apoptosis of infected columnar absorptive cells has also been observed but occurs more slowly and less frequently than that seen in macrophages (Kim *et al*. 1998), prompting the question of why each cell responds differently to invasion by *S. typhimurium*. Interestingly, *S. typhimurium* does not cause apoptosis in cultured epithelial cells. Whether this is a result of using immortalized epithelial cell lines, or because there are fundamental differences between epithelial cells and macrophages that *Salmonella* exploits, remains to be determined.

3. COLONIZATION OF MACROPHAGES BY *S. TYPHIMURIUM* IN A SYSTEMIC MOUSE MODEL OF INFECTION

Following invasion of host cells *in vitro*, *S. typhimurium* is localized within a membrane compartment known as the *Salmonella*-containing vacuole (SCV). These bacteria are

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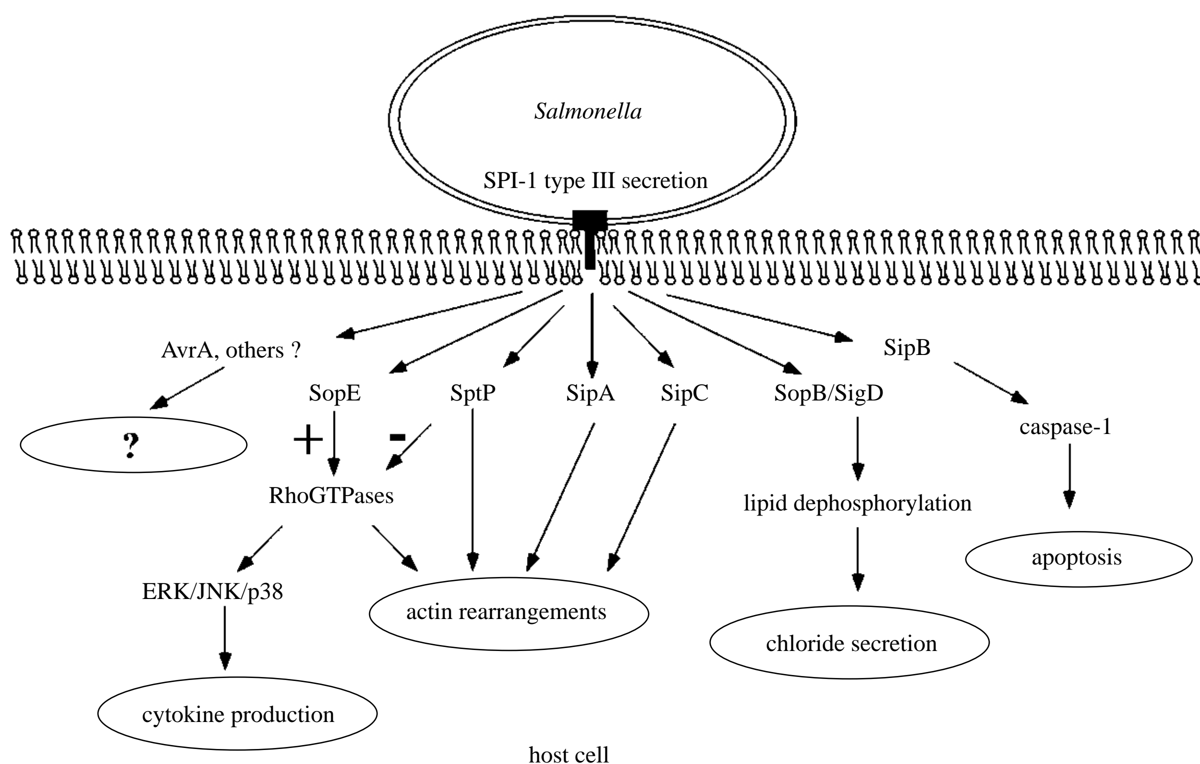

Figure 3. Invasion of epithelial cells requires delivery of effector molecules into the host cell. Depicted are the translocated effectors of the SPI-1-encoded type III secretion system and the host signalling systems with which they directly interact. These effectors have numerous effects on the host cell, including actin rearrangements (for invasion), production of pro-inflammatory cytokines, chloride secretion, and programmed cell death (apoptosis). Artwork modified from Brumell *et al*. (1999).

capable of survival and replication within the SCV, eventually killing the host cell and being released into the extracellular medium to infect other cells. The ability of *S. typhimurium* to survive and replicate within macrophages, which possess an arsenal of antimicrobial defences, indicates that these are the host cells colonized during the systemic phase of disease. Indeed, mutants that are incapable of survival in macrophages *in vitro* are avirulent in the mouse model of infection (Fields *et al*. 1986).

To analyse the localization of *S. typhimurium* in a relevant infection model, a low-dose murine infection was used (Richter-Dahlfors *et al*. 1997). This model entails the injection of approximately 100 colony forming units of wild-type *S. typhimurium* into the tail vein of experimental mice and examination of thick liver sections (30 μm) by confocal microscopy at different times following injection. This dose is similar to the lethal dose. A major advantage of this method is that it precludes the need for an artificially high inoculum at late infection times to visualize bacteria in the organs using normal electron microscopic methods, and allows a much larger area of study than conventional electron microscopy. Shortly after appearance in the liver, *S. typhimurium* were localized at inflammatory foci, where infiltrating neutrophils could be seen. At the later stages of infection, three-dimensional confocal projections were used to conclusively establish that the bacteria reside within (as opposed to on) macrophages. Thus, *S. typhimurium* remains a facultative intracellular pathogen during initial infection of epithelial cells in the gut and throughout the duration of infection of solid organs such as the liver.

Infections were found to induce apoptosis of macrophages within the liver, possibly allowing evasion of host immune cell responses in these tissues (Richter-Dahlfors *et al*. 1997). As noted above, *Salmonella* triggers apoptosis in cultured macrophages using the SPI-1 system, with direct contact being required for this event. The apoptosis observed *in vivo* may represent this process, although apoptosis was also observed in liver macrophages that were not infected with bacteria, perhaps suggesting that a secreted factor (cytokine?) may be involved in this process. By triggering host cell apoptosis, the pathogen may be dampening the immune response to the infection (apoptotic cells do not trigger inflammation). Since apoptotic cells are phagocytosed by other macrophages, the pathogen may also be acquiring a fresh host cell to exploit. Alternatively, the host may be depleting or removing the cellular reservoir (macrophages), thereby limiting pathogen spread as a mechanism to control disease. Experimentally distinguishing the role of these events is near to impossible at present, although transgenic mouse lines that are affected in apoptosis (if viable) may help answer this question.

4. INTRACELLULAR TRAFFICKING OF THE SCV

Intracellular trafficking of the SCV differs from that of other internalized particles and may represent a key mechanism for survival and replication within host cells (see figure 4). Shortly following invasion, interaction with early endosomes is revealed by delivery of early endosome antigen-1 and the transferrin receptor in cultured epithelial

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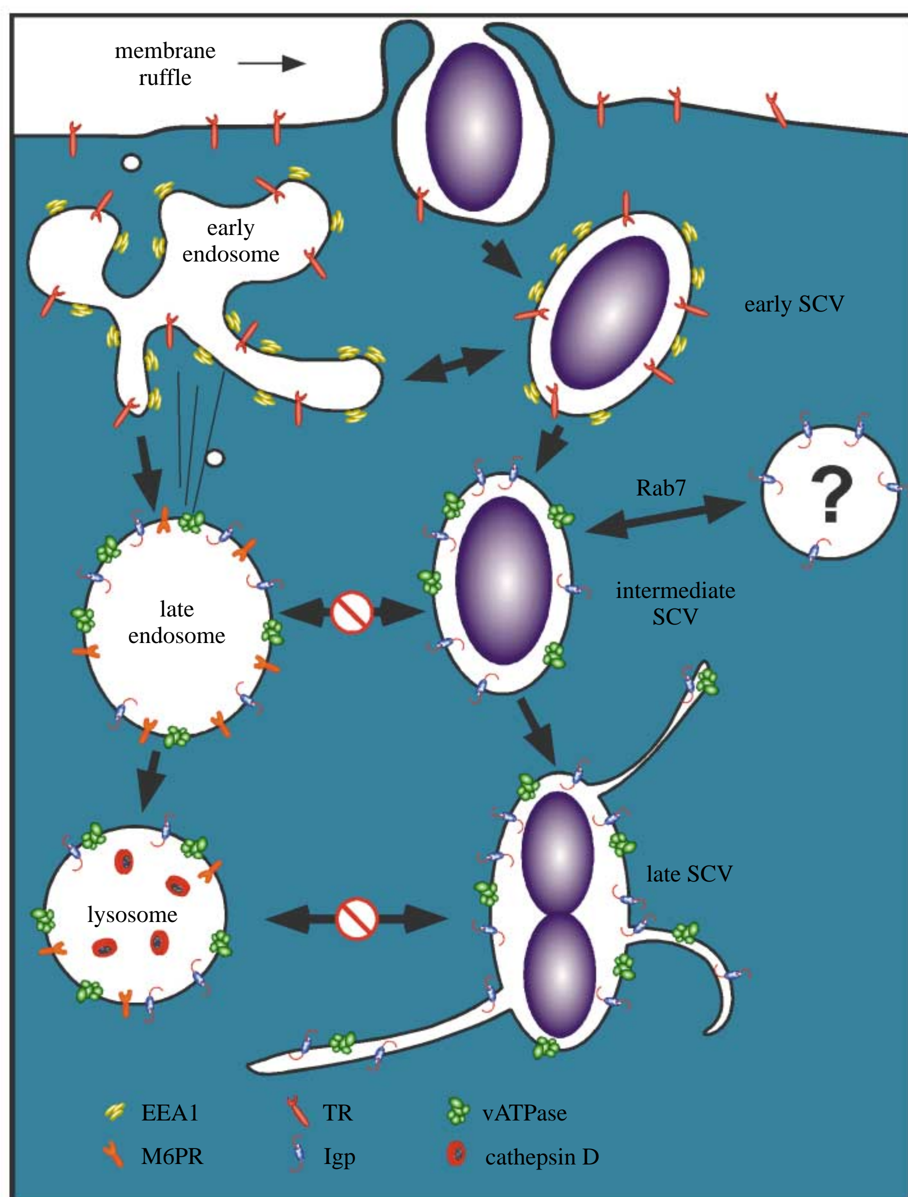

Figure 4. Intracellular trafficking of *S. typhimurium* SCVs in host cells. Upon entry into host cells, *S. typhimurium* remain in an SCV that interacts transiently with early endosomes. However, these vacuoles do not undergo further interactions with the endosomal system and do not fuse with lysosomes. Instead, the SCV appear to interact with an unknown compartment that mediates delivery of Lgp (such as LAMP-1) but not degradative lysosomal enzymes such as cathepsin D (Steele-Mortimer *et al.* 1999). After several hours, intracellular *S. typhimurium* begin to replicate and long, contiguous tubules (Sifs) are formed. Artwork provided by O. Steele-Mortimer and modified from Steele-Mortimer *et al.* (1999).

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cells (Steele-Mortimer *et al.* 1999). Within 30 min, however, SCVs become uncoupled from the endocytic pathway of the host. As such, the SCV do not fuse with lysosomes and avoid exposure to the harsh degradative enzymes that they contain. While not fusing with lysosomes, *S. typhimurium* SCVs do acquire lysosomal glycoproteins (Lgp). Recent findings suggest that Lgp are acquired through interaction with an unknown compartment and that Rab7 mediates this processing of the SCV in cultured epithelial cells (Méresse *et al.* 1999). Uncoupling from the endocytic pathway is witnessed only with live *S. typhimurium* and is similar in both epithelial (Garcia-del Portillo & Finlay 1995) and macrophage cell lines (Mills & Finlay 1998; Rathman *et al.* 1997), suggesting that these pathogens actively use a common mechanism for altering intracellular trafficking. Confirming the intracellular route taken by SCVs as bacteria penetrate the intestine and live within deeper tissues is extremely difficult. We have been able to visualize SCVs in liver macrophages that stain with Lgp (A. Richtor-Dahlfors, unpublished data), but the resolution and background fluorescence present significant difficulties. Furthermore, synchronizing the infection *in vivo* is difficult. Perhaps the best method to confirm the trafficking of the SCV is to follow it in primary cells such as macrophages *in vitro*.

In both cultured epithelial cells and macrophages, invasion is followed by a lag phase of 3–4 h, after which the intracellular bacteria begin replication. At this time, *S. typhimurium* are localized within tubular structures known as Sifs (*Salmonella*-induced filaments). These

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filamentous structures can be visualized in cells by immunostaining for Lgp and appear to require bacterial replication for their formation (Garcia-del Portillo & Finlay 1994). The role of these structures for *S. typhimurium* pathogenesis remains unknown, and current confocal techniques are not of sufficient resolution to see such structures in thick tissue sections, and thus the trafficking and presence of Sifs have not been studied *in vivo*.

5. MECHANISMS FOR SURVIVAL AND REPLICATION OF INTRACELLULAR *S. TYPHIMURIUM*

Many factors have been identified in *S. typhimurium* that are required for survival and replication within host cells and for causing disease (Groisman & Ochman 1997; Salama & Falkow 1999). These include factors which mediate nutrient biosynthesis within the host cell (Hoiseth & Stocker 1981), resistance to reactive intermediates of oxygen and nitrogen in the SCV (Fang *et al*. 1999; Lundberg *et al*. 1999) and DNA repair (Buchmeier *et al*. 1993). Of particular interest for the study of typhoid, is the recent discovery of a type III secretion system within the second pathogenicity island of *S. typhimurium* (SPI-2) (Ochman *et al*. 1996; Shea *et al*. 1996). Many genes encoded within SPI-2 are induced following invasion of host cells and play a critical role in allowing replication of the bacteria within the SCV; type III secretion system apparatus or putative translocated effector mutants are highly attenuated in mouse models of infection (Cirillo *et al*. 1998; Hensel *et al*. 1998). Recent evidence suggests that one putative effector of SPI-2, SpiC, is translocated into the cytosol of infected macrophages and may influence intracellular trafficking of the SCV (Uchiya *et al*. 1999). It is anticipated that the functions of other SPI-2 effectors will be as diverse and complex as those of the SPI-1 effectors. Interestingly, the role of SPI-2 appears limited to systemic phases of disease, since mutants of this type III secretion system had no effect in a diarrhoea model in rabbits (Everest *et al*. 1999).

6. *SALMONELLA* AND THE IMMUNE SYSTEM

The interactions of *Salmonella* species with the immune system are numerous and complex. The use of simple tissue culture cells have yielded a significant amount of (often conflicting) data about cytokine induction, etc. However, by using these cells in isolation, the true nature of bacterial effects on the immune system cannot be easily studied *in vitro*. Some investigators have had significant success by combining a polarized epithelial monolayer with PMNs to study chemotaxis and other events. However, defining the complex events that occur in the immune system following *Salmonella* infection will require more sophisticated technology, such as cytokine arrays, etc. (see §7). It is doubtful that an *in vitro* 'immune response' system can be used to model the complex events of the immune response, and relevant disease models will need to be used to understand further such complex interactions. As discussed below (§7), the choice of the host model is critical for these studies. For example, the standard mouse model for *Salmonella* infections uses a susceptible host, for which the cytokine response differs greatly from that of a resistant host (Lalmanach & Lantier 1999).

7. THE FUTURE

The biggest challenge to this field in the immediate future is to establish which events, identified using tissue culture models, actually occur *in vivo*, and more importantly, their contribution to disease. Continuing to use techniques such as *in vivo* expression technology (IVET), signature-tagged mutagenesis, and differential fluorescence induction to search for genes that are expressed *in vivo* will certainly increase the number of genes that need to be studied. Tissue culture cells will no doubt be extremely useful in delineating how these molecules function at a molecular level. However, clever experiments will be needed to determine how these molecules actually function *in vivo*, and to define their contribution to disease. The use of transgenic animals will certainly provide additional tools to study these events and confirm their functions. Perhaps even expressing individual bacterial virulence factors in a transgenic setting will enhance these studies.

Another major technological advance that will impact significantly on the study of pathogenesis is genomics (pathogenomics). It is anticipated that we will soon have the entire genomic sequence of *S. typhi* and *S. typhimurium*. This will enable arrays to be constructed to follow the expression of the entire bacterial genome during interactions with host cells. However, such a technique will encounter many technical hurdles. For example, isolating bacterial mRNA from a small number of *Salmonella* in the context of infected tissue or in the intestine (where significant normal flora are present) will be a major challenge. Synchronizing the infection will be difficult, and arrays will only give an average expression profile. As was learned using IVET, just because a particular gene is expressed *in vivo*, it does not necessarily follow that it has a critical role in disease. Perhaps the biggest problem will be the vast amount of information these techniques will generate, and deciding which leads to follow. Transforming this information to the study of individual genes will be an immense task. Given that several dozen virulence factors have been identified in *Salmonella* already, yet we only know the molecular function of a handful, perhaps examining the expression of a 'mini-array' of *Salmonella* virulence factors would be more effective (and technically realistic). Within a few years, the entire genome of mice and humans will also be available. This will enable the host response to a pathogen to be examined at a genomic level. Arrays already exist containing several hundred host genes of various gene families. Again, determining the interplay of individual host genes that are induced during infection will require significant effort. However, by using defined *Salmonella* mutants, determining the host response to a particular virulence factor should be possible. Array technology will also miss those factors that are modified post-transcriptionally (such as phosphorylation events) during infection, so alternative techniques will need to be developed to detect these modifications.

As discussed above, the choice of disease model is critical to extending *in vitro* observations to *in vivo* settings. The most popular model of *Salmonella* infection is the murine typhoid model. However, this model is not a diarrhoeal model, which is the predominant *Salmonella* disease in humans in developed countries, especially as typhoid

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fever decreases due to use of the successful vaccines. Additionally, this model uses a susceptible animal with a defective host resistance gene, NRAMP-1. Ironically, no *Salmonella* virulence factor has ever been identified in a resistant (functional NRAMP-1) murine host! Clearly this is an issue that needs to be addressed.

Salmonella species show varying degrees of host specificity. For example, *S. typhi* is a human-specific pathogen with no known animal infections. In contrast, *S. typhimurium* causes enteritis in humans and systemic disease in susceptible mice. Other *Salmonella* species are animal specific (e.g. poultry pathogens), yet others cause a similar disease in a wide range of animal hosts. Finally, some animals are carriers of *Salmonella* species and cause no disease symptoms in these hosts. Clearly different virulence factors are at play in these different settings, and elucidating these differences between hosts remains a major challenge. It is obvious that infected tissue culture models will not provide the answers for such a diverse range of host infection strategies, emphasizing that we have only begun to appreciate the complexity and diversity of *Salmonella* pathogenesis.

8. CONCLUSIONS

Salmonella species cause a wide range of effects on different host cells. An immense amount has been learned about how these pathogens interact with isolated cultured host cells. However, the major challenge will be to extend and confirm these findings to relevant infection and disease models, and define the contribution to disease of these events. Only by using this approach will we truly begin to define the important molecular mechanisms of diseases caused by *Salmonella*.

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Discussion

C. W. Keevil (*Centre for Applied Microbiology and Research, Porton Down, Wiltshire, UK*). The mouse model of *S. typhimurium* infection has provided clear benefits in elucidating the role of potential virulence determinants at the molecular level. As you have pointed out, however, the model does not describe gastroenteritis. Recent studies show that *S. typhimurium* mutants which are avirulent in the mouse model continue to colonize cattle. Consequently, can you comment on the continued suitability of the mouse model for studying infection in man?

B. B. Finlay. *S. typhimurium* infection in mice is a relatively good model of typhoid fever. However, it is not a good model for gastroenteritis. Despite this, it has led to some significant advances in virulence determinants and there is a surprising amount of overlap in virulence determinants between those needed to cause gastroenteritis and those needed for systemic typhoid disease. Both mouse and cattle are complementary models, and I believe that studies comparing and contrasting results with both models will elucidate further the true virulence determinants of salmonellosis.

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