PAPER

Host–pathogen interactions: the seduction of molecular cross talk

P Sansonetti

Gut 2002;**50**(Suppl III):iii2–iii8

Bacterial pathogens have evolved two major strategies to colonise the intestinal epithelium. Adherent microorganisms bind to the apical pole of the intestinal epithelium, whereas invasive microorganisms disrupt and invade the epithelium. Recognition of the genetic bases of bacterial pathogenicity and analysis of the molecular cross talks established between pathogens and their mammalian target cells have illuminated this diversity of interactions. We have compared the strategies of enteroinvasive pathogens, with emphasis on bacterial species such as *Shigella*, *Yersinia*, and *Salmonella*, that represent paradigms of interaction. Cross talks leading to alteration of the epithelial cell actin cytoskeleton appear as a recurrent theme during entry and dissemination into epithelial cells. Other cross talks alter the trafficking of cellular vesicles and induce changes in the intracellular compartment in which they reside, thus creating niches favourable to bacterial survival and growth. Finally, a variety of strategies also exist to deal with other components of the epithelial barrier, such as macrophages. Pro-phagocytic, anti-phagocytic, and pro-apoptotic processes appear to be of particular importance.

The intestinal epithelium, in addition to its absorptive and digestive properties, represents an efficient barrier against the commensal flora and pathogens. Exclusion of the pathogens is not only a result of the continuous physical barrier formed by the tightly bound epithelial cells[1]; it also reflects the presence of the apical brush border microvilli and their prolongation by a layer of heavily glycosylated, membrane associated mucins forming the glycocalyx.[2] Other associated factors are also involved in the defence process such as the mucus layer, intestinal peristalsis, and an array of innate antibacterial factors such as lactoferrin, lysozyme, and cryptdins, a family of short hydrophobic antibacterial peptides produced by Paneth cells in intestinal crypts.[3]

In addition, the intestinal mucosa has a function of specific immunological protection that is largely mediated by secretory IgAs. Induction of this function requires sampling of microbial antigens through sites in the epithelium which translocate those antigens or the microbes themselves to the antigen presenting cells associated with the lymphoid follicles that are components of the inductive arm of the mucosal immune system. These sites correspond to the follicle associated epithelium (FAE) characterised by the presence of M cells which are derived from regular villous epithelial cells[4]; they lack microvilli, produce very little glycocalyx, and express high endocytic activity which accounts for active translocation of particulate antigens to the underlying lymphoid tissue. Differentiation of FAE into M cells is a complex process that involves close interaction between epithelial and lymphoid cells.[5]

MECHANISMS OF EPITHELIAL CELL INVASION

Entry of enteroinvasive bacteria into the intestinal epithelial cell is key to a successful invasive process. We will consider it first, in order to review the major signalling processes that an invasive microorganism may elicit to force its way into a non-phagocytic cell. We will then incorporate this essential step into the more global picture of these invasive microorganisms disrupting and invading the intestinal barrier, a process that involves interaction with other cellular components of this barrier, as reviewed above.

There are essentially two major mechanisms of bacterial internalisation.[6] The "zippering" process corresponds to tight envelopment of the bacterial body by the mammalian cell membrane, involving a surface bound bacterial protein binding an adherence molecule of the mammalian cell surface with high affinity—that is, the invasin (Inv) of *Yersinia* binding integrins of the β1 family,[7] or internalin A of *Listeria monocytogenes* binding to E cadherin.[8] The "trigger" process corresponds to bacteria inducing massive cytoskeletal changes in the mammalian cell underneath its site of contact, thereby causing a ruffling process that internalises the bacterial body in a macropinocytic vacuole.[9]

Y pseudotuberculosis is a paradigm of "zippering" entry (fig 1[1]) requiring Inv, an outer membrane protein of 986 aa.[10] Inv interacts with β1 integrins (α3β1, α4β1, α5β1, α6β1, and αVβ1) which are involved in adherence of epithelial cells to the extracellular matrix.[7] The C terminal domain of

See end of article for authors' affiliations

Correspondence to: Dr P Sansonetti, Unité de Pathogénie Microbienne Moléculaire, INSERM U389, Institut Pasteur, 28 Rue du Docteur Roux, 75724, Paris cedex 15, France; psanson@pasteur.fr

Abbreviations: CT, cholera toxin; EHEC, enterohaemorrhagic *E coli*; EPEC, enteropathogenic *E coli*; ETEC, enterotoxigenic *E coli*; FAE, follicle associated epithelium; GAP, GTPase activating protein; GRR, glycine rich repeat; LT, thermolabile; PMN, polymorphonuclear leucocyte; SLT, Shiga like toxin; ST, thermostable; TTSS, type III secretory system

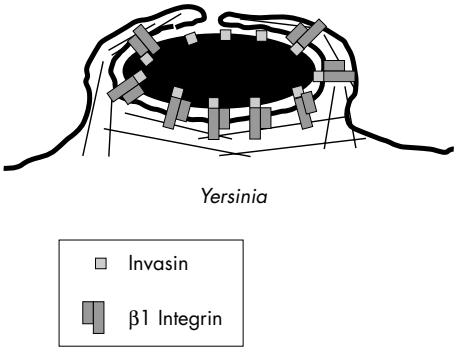

Figure 1 A paradigm of "zippering" entry of a bacterial pathogen into epithelial cells. Invasin mediated binding of *Yersinia pseudotuberculosis* to β1 integrins and internalisation.

Inv ("super domain") interacts with the integrin molecule. In spite of its lack of an RGD motif that is characteristic of the binding site of matrix proteins to integrins, it is a competitive inhibitor of fibronectin binding to β1 integrins. Inv is able to oligomerise and to bind β1 integrins with an affinity constant much higher than fibronectin.[11] These two properties that allow strong engagement of integrins, compared to matrix proteins, may account for the transition between adherence and internalisation. The cytoplasmic domain of β1 integrins transmits signals to the cell cytoskeleton that mediate internalisation. In physiological conditions, it interacts with components of focal complexes, adherence plaques, and p125FAK, the major tyrosine kinase of adherence plaques is involved.[12] The cytoplasmic domain of the integrin α chain is not required for internalisation. Surprisingly, certain alterations of the cytoplasmic domain of the β chain increase internalisation, suggesting that loosening of these interactions may increase receptor motility in the membrane and consequently facilitate internalisation.

Shigella and *Salmonella* (fig 2) are paradigms of "triggering" entry involving a type III secretory system (TTSS). However, they show strinkingly different intracellular behaviours. TTSS have been visualised in both species: Mxi-Spa in *Shigella*[13] and Inv-Spa in *Salmonella*,[14] and have started to be characterised with regard to their protein components in both species.[14–16] These TSSS (fig 3) are composed of a cytoplasmic bulb followed by a disk like structure that spans the inner and outer membranes. A needle like structure crosses the previous domains and extends outside the outer membrane with an average length of 60 microns and internal diameter of 2–3 nm. They are involved in the secretion of a series of bacterial effectors on contact between bacteria and their target cells. The first event is the insertion of two of these effectors in the eukaryotic cell membrane: IpaB and IpaC in the case of *Shigella*, SipB and SipC in the case of *Salmonella*. These proteins form a pore like translocator that accounts for the intracellular transfer of the other effectors.[13 17]

Effectors of *Salmonella* entry into epithelial cells are delivered via the TTSS Inv-Spa (fig 2). Two homologues, SopE1 and SopE2, are exchange factors (GEF) for the small GTPases Cdc42 and Rac.[18] They catalyse the exchange of GDP for GTP on these small GTPases, leading to a cascade of activation signals causing actin polymerisation.[19] Activated, GTP binding, GTPases then interact with proteins of the WASp family that, in turn, bind and activate Arp2/3, a complex of seven proteins that induces actin nucleation.[20] Alternatively, or in a coordinated manner, SipC, an effector protein encoded by SIP1 that gets inserted into the eukaryotic cell membrane, induces direct actin nucleation in vitro.[21] SipA, another product of SPI1, binds and stabilises actin filaments, thereby improving organisation of the entry focus.[22] SopB, an inositol phosphatase, is also transferred through the TTSS, although its exact role in the entry process is unknown.[23] In order to be able to complete its entry process and repair local cytoskeletal alterations, *Salmonella* initiates actin depolymerisation through the translocation of another effector protein, SptP. This protein is a hybrid of a tyrosine phosphatase and a GAP (GTPase activating protein) that down regulates the function of Cdc42 and Rac by stimulating their GTPase activity, thus producing their inactive GDP binding form.[24]

In spite of a certain degree of homology between the *Salmonella* Sip proteins and the Ipa proteins in *Shigella*, their entry mechanisms into epithelial cells show clear differences (fig 2). Recent sequence of the *Shigella* virulence plasmid that is necessary and sufficient to promote entry into epithelial cells has not shown homologues to *sop*E or *spt*P.[25] Three proteins have been shown so far to induce the signals required for entry via cytoskeletal rearrangements that cause the formation of a macropinocytic vacuole. IpaC, which is a component of the pore allowing the translocation of effector proteins, is also involved, through its C terminal domain exposed into the host cell cytoplasm, in triggering actin nucleation/polymerisation.[26] The mechanism by which IpaC operates is still unknown and does not involve a GEF activity on Cdc42 and Rac. IpaA is involved in entry by inducing maturation of the entry focus. IpaA binds to vinculin and activates functions of this protein that belongs to focal adherence plaques and orchestrates organisation of the actin filaments.[27] High affinity binding of IpaA to the N terminal head of vinculin triggers its unfolding, thereby promoting its actin binding capacity on its C terminus. This leads to the formation of an actin cup, a focal plaque like structure that seems essential to carry out *Shigella* entry.[28] Surprisingly, the ultimate consequence of this interaction is actin depolymerisation,[29] which carries out the transition from filopodial/lamellipodial structure to actin cup formation and final repair of the entry focus once entry is completed. IpgD, another secreted effector protein has a phosphatidyl inositol phosphatase activity that seems to account for the relaxation of the membrane–cytoskeletal association, thus facilitating extension of actin filaments at the early stage of the entry process.[30] Organisation of the signalling process induced by *Shigella* is orchestrated by c-src which is recruited at the entry site, and depending on the stage of the entry process, either enhances or down regulates the development of actin filaments.[31 32]

The differential intracellular behaviour of *Salmonella* and *Shigella* accounts for differential pathogenic properties. Once intracellular, *Salmonella* remain trapped in a vacuolar compartment, whereas *Shigella* disrupt their vacuole and escape into the cytoplasm.[33] *Shigella* is thereby allowed to express intracellular motility and cell to cell spread, permitting efficient epithelial invasion. Mutants that have lost this capacity are strongly attenuated, both in vitro and in vivo.[34–36] Intracellular motility of *Shigella* is caused by the polar expression of IcsA, an outer membrane protein of 1102 aa that is also encoded by the virulence plasmid of *Shigella*.[35] The N terminal portion of IcsA, through a series of glycine rich repeats (GRRs), binds N-WASP[37] and activates this protein by causing its unfolding in a way that makes its C terminal domain (the VCA domain) available for recruitment and binding of the Arp2/3 complex, thereby causing actin nucleation and polymerisation.[38] In consequence, this complex (IcsA, N-WASP, and Arp2/3) appears necessary and sufficient to cause actin nucleation–polymerisation and to promote bacterial motility in the cytoplasm. Cell to cell spread also involves engagement by *Shigella* of components of the intermediate junction.[39] The protrusion formed by the spreading microorganism is actively endocytosed by the adjacent cell in a process that requires activation of myosin II.[40] Following internalisation of the protrusion, the two membranes are destroyed following secretion of the pore forming IpaB and IpaC proteins.[41–43]

A majority of studies have addressed the intracellular behaviour of *Salmonella* inside macrophages.[44] *Salmonella* containing

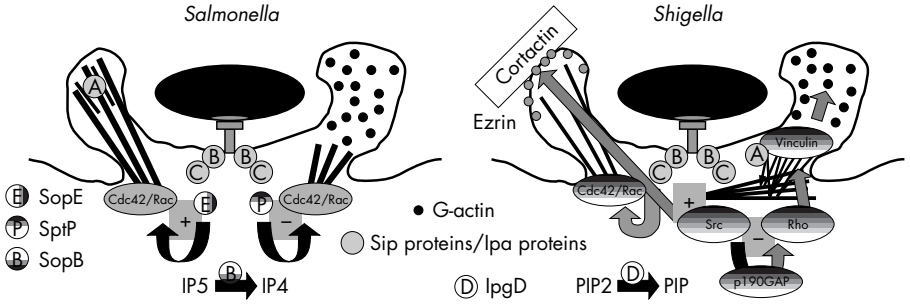

Figure 2 A paradigm or "triggering" entry of pathogens into epithelial cells: TTSS mediated translocation of *Salmonella* and *Shigella* effectors of entry inducing the formation of a macropinocytic vacuole.

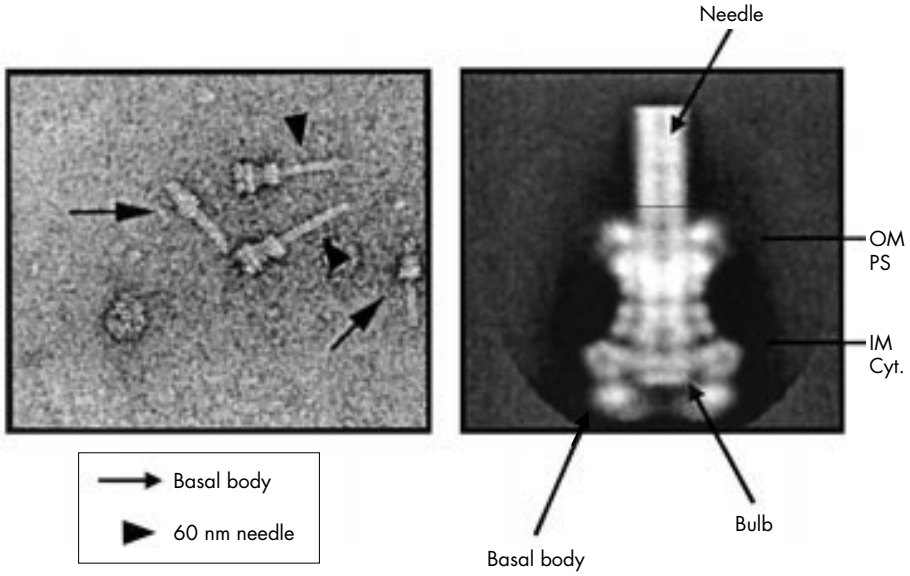

Figure 3 Structure of the TTSS of *Shigella flexneri*. OM, outer membrane; PS, periplasmic space; IM, inner membrane; Cyt, cytoplasm.

vacuoles, in HeLa cells, were initially shown to form two populations: one that appeared separated from the endocytic route and one that followed a "classical" phagocytic pathway.[45] Both compartments may be favourable to *Salmonella* survival and intracellular growth. However, live pathogenic *Salmonella* are now associated with an atypical compartment that acquires lysosomal markers such as Lgp or Lamp, but not the mannose-6-phosphate receptors and cathepsin D/L which are markers of late maturation towards terminal lysosomes.[46] These data indicate that S*almonella* interrupt the maturation of their compartments in order to survive and grow intracellularly. Rab-7 may control addition of the membranous material constituting this compartment by recruiting and fusing vesicles rich in Lpg and poor in cathepsins.[46] This compartment is also characterised by its capacity to form tubular structures in HeLa cells.[47] The SPI2 pathogenicity island of *Salmonella* is essential for controlling this maturation process.

PATHOGEN INTERACTIONS WITH THE INTESTINAL EPITHELIAL BARRIER

Intestinal pathogens can either adhere to the intestinal epithelium and colonise its surface, or invade and cause inflammatory lesions. *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC) are representative of the first category. They express surface adhesins that specifically bind carbohydrates linked to glycoproteins or glycolipids of the brush border membrane, without causing significant alteration of the cytoskeleton of the microvilli. They also produce toxins (cholera toxin (CT) or thermolabile (LT) and thermostable (ST) toxins) that act as pharmacological antagonists of sodium/water reabsorption and agonists of chloride/water secretion, thus causing the net hydroelectrolytic flux that accounts for diarrhoea. Enteropathogenic *E coli* (EPEC) and enterohaemorrhagic *E coli* (EHEC) belong to the same category as they remain extracellular. However, the type of interaction they establish with the apex of epithelial cells is closer to that of the invasive microorganisms as they secrete protein effectors which mediate intimate adherence to and effacement of the microvilli of the brush border involving major rearrangement of the actin cytoskeleton.[48] EHEC, in addition, secrete Shiga like toxins (SLT1 and 2) that act as potent cytotoxins, both locally and systemically. The category of enteroinvasive pathogens encompasses bacterial species such as *Shigella*, *Salmonella*, and *Yersinia*. From their initial site of invasion, several scenarios are observed: *Shigella* remains essentially local, causing major inflammatory destruction of the colonic and rectal mucosa. *Yersinia* proceeds to loco-regional infection, involving the mesenteric lymph nodes draining the ileum that is generally infected. *Salmonella* can subsequently proceed to systemic dissemination, such as in the case of typhoid fever.

These different patterns reflect genetic differences among these invasive pathogens that dictate particular patterns of infection. Horizontal transmission of genes by plasmids, transposons, and bacteriophages, integration of pathogenicity islands in these genomes characterise the speciation of these microbes towards a particular pathogenicity profile. This review focuses on the strategies used by enteroinvasive pathogens to disrupt, invade, and proceed to the inflammatory destruction of the intestinal mucosa.

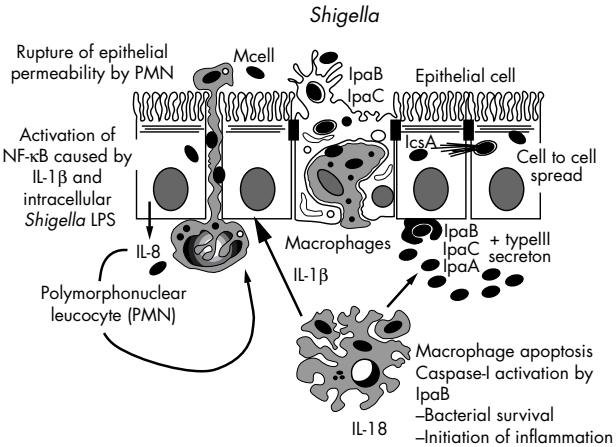

Figure 4 Physiopathological scheme of *Shigella* infection.

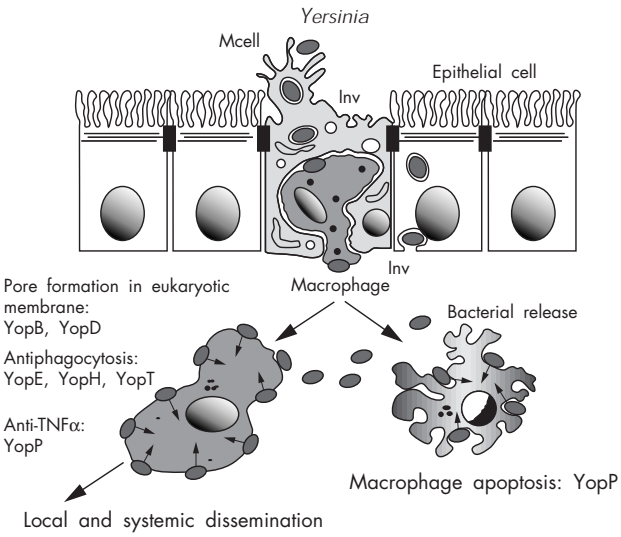

Figure 5 Physiopathological scheme of *Yersinia* infection.

CELLULAR ROUTES OF TRANSLOCATION OF THE EPITHELIAL BARRIER BY ENTERIC PATHOGENS

Recent contributions combining cell assay systems and in vivo models of intestinal invasion by bacteria have revealed a complex picture. Three routes of invasion can currently be considered: M cells, villous epithelial cells, and a "CD18 dependent" pathway.[49]

M cells of the FAE as a route of translocation

A variety of bacterial species, viruses, and protozoans translocate through the intestinal epithelium via M cells.[50 51] These pathogens take advantage of a physiological route of mucosal sampling of antigens to cross the epithelial barrier. Unlike the classic concept involving initial translocation through the villous epithelium, a process that appears difficult to achieve, even for invasive microbes,[52] here the pathogens need primarily to survive the deleterious effect of resident phagocytic cells that prevail in the follicular dome, instead of entering straight into epithelial cells via their apical pole. Invasion of the villous epithelium thus becomes the second event in the chronology of intestinal invasion.

Shigella infection (fig 4): prior to development of dysentery, early inflammatory lesions of the colorectal mucosa often resemble aphthoid ulcers with the presence of a lymphoid follicle.[53] Experimental infection in macaque monkeys[36] and in the rabbit ligated intestinal loop model of infection,[54] confirm these clinical observations. In the rabbit, bacteria selectively translocate through M cells.[55] No specific adherence system mediating the interaction between *Shigella* and the luminal side of M cells has been identified so far. However, invasive *Shigella* translocate much more efficiently through M cells than a non-invasive mutant, indicating that expression of an invasive phenotype plays a major role in *Shigella*–M cell interaction. Following translocation, shigellae are phagocytosed by dendritic cells and resident macrophages present in the dome. The survival strategy of *Shigella* is to cause apoptosis of the macrophage,[55 56] thereby allowing access to the basal side of epithelial cells where bacteria can efficiently enter. Apoptotic killing of macrophages by *Shigella* involves activation of caspase 1,[57 58] which also initiates inflammation by causing the maturation of two inflammatory cytokines: interleukin 1β and interleukin 18.[59] This early inflammatory process leads to quick disruption of the epithelial barrier, thereby facilitating further *Shigella* invasion.

Yersinia enterocolitica (fig 5) usually causes a diarrhoeal disease, whereas *Y pseudotuberculosis* causes mild enteric symptoms that may be followed by mesenteric lymphadenitis and sometimes systemic diffusion. Yersiniae cross the intestinal epithelium primarily through the FAE, in the Peyer's patches of the ileum.[60] Invasin (Inv), a 103 kDa outer membrane protein of *Y pseudotuberculosis* binds β1 integrins that are also expressed apically on M cells. Inv negative mutants still adhere to and invade M cells, but at a much lower level than the wild type strain and their colonisation potential for Peyer's patches is considerably reduced.[61] Other *Yersinia* surface proteins such as Ail, PsaA, and YadA may account for residual invasion of *inv* mutants.[62] Once the dome is reached, yersiniae survive attack by resident macrophages by expressing an antiphagocytic strategy caused by the injection, through a plasmid encoded type III secreton, of three protein effectors, YopH, T, and E, that disrupt cytoskeletal assembly.[63 64] YopH, a tyrosine phosphatase, dephosphorylates paxilin, p130^{cas}, and FAK that are involved in the assembly of cytoskeletal complexes required for phagocytosis.[65] YopT provokes the depolymerisation of actin filaments by inducing redistribution of the RhoA GTPase.[66] YopE expresses a GAP function that inhibits the small GTPases of the Rho family involved in phagocytosis.[67] Yersiniae therefore remain essentially extracellular in infected Peyer's patches and mesenteric lymph nodes. This allows their extracellular survival and possible Inv mediated entry into epithelial cells.

Salmonella typhimurium (fig 6) crosses the epithelial barrier and causes systemic dissemination, resulting in fatal septicaemia in mice. A similar situation is observed in humans infected by *Salmonella typhi*. *S typhimurium* binds to M cells and translocates through the FAE in the murine intestine.[68 69] It is cytotoxic for M cells.[69 70] Long Lpf fimbriae mediate adherence to murine M cells.[71] However, Lpf is likely to be one component of a repertoire of adherence factors also comprising a carbohydrate containing galactose-β(1–3)-galactosamine shown on Caco-2 cells.[72] Adherence of *S typhimurium* to M cells is followed by ruffling of the cell membrane and macropinocytosis, reflecting cytoskeletal changes similar to those occuring in vitro in cultivated cells.[73] The invasion system encoded by *Salmonella* pathogenicity island 1 (SPI1) and additional proteins secreted through the TTSS encoded by SPI1 contribute to invasion of M cells. SPI1 negative mutants are not cytotoxic for M cells and their capacity to cross the intestinal barrier is impaired, whereas their virulence remains intact following systemic infection.[74] Once it has reached the dome of lymphoid follicles, following its phagocytosis by resident macrophages and dendritic cells,[75] *S typhimurium*, via SPI1, causes SipB dependent apoptotic killing of these macrophages following activation of caspase-1.[76] However, *Salmonella* has also evolved a strategy of survival inside phagocytes, particularly macrophages, which may facilitate its systemic dissemination. SPI2, another pathogenicity island encoding an alternative type III secreton and its dedicated effector

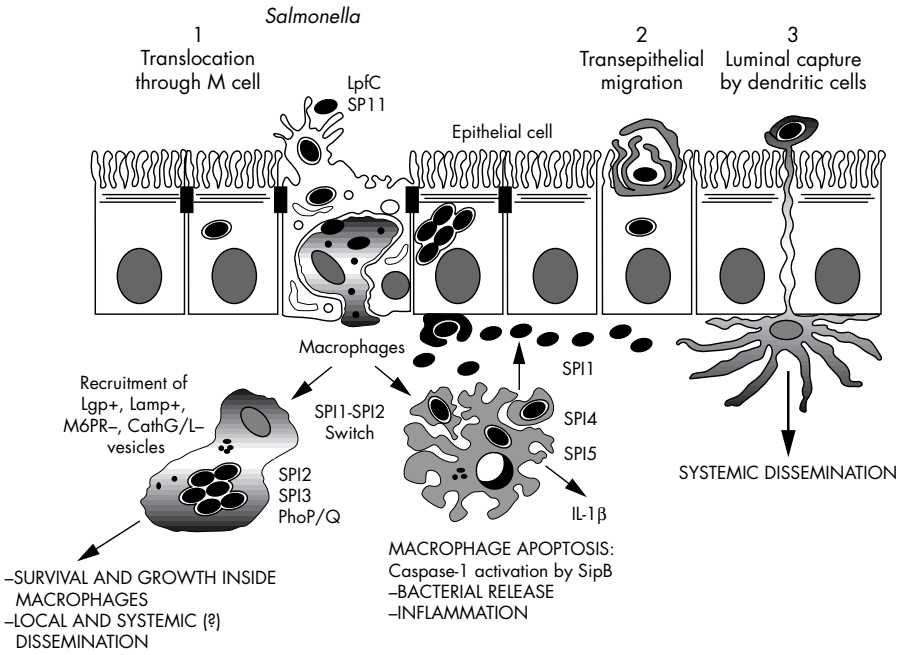

Figure 6 *Salmonella* routes for crossing the intestinal barrier and physiopathological scheme of infection.

proteins,[77] as well as a series of phoP/phoQ regulated genes,[78] are essential for *Salmonella* survival and growth inside macrophages. How a balance is achieved between macrophage killing and survival in a preserved cell is as yet unknown.

A transepithelial route of invasion

Although M cells account for a large part of the initial crossing of the intestinal epithelium by enteroinvasive microorganisms, alternative routes are likely to exist. Electron microscopic evidence exists for apical invasion of villous epithelial cells by *Salmonella* in vivo.[79] *Shigella* invade intestinal villi in the absence of Peyer's patch in the rabbit ligated loop model of infection.[55 80 81] However, invasion of the villous epithelium appears later (8 hours instead of 2–4 hours for the FAE) and is seen with bacterial inocula that are much higher than those known to cause the natural disease in humans. In addition, intestinal epithelial cells respond to invasive pathogens by expressing proinflammatory cytokines and chemokines.[82] This programming of epithelial cells to produce proinflammatory molecules leads to attraction and transepithelial migration of polymorphonuclear leucocytes (PMN), thereby disrupting the permeability of the epithelium.[83] Direct interaction of *Salmonella* without internalisation is sufficient to trigger transepithelial migration of PMNs.[84] It is possible that in *Salmonella* and *Shigella*, some of the proteins injected by the TTSS trigger the activation of proinflammatory transcription factors such as NF-κB. In the case of *Shigella*, this process facilitates bacterial invasion of epithelial cells via their basolateral pole which is more permissive to bacterial entry.[80 85] It seems therefore that transepithelial signalling induced by these bacteria may ultimately allow epithelial invasion in areas that do not possess FAE and lymphoid structures. Recent demonstration that intracellular *Shigella* LPS is able to induce rapid and prolonged activation of NF-κB and Jun terminal kinase through Nod1, thus causing production of IL-8 by epithelial cells, is another signalling process that may locally subvert the villous epithelium and facilitate its invasion.[86]

A CD18 dependent route of infection for *Salmonella* (and other invasive bacteria?)

Even though SPI1 deficient mutants of *S typhimurium* are deficient in invading both M cells and villous epithelial cells, they conserve their capacity to disseminate and to kill infected mice.[87] Likewise, Inv negative mutants of *Y enterocolitica* are unable to colonise Peyer's patches and the corresponding mesenteric lymph nodes, but they still retain their capacity of systemic dissemination.[88] These observations have suggested that alternative routes to M cells and villous epithelial cells may exist for crossing the epithelial barrier. Recent evidence indicates that in the case of *S typhimurium*, such an alternative route exists that permits systemic dissemination. This route involves CD-18 expressing mononuclear phagocytes.[89] Bacterial uptake appears to be mediated by dendritic cells which open the tight junctions and send dendrites on the luminal side of the epithelium where they take up the bacteria. These cells express tight junction proteins, possibly involved in resealing of the epithelium, thereby preserving impermeability of the epithelial barrier.[90]

CONCLUSION

The complex processes by which enteroinvasive bacterial pathogens disrupt and invade the intestinal epithelium have been reviewed with a strong emphasis on the cross talks that are established between the bacteria and their cellular targets. Much has been learned over the past years about the signals occurring at the interface between bacteria and the cells, but more remains to be learned about the more global signalling processes that lead to tissue damage and repair in the course of an infectious process. We are currently witnessing a transition period between the now classical concept of "cellular microbiology" and the new concept of "tissular microbiology".

ACKNOWLEDGEMENT

I wish to thank my colleagues of Unité de Pathogénie Microbienne Moléculaire for discussions on this topic and Colette Jacquemin for the edition of this manuscript. PS is a Howard Hughes Medical Institute scholar.

Authors' affiliations

P Sansonetti, Unité de Pathogénie Microbienne Moléculaire, INSERM U389, Institut Pasteur, Paris, France

REFERENCES

1 **Madara JL**, Nash S, Moore R, *et al*. Structure and function of the intestinal epithelial barrier in health and disease. *Monogr Pathol* 1990;**31**:306–24.

2 **Maury J**, Nicoletti C, Guzzo-Chambraud I, *et al*. The filamentous brush border glycocalyx, a mucin-like marker of enterocyte hyperpolarisation. *Eur J Biochem* 1995;**228**:323–31.
3 **Sanderson IR**, Walker WA. Mucosal barrier, an overview. In: Ogra PL, Lamm ME, Bienenstock J, *et al*, eds. *Mucosal immunology*, 2nd edn. San Diego: Academic Press, 1999:5–17.
4 **Neutra MR**. M cells in antigen sampling. *Curr Top Microbiol Immunol* 1999;**236**:17–32.
5 **Kerneis S**, Bogdanova A, Kraehenbuhl JP. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science* 1996;**277**:949–52.
6 **Isberg RR**. Discrimination between intracellular uptake and surface adhesion of bacterial pathogens. *Science* 1991;**252**:934–8.
7 **Isberg RR**, Barnes P. Subversion of integrins by enteropathogenic Yersinia. *J Cell Science* 2001;**114**:21–8.
8 **Mengaud J**, Ohayon H, Gounon P, *et al*. E-cadherin is the receptor for internalin, a surface protein required for entry of Listeria monocytogenes into epithelial cells. *Cell* 1996;**84**:924–32.
9 **Finlay BB**, Cossart P. Exploitation of mammalian host cell functions by bacterial pathogens. *Science* 1997;**276**:718–25.
10 **Isberg RR**. Uptake of enteropathogenic Yersinia by mammalian cells. *Curr Top Microbiol Immunol* 1996;**209**:1–24.
11 **Tran Van Nhieu G**, Isberg RR. The Yersinia pseudotuberculosis invasin protein and human fibronectin bind to mutually exclusive sites on the α5β1 integrin receptor. *J Biol Chem* 1991;**266**:24367–75.
12 **Alrutz M**, Isberg RR. Involvement of focal adhesion kinase in invasin-mediated uptake. *Proc Natl Acad Sci U S A* 1998;**95**:13658–63.
13 **Blocker A**, Gounon P, Larquet E. Role of Shigella's type III secretion system in insertion of IpaB and IpaC into the host membrane. *J Cell Biol* 1999;**147**:683–93.
14 **Kubori T**, Matsushima Y, Nakamura D, *et al*. Supramolecular structure of the Salmonella typhimurium type III protein secretion system. *Science* 1998;**280**:602–5.
15 **Tamano K**, Aizawa, AC, Katayama E, *et al*. Supramolecular structure of the Shigella type III secretion machinery: the needle part is changeable in length and is essential for delivery of effectors. *EMBO J* 2000;**19**:3876–87.
16 **Blocker A**, Jouihri N, Larquet E, *et al*. Structure and composition of the Shigella flexneri "needle complex" a part of its type III secreton. *Mol Microbiol* 2001;**39**:652–63.
17 **De Geyter C**, Wattiez R, Sansonetti PJ. Characterization of the interaction of IpaB and IpaD, proteins required for entry of Shigella *flexneri* into epithelial cells, with a lipid membrane. *Eur J Biochem* 2000;**267**:5769–76.
18 **Galan J**, Zhou D. Striking the balance: modulation of the actin cytoskeleton by Salmonella. *Proc Natl Acad Sci U S A* 2000;**97**:8754–61.
19 **Hall A**. Rho GTPases and the actin cytoskeleton. *Science* 1998;**279**:509–14.
20 **Welch MD**. The world according to Arp: regulation of actin nucleation by the Arp2/3 complex. *Trends Cell Biol* 1999;**9**:423–7.
21 **Hayward R**, Koronakis V. Direct nucleation and bundling of actin by the SipC protein of invasive Salmonella. *EMBO J* 1999;**18**:4926–34.
22 **Zhou D**, Mooseker MS, Galan JE. Role of the Salmonella typhimurium actin-binding protein SipA in bacterial internalisation. *Science* 1999;**283**:2092–5.
23 **Zhou D**, Hernandez L, Shears S, *et al*. A Salmonella inositol phosphatase acts in conjunction with other bacterial effectors to promote host cell actin cytoskeleton rearrangements and bacterial internalisation. *Mol Microbiol* 2001;**39**:248–59.
24 **Fu Y**, Galan JE. A Salmonella protein antagonises Rac-1 and Cdc42 to mediate host cell recovery after bacterial invasion. *Nature* 1999;**401**:293–7.
25 **Buchreiser C**, Glaser P, Rusniok C, *et al*. The virulence plasmid pWR100 and the repertoire of proteins secreted by the type III secretion apparatus of Shigella flexneri. *Mol Microbiol* 2000;**38**:1–14.
26 **Tran Van Nhieu G**, Caron E, Hall A. IpaC determines filopodia formation during Shigella entry into epithelial cells. *EMBO J* 1999;**18**:3249–62.
27 **Tran Van Nhieu G**, Ben Ze'ev A, Sansonetti PJ. Modulation of bacterial entry in epithelial cells by association between vinculin and the Shigella IpaA invasin. *EMBO J* 1997;**16**:2717–29.
28 **Bourdet-Sicard R**, Rudiger M, Sansonetti PJ. Vinculin is unfolded by the Shigella protein IpaA, and the complex promotes F-actin depolymerization. *EMBO J* 1999;**18**:5853–62.
29 **Bourdet-Sicard R**, Egile C, Sansonetti PJ. Diversion of cytoskeletal process by Shigella during invasion of epithelial cells. *Microbes & Infection* 2000;**2**:813–19.
30 **Niebuhr K**, Jouihri N, Allaoui A. IpgD, a protein secreted by the type III secretion machinery of Shigella flexneri, is chaperoned by IpgE and implicated in entry focus formation. *Mol Microbiol* 2000;**38**:8–19.
31 **Tran Van Nhieu G**, Bourdet-Sicard R, Duménil G, *et al*. Bacterial signals and cell responses durign Shigella entry into epithelial clells. *Cell Microbiol* 2000;**2**:187–93.
32 **Duménil G**, Sansonetti PJ, Tran Van Nhieu G. Src tyrosine kinase activity down-regulates Rho-dependent responses during Shigella entry into epithelial cells and stress fiber formation. *J Cell Sci* 2000;**113**:71–80.
33 **Sansonetti PJ**, Ryter A, Clerc P. Multiplication of Shigella flexneri within HeLa cells: lysis of the phagocytic vacuole and plasmid-mediated contact hemolysis. *Infect Immun* 1986;**51**:461–9.
34 **Makino S**, Sasakawa C, Kamata K. A genetic determinant required for continuous reinfection of adjacent cells on large plasmid in S. flexneri 2a. *Cell* 1986;**46**:551–5.
35 **Bernardini ML**, Mounier J, d'Hauteville H. Identification of *icsA*, a plasmid locus of Shigella flexneri which governs bacterial intra- and intercellular spread through interaction with F-actin. *Proc Natl Acad Sci U S A* 1989;**86**:3867–71.
36 **Sansonetti PJ**, Arondel J, Fontaine A, *et al*. OmpB (osmo-regulation) and *icsA* (cell to cell spread) mutants of Shigella flexneri. Evaluation as vaccine candidates. Probes to study the pathogenesis of shigellosis. *Vaccine* 1991;**9**:416–22.
37 **Miki H**, Suetsugu S, Takenawa T. WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. *EMBO J* 1998;**17**:6932–41.
38 **Egile C**, Loisel TP, Laurent V. Activation of the CDC42 effector N-WASP by the Shigella *icsA* protein promotes actin nucleation by Arp2/3 complex and bacterial actin-ased motility. *J Cell Biol* 199;**146**:1319–32.
39 **Sansonetti PJ**, Mounier J, Prévost MC. Cadherin expression is required for the spread of Shigella flexneri between epithelial cells. *Cell* 1994;**76**:829–39.
40 **Rathman M**, De Lanerolle M, Ohayon H. Myosin light chain kinase plays an essential role in S. flexneri dissemination. *J Cell Sciences* 2000;**113**:3375–86.
41 **Page AL**, Ohayon H, Gounon P. Role of IpaB and IpaC in the intercellular dissemination of Shigella flexneri. *Cell Microbiol* 1999;**1**:183–93.
42 **Schuch R**, Sandlin RC, Maurelli AT. A system for identifying post-invasion functions of invasion genes: requirements for the Mxi-Spa type III secretion pathway of Shigella flexneri in intercellular dissemination. *Mol Microbiol* 1999;**34**:675–89.
43 **Rathman M**, Jouirhi N, Allaoui A. The development of a FACS-based strategy for the isolation of Shigella flexneri mutants that are deficient in intercellular spread. *Mol Microbiol* 2000;**35**:974–90.
44 **Haas A**. Reprogramming the phagocytic pathway—intracellular pathogens and their vacuoles (review). *Mol Membr Biol* 1998;**15**:103–21.
45 **Garcia del Portillo F**, Finlay BB. Targetting of Salmonella typhimurium to vesicles containing lysosomal membranes glycoproteins bypasses compartments with mannose-6-phosphate receptors. *J Cell Biol* 1995;**129**:81–97.
46 **Méresse S**, Steele-Mortimer O, Finlay BB, *et al*. The Rab-7 GTPase controls the maturation of Salmonella typhimurium-containing vacuoles in HeLa cells. *EMBO J* 1999;**18**:4394–403.
47 **Garcia del Portillo F**, Zwick MB, Leung KY, *et al*. Salmonella induces the formation of filamentous structures containing lysosomal membrane glycoproteins in epithelial cells. *Proc Natl Acad Sci U S A* 1993;**90**:10544–8.
48 **De Vinney R**, Knoechel DG, Finlay BB. Enteropathogenic Escherichia coli: cellular harassement. *Curr Opin Microbiol* 1999;**2**:83–8.
49 **Vasquez-Torrez A**, Fang F. Cellular routes of invasion by enteropathogens. *Curr Opin Microbiol* 2000;**3**:54–9.
50 **Owen RL**. Uptake and transport of intestinal macromolecules and microorganisms by M cells and Peyer's patches—a historical and personal perspective. *Semin Immunol* 1999;**11**:157–63.
51 **Sansonetti P**, Phalipon A. M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. *Semin Immunol* 1999;**11**:193–203.
52 **Mounier J**, Vasselon T, Hellio R, *et al*. Shigella flexneri enters human colonic Caco-2 cells through the baso-lateral pole. *Infect Immun* 1992;**60**:237–48.
53 **Mathan MM**, Mathan VI. Intestinal manifestations of invasive diarrheas and their diagnosis. *Rev Infect Dis* 1991;**13**(suppl 4):S314–18.
54 **Wassef JS**, Keren DF, Mailloux JL. Role of M cells in initial antigen uptake and in ulcer formation in the rabbit intestinal loop model of shigellosis. *Infect Immun* 1989;**57**:858–63.
55 **Sansonetti PJ**, Arondel J, Cantey RJ, *et al*. Infection of rabbit Peyer's patches by Shigella flexneri: effect of adhesive and invasive bacterial phenotypes on follicular-associated epithelium. *Infect Immun* 1996;**64**:2752–64.
56 **Zychlinsky A**, Prévost MC, Sansonetti PJ. Shigella flexneri induces apoptosis in infected macrophages. *Nature* 1992;**358**:167–69.
57 **Chen Y**, Smith MR, Thirumalai K, *et al*. A bacterial invasin induces macrophages apoptosis directly to ICE. *EMBO J* 1996;**15**:3853–60.
58 **Hilbi H**, Moss JE, Hersh D, *et al*. Shigella-induced apoptosis is dependent on caspase-1 which binds to IpaB. *J Biol Chem* 1998;**273**:32895–900.
59 **Sansonetti PJ**, Phalipon A, Arondel J. Caspase-1 activation of IL1β and IL-18 are essential for Shigella flexneri induced inflammation. *Immunity* 2000;**12**:581–90.
60 **Grutzkau A**, Hanski C, Hanhn H, *et al*. Involvement of M cell in the bacterial invasion of Peyer's patches: a common mechanism shared by Yersinia enterocolitica and other enteroinvasive bacteria. *Gut* 1990;**31**:1011–15.
61 **Clark MA**, Hirst BH, Jepson MA. M-cell surface β1 integrin expression and invasin-mediated targeting of Yersinia pseudotuberculosis to mouse Peyer's patch M cells. *Infect Immun* 1998;**66**:1237–43.
62 **Marra A**, Isberg RR. Invasin-dependant and invasin-independant pathways for translocation of Yersinia pseudotuberculosis across the Peyer's patch intestinal epithelium. *Infect Immun* 1997;**65**:3412–21.
63 **Fällmann M**, Persson C, Wolf-Watz H. Yersinia proteins that target host cell signalling pathways. *J Clin Invest* 1997;**99**:1153–7.

64 **Cornelis G**. The Yersinia deadly kiss. *J Bacteriol* 1998;**180**:5495–504.
65 **Persson C**, Carballeira N, Wolf-Watz H, *et al*. The PTPase YopH inhibits uptake of Yersinia, tyrosine phosphorylation of p130^{cas} and FAK, and the associated accumulation of these proteins in peripheral focal adhesions. *EMBO J* 1997;**16**:2307–18.
66 **Zumbihl R**, Aepfelbacher M, Andor A, *et al*. The cytotoxin YopT of Yersinia enterocolitica induces modifications and cellular redistribution of the small GTP-binding protein RhoA. *J Biol Chem* 1999;**274**:29289–93.
67 **Black D**, Bliska J. The RhoGAP activity of the Yersinia pseudotuberculosis cytotoxin YopE is required for antiphagocytosis function and virulence. *Mol Microbiol* 2000;**37**:515–27.
68 **Clark MA**, Jepson MA, Simmons NL, *et al*. Preferential interaction of Salmonella typhimurium with mouse Peyer's patch M cells. *Res Microbiol* 1994;**145**:543–52.
69 **Jones BD**, Ghori N, Falkow S. Salmonella typhimurium initiates murine infection by penetrating and destroying the specialised epithelial M cells of Peyer's patches. *J Exp Med* 1994;**180**:15–23.
70 **Kohbata S**, Yokobata H, Yabuuchi E. Cytopathogenic effect of Salmonella typhi GIFU10007 on M cells of murine ileal Peyer's patches in ligated ileal loops: an ultrastructural study. *Microbiol Immunol* 1986;**30**:1225–37.
71 **Baumler AJ**, Tsolis RM, Heffron F. The lpf fimbrial operon mediates adhesion of Salmonella typhimurium to murine Peyer's patch. *Proc Natl Acad Sci U S A* 1996;**93**:279–83.
72 **Giannasca KT**, Giannasca PJ, Neutra MR. Role of apical membrane glycoconjugates in adherence of Salmonella typhimurium to intestinal epithelial cells. *Infect Immun* 1996;**64**:135–45.
73 **Finlay BB**, Falkow S. Salmonella interaction with polarised human intestinal Caco-2 epithelial cells. *J Infect Dis* 1990;**162**:1096–106.
74 **Penheiter KL**, Mathur N, Giles D, *et al*. Non-invasive Salmonella typhimurium mutants are avirulent because of an inhability to enter and destroy M cells of ileal Peyer's patches. *Mol Microbiol* 1997;**24**:697–709.
75 **Hopkins SA**, Niedergang F, Corthesy-Theulaz IE, *et al*. A recombinant Salmonella typhimurium vaccine strain is taken up and survives within murine Peyer's patch dendritic cells. *Cell Microbiol* 2000;**2**:59–68.
76 **Hersh D**, Monack DM, Smith MR, *et al*. The Salmonella invasin SipB induces macrophage apoptosis by binding to Caspase-1. *Proc Natl Acad Sci U S A* 1999;**96**:2396–401.
77 **Shea J**, Hensel M, Gleeson C, *et al*. Identification of a virulence locus encoding a second type III secretion system in Salmonella typhimurium. *Proc Natl Acad Sci U S A* 1996;**93**:2593–7.
78 **Miller SI**. PhoP/PhoQ: macrophage-specific modulator of Salmonella virulence? *Mol Microbiol* 1991;**5**:2073–8.
79 **Takeuchi A**. Electron microscope studies of experimental Salmonella infection. *Am J Pathol* 1966;**50**:109–36.
80 **Perdomo JJ**, Cavaillon JM, Huerre M, *et al*. Acute inflammation causes epithelial invasion and mucosal destruction in experimental shigellosis. *J Exp Med* 1994;**180**:1307–19.
81 **Sansonetti PJ**, J Arondel, JM Cavaillon, *et al*. Role of IL-1 in the pathogenesis of experimental shigellosis. *J Clin Invest* 1995;**96**:884–92.
82 **Jung HC**, Eckmann I, Yang SK, *et al*. A distinct array of pro-inflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. *J Clin Invest* 1995;**95**:55–65.
83 **McCormick BA**, Miller SI, Carnes D, *et al*. Transepithelial signaling to neutrophils by salmonellae: a nouvel virulence mechanism for gastroenteritis. *Infect Immun* 1995;**63**:2302–9.
84 **Gewirtz AT**, Siber AM, Madara JL. Orchestration of neutrophil movement by intestinal epithelial cells in response to Salmonella typhimurium can be uncoupled from bacterial internalization. *Infect Immun* 1999;**67**:608–17.
85 **Perdomo JJ**, Gounon P, Sansonetti PJ. Polymorphonuclear leukocyte transmigration promotes invasion of colonic epithelial monolayer by Shigella flexneri. *J Clin Invest* 1994;**93**:633–43.
86 **Girardin S**, Tournebize R, Mavris M, *et al*. CARD4/Nod1mediates NF-kB and JNK activation by invasive Shigella flexneri. *EMBO Reports* 2001. In press. **[AQ:1]**
87 **Everest P**, Ketley J, Hardy S, *et al*. Evaluation of Salmonella typhimurium mutants in a model of experimental gastroenteritis. *Infect Immun* 1999;**67**:2815–21.
88 **Pepe J**, Miller VL. Yersinia enterocolitica invasin: a primary role in the initiation of infection. *Proc Natl Acad Sci U S A* 1993;**90**:6373–7.
89 **Vasquez-Torres A**, Jones-Carson J, Baumler AJ, *et al*. Extraintestinal dissemination of Salmonella via CD18-expressing phagocytes. *Nature* 1999;**401**:804–8.
90 **Rescigno M**, Urbano M, Valzasina B, *et al*. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunol* 2001;**2**:361–7.