Institut Pasteur
Microbes and Infection 17 (2015) 173–183
www.elsevier.com/locate/micinf

Review

How bacterial pathogens colonize their hosts and invade deeper tissues

David Ribet [a,b,c,*], Pascale Cossart [a,b,c,*]

[a] *Institut Pasteur, Unité des Interactions Bactéries-Cellules, Département de Biologie Cellulaire et Infection, F-75015 Paris, France*
[b] *INSERM, U604, F-75015 Paris, France*
[c] *INRA, USC2020, F-75015 Paris, France*

Received 25 September 2014; accepted 19 January 2015
Available online 29 January 2015

Abstract

Bacterial pathogens have evolved a wide range of strategies to colonize and invade human organs, despite the presence of multiple host defense mechanisms. In this review, we will describe how pathogenic bacteria can adhere and multiply at the surface of host cells, how some bacteria can enter and proliferate inside these cells, and finally how pathogens may cross epithelial or endothelial host barriers and get access to internal tissues, leading to severe diseases in humans.

Keywords: Bacterial invasion; Bacterial adhesion; Microbiota; Host barrier; Host–pathogen interactions; *Listeria*

1. Introduction

The human body harbors a large number of bacteria but their localization in healthy individuals is normally restricted to certain body areas such as the skin, the mucosae of buccal and nasal cavities, vagina and, most importantly, the gastrointestinal tract [1–6]. The internal tissues are normally sterile. In some circumstances, however, some opportunistic pathogens are able to enter the host by taking advantage of injuries or breaches in one of the different host barriers. In addition, *bona fide* pathogens have evolved mechanisms to cross host barriers and reach deeper organs where they proliferate and lead to severe disease for their host.

In this review, we will describe the diversity of mechanisms used by bacterial pathogens to colonize and invade human organs. We will first focus on the capacity of these bacteria to adhere and to proliferate at the surface of host cells and tissues, despite a wide-range of defense mechanisms used by the host. We will then present how some bacteria are able to enter and to proliferate inside host cells. Finally we will discuss how some pathogens can cross host barriers and get access to deeper tissues thereby promoting their dissemination inside their host.

2. Colonization of host surfaces

The respiratory, digestive and urogenital mucosa represent a surface area of approximately 300–400 square meters (*i.e.* 200-fold larger than that of the skin) and thus constitute major sites of contact with bacteria. These mucosa are composed of three layers: an epithelium, a layer of loose connective tissue called lamina propria, and a thin layer of smooth muscles. These surfaces constitute frontline barriers limiting the invasion by both commensal and pathogenic bacteria. Despite the different defense mechanisms occuring at the level of these barriers, pathogenic bacteria have evolved various molecular strategies to adhere to these epithelia and to proliferate at their surface.

2.1. Host epithelia and associated defense mechanisms

Epithelia of diverse organs in contact with the extracellular milieu, and thus with environmental bacteria, are covered by a

* Corresponding authors. Institut Pasteur, Unité des Interactions Bactéries-Cellules, Département de Biologie Cellulaire et Infection, F-75015 Paris, France.
E-mail addresses: david.ribet@pasteur.fr (D. Ribet), pascale.cossart@pasteur.fr (P. Cossart).

http://dx.doi.org/10.1016/j.micinf.2015.01.004

mucus layer that allows a protection against intruders. The intestinal mucus layer, for example, plays a key role in limiting invasion by commensal bacteria of the microflora or by foodborne pathogenic bacteria [7] (Figs. 1 and 2). This mucus is mainly composed of glycoproteins called mucins, digestive enzymes, antimicrobial peptides and immunoglobulins. Bacteria are often found at the top of this intestinal mucus layer, where they interact with mucins, whereas the inner layer of mucus, where the concentration of antimicrobial compounds is high, is normally devoid of bacteria [8]. Mucins are produced and secreted in the intestine by goblet cells, a specialized cell-type of the intestinal epithelium. Their production can be modulated in response to microbial products or inflammation [7]. The level of antimicrobial peptides, predominantly secreted by Paneth cells from intestinal crypts, can also be regulated by the presence of microorganisms. Indeed, whereas α-defensins are constitutively expressed, other antimicrobial peptides such as REG3γ (Regenerating islet-derived protein 3γ) or cryptdins are produced in response to the detection of pathogen-associated molecular patterns (PAMPs) that activates TLR (Toll-like receptors) or NOD (nucleotide-binding oligomerization domain-containing protein) signaling pathways [9–12]. IgA, produced by B cells in the lamina propria and secreted into the mucus via epithelial cells, are

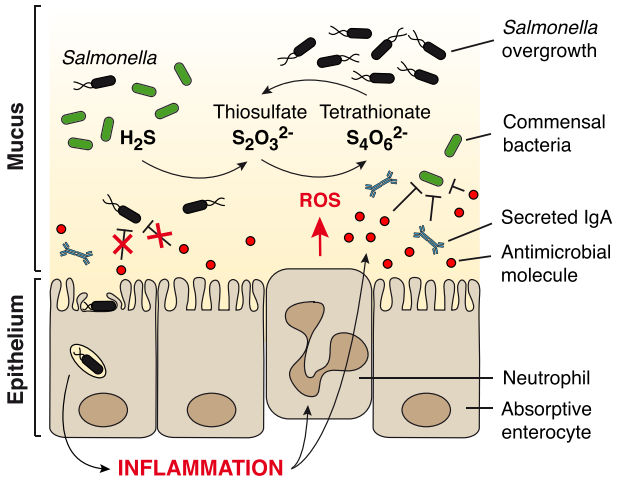

Fig. 2. Schematic representation of events leading to *Salmonella* overgrowth in the intestine. Invasion of intestinal epithelial cells by *Salmonella* triggers an inflammatory response leading to the release of antimicrobial peptides and the production of ROS (*R*eactive *O*xygen *S*pecies) by neutrophils. H_2S, a fermentation end product generated by commensal bacteria, is oxidized into thiosulfate by the colonic epithelium and then into tetrathionate by ROS. In contrast to fermenting bacteria of the microbiota, *Salmonella* can use this tetrathionate as a terminal electron acceptor to support growth in anaerobic conditions. The use of tetrathionate, in addition to *Salmonella* resistance to antimicrobial molecules, allow this pathogen to out-compete commensal bacteria in this inflamed context.

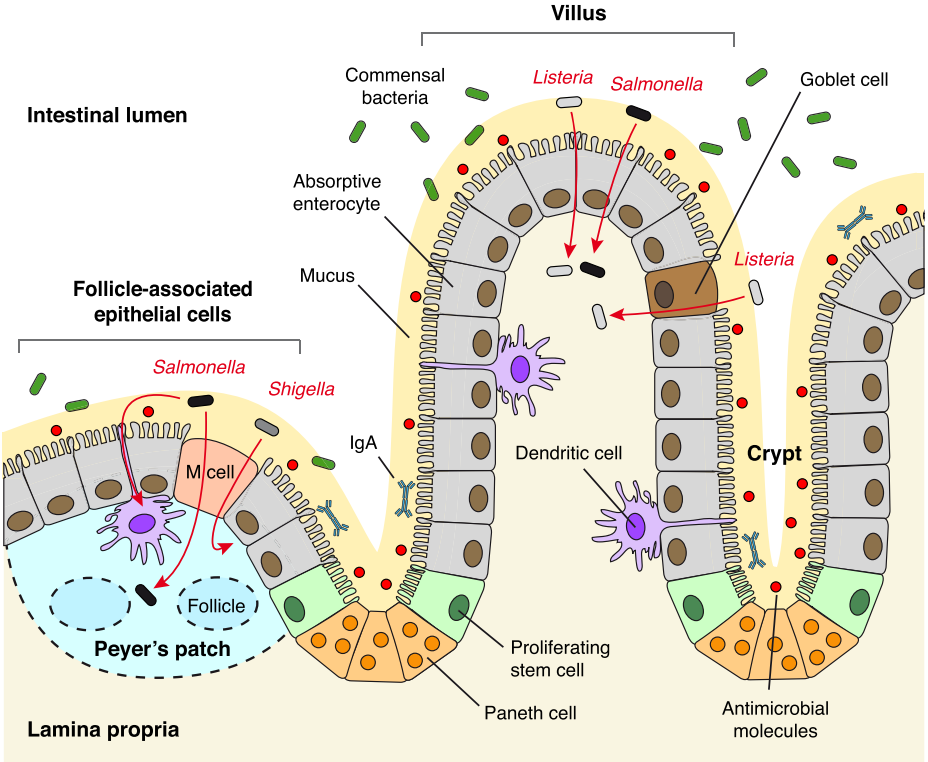

Fig. 1. Routes of invasion by enteric pathogens in the human small intestine. The epithelium of the small intestine is composed of absorptive enterocytes, mucus-producing goblet cells, M cells, as well as proliferating stem cells and Paneth cells located in intestinal crypts. The intestinal epithelium is covered by a mucus layer containing secreted IgA, antimicrobial peptides and other types of antimicrobial compounds that limit the colonization by commensal bacteria or foodborne pathogens. Peyer's patches and the overlaying follicle-associated epithelium, M cells and dendritic cells constitute specialized regions of the intestine that continuously sample the intestinal luminal content (adapted from Ref. [99]). *Listeria monocytogenes* can cross the host intestinal barrier at sites of cell extrusion at the tip of the villi or at junctions between goblet and absorptive epithelial cells. *Salmonella* Typhimurium can cross the intestinal epithelium by targeting absorptive cells, M cells of Peyer's patches or dendritic cells sampling the intestinal lumen. *Shigella flexneri* also target M cells for crossing the intestinal barrier and then re-infect epithelial cells basolaterally.

also involved in limiting bacterial association with the intestinal epithelium and intestinal barrier crossing [13,14]. Finally, in addition to these molecules with antimicrobial activities, shedding of mucus can be another mechanism to prevent bacterial adhesion to epithelial surface, as reported in the case of gastric mucus colonized by the pathogenic bacterium *Helicobacter pylori* [15]. Interestingly, some bacterial pathogens have evolved mechanisms to go through this mucus layer in order to reach epithelial cells. They either produce proteases and directly target host mucins, locomote via flagella-based motility or resist to antimicrobial products [16–21], (Fig. 2). Interactions between pathogenic bacteria and host mucus thus constitute a challenging issue during host infection.

Besides mucus, an important actor in the control of pathogen invasion is the microbiota, which is mainly constituted of commensal bacteria living on human mucosal surfaces. In the human intestine, this microbiota plays fundamental roles in digestion, as well as in intestinal epithelial metabolism and proliferation. In addition, it plays a key role in the resistance to foodborne infections by directly competing with enteric pathogens [22]. Indeed, inhibitory metabolites, such as acetate or butyrate, can be released by different species of commensal bacteria. These bacteria can furthermore utilize various nutrients of the intestinal lumen, which would then be no longer available for incoming pathogens. Finally, the microbiota is involved in the regulation of the host immune system [23]. It has been shown in particular that germ-free mice, *i.e.* animals devoid of normal microbiota, display poorly developed intestinal mucosal lymphoid follicles, called Peyer's patches. They also have an altered composition of $CD4^+$ T cells and IgA-producing B cells in the lamina propria [24,25]. Despite the protection provided by the microbiota, some enteric pathogens are able to efficiently colonize the gut and even cross the intestinal barrier. Triggering of inflammation can be considered as a mechanism used by pathogens to alter the microbiota composition, thereby allowing them to outcompete luminal commensals [26]. Inflammation of the gut is characterized by an increase in the quantity of mucosal antimicrobial peptides to which pathogens may exhibit higher resistance compared to commensals [20–22]. Mucosal inflammation also leads to the production of specific compounds that can be used by pathogens such as particular glycosylated proteins or tetrathionate [27,28]. This last molecule is indeed used by the murine enteric bacterial pathogen *Salmonella* Typhimurium, which uses it as a terminal electron acceptor during anaerobic respiration, giving to this pathogen a growth advantage over fermenting commensal bacteria in this inflamed environment [28] (Fig. 2). Together, the overall crosstalks and interactions between commensal bacteria, enteric pathogens and host are crucial in the establishment and progression of intestinal diseases [26].

Finally, in addition to mucus and to the microbiota, epithelial cell renewal plays an important role in the control of bacterial colonization [29]. In the gut, cells of the intestinal epithelium have a very high turnover rate. Indeed, new epithelial cells are constantly generated at the level of intestinal crypts. These cells are then migrating along the villi to be finally extruded at the tip of these villi after about one week. There is a tight balance between the self-renewal of cells and their elimination that is crucial to homeostasis and epithelium integrity. Induction of epithelial cell death has been characterized as a defensive mechanism used by the host to limit infection by enteric pathogens [29]. Cell death indeed allows the elimination of damaged cells and limits persistent bacterial colonization. The global upregulation of the epithelial turnover furthermore facilitates the repair of epithelial injuries and decreases the intestinal permeability induced by some pathogens. Consistently, the intestinal epithelium turnover rate constitutes a target for pathogens. Some bacteria can indeed block epithelial cell death to preserve their replication niches, whereas others trigger cell death to facilitate their egress or induce breaches in the epithelial barrier in order to access the underlying tissues [29] (Fig. 3).

2.2. Mechanisms of bacterial adhesion to host cells

Adhesion of bacteria to host surfaces is a crucial aspect of host colonization as it prevents the mechanical clearing of pathogens and confers a selective advantage towards bacteria of the endogenous flora. Accordingly, bacteria have evolved a very large arsenal of molecular strategies allowing them to target and adhere to host cells.

Pili, which are polymeric hair-like organelles protruding from the surface of bacteria, represent a first class of structures involved in the binding of bacteria to host cells [30,31]. The base of these structures, initially discovered in gram-negative bacteria, is anchored to the bacterial outer membrane, whereas the tip is usually an adherence factor conferring the binding specificity of these structures. For example, UPEC, which are uropathogenic strains of *Escherichia coli* colonizing the urinary tract and involved in kidney infections, display pyelonephritis-associated (P) pili at their surface. The tip of these pili is constituted by an adhesion factor called PapG, that binds to glycosphingolipids of the kidney epithelium [32]. Some UPEC strains also possess Type I pili at their surface, which have binding specificity to D-mannosylated receptors, such as the uroplakins of the bladder [33]. Type IV pili constitute another class of polymeric adhesive surface structures expressed by different gram-negative bacteria [34]. These pili are composed of thousands of copies of the major pilin protein that are first synthetized in the bacterial cytoplasm and then translocated across the inner membrane and proteolytically processed. Only the processed forms of pilin are competent for polymerization. The assembled pili then pass through the outer membrane via a channel formed by the secretin protein [34]. Once formed, these pili can aggregate laterally to form bundles. Type IV pili have the ability to retract through the bacterial cell wall, while the pilus tip remains attached to its target surface, allowing the so-called "twitching motility", a flagella-independent mode of motility important for efficient colonization of host surfaces [35]. In the case of *Neisseria meningitidis*, a bacterium found in the human nasopharynx but which may occasionally get access to the host bloodstream leading to sepsis and meningitis, type IV

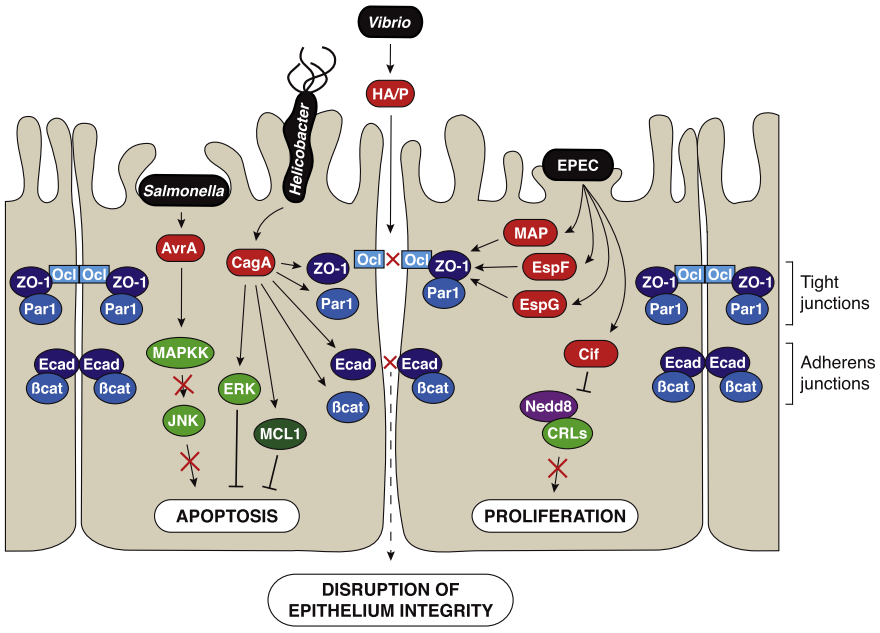

Fig. 3. Bacterial virulence factors manipulating epithelial cell functions. Examples of bacterial virulence factors targeting host proteins involved either in epithelial cell–cell junctions or in cell death and turnover. CagA, an effector of *Helicobacter pylori*, interacts with ZO-1, a component of tight junctions, and Par1, a regulator of cell polarity, and triggers disruption of epithelial tight junctions. This virulence factor also disrupts adherens junctions by targeting E-cadherin (Ecad) and promoting the release of β-catenin (βcat) from the adherence complex. *Vibrio cholerae* secretes the metalloprotease HA/P which degrades the extracellular domain of occluding (Ocl), another component of tight junctions. EPEC injects different effectors in the host cytoplasm, such as MAP, EspF or EspG that also target and disrupt tight junctions. AvrA, a factor delivered by *Salmonella* Typhimurium possesses acetyltransferase activity towards MAP kinase (MAPKK) and plays a major role in the downregulation of both inflammatory and c-Jun N-terminal kinase (JNK)-mediated epithelial cell death responses to infection. CagA, in addition to its alteration of cell–cell junctions, promotes cell proliferation by upregulating ERK, a pro-survival factor, and Mcl1, an anti-apoptotic factor. In contrast to AvrA or CagA, some pathogenic *E. coli* strains express Cif, an effector that blocks the cell cycle by inactivating Nedd8-conjugated Cullin-RING E3 Ubiquitin ligases (CRLs).

pili play a role in the formation of microcolonies attached to vascular endothelial cells [36]. Adhesion of bacteria to vascular endothelium is normally impaired by the shear stress, which represents the hydrodynamic forces generated by the blood components circulating at high speed through the vessels. Local and temporal drops in this shear stress, such as those observed in some brain capillaries, may however be compatible with an attachment of "seeding" *N. meningitidis* to the vascular endothelium [37]. Once attached, these bacteria are much more resistant to shear stress and can start to proliferate leading to the formation of microcolonies. This resistance to shear stress is in part due to the induction of filopodia-like cell projections, that can be observed inside or surrounding *Neisseria* microcolonies, and to the recruitment of several host proteins underneath bacteria [38]. Furthermore, upon divisions, bacteria remain aggregated via their type IV pili. It is interesting to note that, in some instance, some bacteria may modify their pili (via a post-translational modification of specific pili subunits) in order to destabilize pilus fiber interactions, leading to the detachment of these bacteria from the original microcolony and facilitating their dissemination to distant sites of infection in other vessels or in the cerebrospinal fluid in the cases of blood–brain barrier crossing (see below) [39]. In addition, antigenic variation has been observed for *Neisseria* type IV pili, allowing expression of new variants during infection and escape of this pathogen from the host immune system [34].

In the last decade, pili structures have also been observed in gram-positive bacteria. Two types of pili have been described so far in these species. The first class consists in "sortase-assembled pili", in which successive pilin subunits are linked by isopeptide bonds after translocation across the bacterial membrane. This linkage is catalyzed by bacterial transpeptidases called sortases allowing the formation of completely covalent polymers that are eventually linked to the cell wall peptidoglycan [40]. The second class consists in "type IV-like pili", which are similar to type IV pili of Gram-negative bacteria, even though the lack of outer membranes and the thick peptidoglycan structures of Gram-positive bacteria imply differences in the assembly mechanisms of these filaments [34]. Many studies are now deciphering the role of these pili in the adhesion of gram-positive pathogens to host cells and in pathogenesis.

In addition to pili, a wide range of bacterial surface factors with adhesive properties have been described. These adhesins recognize various classes of host molecules including transmembrane proteins such as integrins or cadherins, or components of the extracellular matrix such as collagen, fibronectin, laminin or elastin [30,31,41,42]. Some of these adhesins, after allowing the binding of bacteria to host cell surfaces, are also triggering the internalization of bacteria inside host cells (see below).

As already mentioned in the case of *Neisseria*, shear stress can decrease adhesion of bacteria bound to host surfaces under

fluid flow. For many bacterial adhesins, the probability of bond breaking increases with the tensile force derived from shear stress. However, in some instances, shear stress does not inhibit but rather promotes adhesion. This counter-intuitive phenomenon can be explained by the existence of specific force-strengthened bonds, called 'catch bonds' [43]. These bonds can be observed for example with the *E. coli* FimH adhesin, which exhibits a shear-enhanced binding to mannose [43].

In parallel to these canonical mechanisms of bacterial adhesion, the EPEC (Entero-Pathogenic *E. coli*) and EHEC (Entero-Hemorragic *E. coli*) pathogens, which are responsible respectively for diarrheal disease in children, and severe foodborne infections, use a very particular mechanism to create an intimate contact with host cells: they inject an effector, called Tir, that inserts into the host cell plasma membrane and serves as an "exogenous" receptor for the bacterial surface protein intimin [44]. Tir is delivered into the host cell cytoplasm via EPEC or EHEC type III secretion system (T3SS), a complex of proteins forming a needle-like structure that traverses the bacterial cell wall and the host-cell plasma membrane [45]. Binding of bacterial intimin to the extracellular domain of Tir is followed by the recruitment of host cell cytoskeleton regulators such as the Wiskott-Aldrich syndrome protein (N-WASP) and the actin-related protein 2/3 (Arp2/3) complex that locally remodels the actin cytoskeleton [44]. This remodeling leads to the retraction of the host cell absorptive microvilli and to the creation of a pedestal under the attached bacterium, thereby creating the characteristic "attaching and effacing" lesions induced by this pathogen. Tir thereby tethers the bacteria to the host epithelial cell surface and provides a direct connection between the bacteria and the host's cytoskeleton. These bacterial factors are essential for pathogenesis as mutants of intimin/Tir interaction do not colonize the intestine and are avirulent in animal models of infection [46].

Adhesion of bacteria to host surfaces is finally a key element in the formation of biofilms, *i.e.* matrix-enclosed microbial assemblies that can adhere to biological or non-biological surfaces. Biofilm formation constitutes a protected mode of growth that allows bacteria to survive in hostile environment. In the context of infectious diseases, biofilms may be critical as matrix-embedded bacterial aggregates are more resistant to host defenses or antibiotic treatments. The exact *in vivo* role of these biofilms during bacterial infections now constitutes an active field of research [47,48].

In conclusion, adhesion represents a crucial step for extracellular bacteria that facilitates their persistence in the host. For intracellular bacteria, it is a first essential step that precedes their internalization within host cells.

3. Establishment and maintenance of an intracellular lifestyle

An intracellular lifestyle provides diverse advantages for bacterial pathogens: they become inaccessible to humoral and complement-mediated attack; they avoid shear stress-induced clearance and get access to a wide range of nutrients, provided they display the metabolic pathways to use them. However, host cells also possess different mechanisms specifically targeting these intracellular bacteria. Intracellular pathogens have therefore developed different strategies to successfully establish and maintain an intracellular infection.

3.1. Getting inside host cells

Professional phagocytes, such as macrophages or M cells of the intestinal Peyer's patches, represent a frontline defense against pathogens. These cells also constitute a niche for bacteria with an intracellular lifestyle, as they naturally internalize foreign particles. After being phagocytosed by macrophages, bacteria such as *Mycobacterium tuberculosis*, the agent of tuberculosis, or *Legionella pneumophila*, the bacterium responsible for Legionnaire's disease, block the acidification of the phagosome and its fusion to lysosomes, thereby avoiding killing and allowing sustained survival in these cells. The ability of some of these phagocytes to migrate through tissues furthermore provides an interesting way for pathogens to disseminate inside their host.

Many bacteria can also induce their internalization into non-professional phagocytes. Two main mechanisms of entry are involved in this case, namely the zipper and the trigger mechanisms. Both of them rely on the activation of signaling cascades leading to the reorganization of the actin cytoskeleton at the level of the host plasma membrane [41,49], (Fig. 4).

In the case of the zipper mechanism, engagement of bacterial proteins with host membrane proteins normally involved in cellular adhesion such as cadherins or integrins, leads to the recruitment of various host factors involved in the strengthening of cell–cell or cell-matrix contacts. Due to the small size of bacteria, induction of a response normally strengthening cell attachment to extracellular matrix or neighboring cells results in this case to bacterial engulfment. *Listeria monocytogenes* (hereafter referred to as *Listeria*), a gram-positive food-borne pathogen responsible for human listeriosis, induces its internalization into non-phagocytic cells via a zipper mechanism [49–51]. Internalization of *Listeria* is mediated by two surface proteins, InlA and InlB, which respectively target E-cadherin and the hepatocyte growth factor receptor Met, which are both host plasma membrane proteins [52,53]. Met is ubiquitously expressed in human cells, whereas E-cadherin is expressed only in specific cell types, such as epithelial cells. Interaction of InlA with E-cadherin triggers the same signaling cascade as the one normally observed for E-cadherin/E-cadherin interactions [50,51]. This leads in particular to the recruitment of different host factors at the site of bacterial entry such as α- and β-catenin, myosin VIIa and vezatin [54]. InlB interaction with its receptor Met results in the recruitment of Gab1, Cbl and Shc, the activation of PI3 kinase and in actin remodeling at the site of entry [51]. Clathrin-mediated endocytosis machinery was shown to be involved in the early steps of *Listeria* internalization after the initial contact between InlA and InlB with their receptors and before cytoskeleton rearrangements [55]. Ultrastructural analysis by electron microscopy revealed the presence of

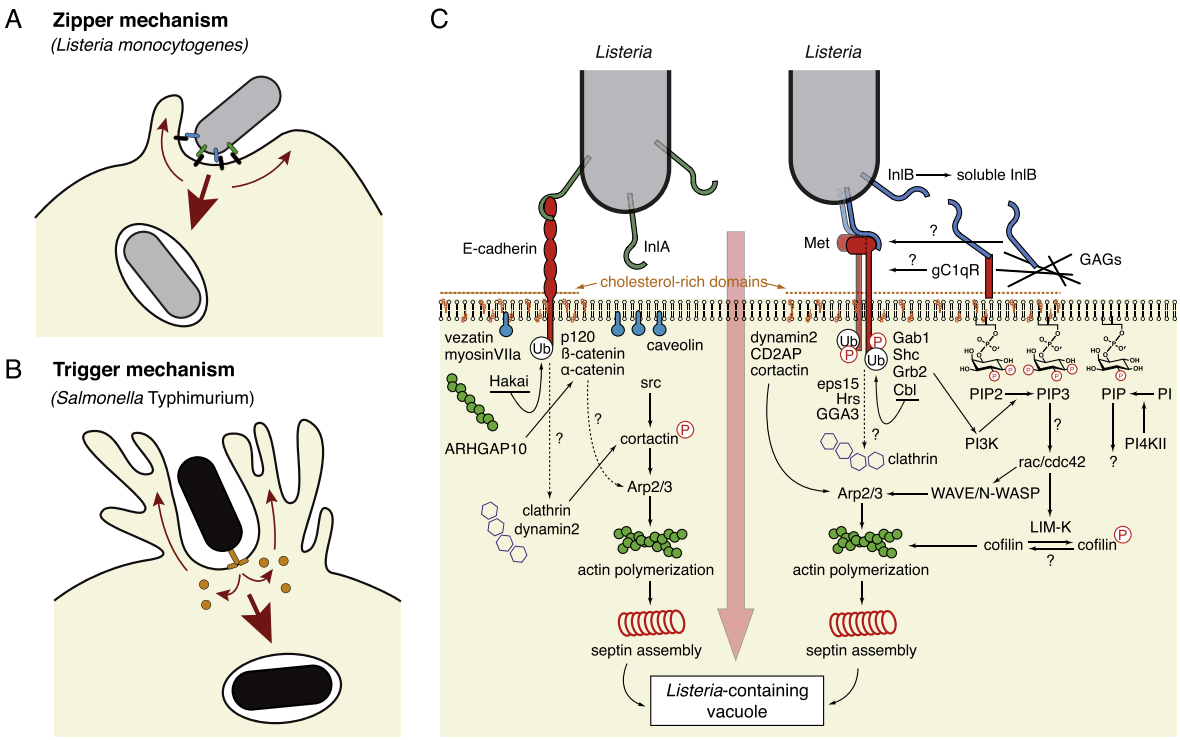

Fig. 4. "Zipper" versus "trigger" mechanisms of bacterial entry inside host cells. Schematic representation of internalization of *Listeria monocytogenes* via a "zipper" mechanism (A), or *Salmonella* Typhimurium via a "trigger" mechanism (B). In the "zipper" mechanism, engagement of bacterial surface proteins with host proteins induce cytoskeleton and membrane rearrangements, leading to the internalization of the bacterium. In the "trigger" mechanism, injection of effectors by the bacterium in the host cell cytoplasm triggers large-scale cytoskeletal rearrangements and ruffles formation allowing the bacterium to be engulfed and internalized. (C) Schematic representation of the multiple molecular pathways activated by bacterial surface proteins (in this case, InlA and InlB from *Listeria monocytogenes)* leading to the internalization of bacteria by a "zipper" mechanism (?: unknown interaction or component) (adapted from Ref. [67]).

isolated clathrin-coated pits assembled at *Listeria* entry foci [56]. Because these structures cannot internalize large particles such as bacteria, it was proposed that the observed clathrin coated pits serve as a platform for cytoskeletal rearrangements at bacteria-host adhesion sites [50,51,56]. Septins, a class of host proteins forming non polar filaments and participating to the cell cytoskeleton, constitute another player involved in the late step of *Listeria* entry process [57,58], (Fig. 4).

In the case of the trigger mechanism, bacteria activate signaling pathways leading to large-scale cytoskeletal rearrangements characterized by the formation of membrane protrusions called ruffles [30,41]. The extending ruffles then fold over and fuse back to the cell surface, thereby entrapping nearby bacteria (Fig. 4). This cellular process can normally be observed in response to soluble growth factors. *Salmonella* induces its internalization into non-phagocytic cells via a trigger mechanism. To do so, it injects directly in the host cell cytoplasm, via one of its two T3SS, a set of sophisticated bacterial effectors that trigger cellular responses [59]. Some of these effectors activate host cell Rho GTPases such as Cdc42 and Rac that spatiotemporally stimulate actin cytoskeleton rearrangements and allow membrane ruffling. Other effectors control these events and permit recovery of the cytoskeleton's normal architecture after infection, by deactivating Rho GTPases [59].

Interestingly, *Salmonella* exhibit target site preferences for internalization in tissue culture. Selection of these preferred loci involve a "near surface swimming" mode, in which flagella-driven motility allows bacteria to land onto and scan the host cell layer for "promising" entry sites [60]. For non-motile bacteria such as *Shigella flexneri*, the bacterial pathogen responsible for bacillary dysentery, it was reported that bacteria can get in contact with the epithelial layer via filopodial-like extensions emanating from the host cells. Upon bacterial contact, filopodia retract and bring *Shigella* in contact with the cell body where invasion occurs [61].

Finally, it should be noted that in the course of infection, one given pathogen can be internalized in different cell types and express different set of virulence genes. In such cases, each infected cell type may have a particular role for disease progression.

3.2. Diversity of intracellular compartments used for bacterial replication

After internalization, intracellular bacteria can replicate in three main classes of compartments. The first class is constituted by lysosomes-like vacuoles, which have an acidic pH and contain hydrolytic enzymes. The second corresponds to intracellular non acidic vacuoles that do not fuse to lysosomes and are usually remodeled by the pathogen. The third

compartment is the cytosol in which some pathogens can reside after escape from their internalization vacuole.

Coxiella burnetti, the agent of Q fever, is a well-known example of an intracellular bacterium able to survive in a lysosomal-like compartment [41,62]. Following internalization, the *Coxiella*-containing phagosome develops into a parasitophorous vacuole harboring lysosomal properties such as acidic pH, the presence of hydrolases and cationic peptides. Despite these harsh environmental conditions, *Coxiella* is able to efficiently replicate in this compartment although the mechanisms used by this pathogen to survive in this type of vacuole are poorly understood [62].

In addition to lysosomal-like vacuoles, there is a great diversity of non-acidic intracellular vacuoles in which pathogenic bacteria may reside [41]. In particular, pathogens are able to remodel the properties of these vacuoles by altering their proteic and lipidic composition or their trafficking and interaction with other vacuolar compartment of the host. *Salmonella*, for example, resides after internalization in vacuoles that undergo acidification but do not behave as lysosomes. Several effectors secreted by the second T3SS of *Salmonella* play important roles in the remodeling of these *Salmonella*-containing vacuoles [63]. Some effectors secreted across the vacuolar membrane remodel locally the actin cytoskeleton, allowing the polymerization of an actin basket surrounding these vacuoles and regulating bacterial virulence [64,65]. Other effectors block the recruitment of NADPH oxidase responsible for the production of bactericidal compounds that normally kill intracellular bacteria [66].

Finally, some pathogens such as *Listeria* are able to escape from their internalization vacuoles and get access to the host cell cytosol [41,67]. In the case of *Listeria*, this escape is mediated by LLO, a pore-forming toxin secreted by the bacteria and the two bacterial phospholipases PC- and PI-PLC (phosphatidylcholine and phosphatidylinositol-specific phospholipases C) [68]. Once in the cytosol, *Listeria* is able to replicate and to move inside cells using actin-based motility [67]. This intracellular motility leads to the formation of bacteria-containing protrusions and cell-to-cell spread. In some cases, protrusion formation is associated with plasma membrane damage due to LLO's pore forming activity. LLO also promotes the release of bacteria-containing vesicles from the host cell, covered with exofacial phosphatidylserine (PS), that can be recognized by PS-binding receptor expressed by macrophages, and phagocytosed. This mechanism, known as efferocytosis and normally used by the host to phagocytose dying or dead cells, is here exploited by *Listeria* to promote cell-to-cell spread and facilitate bacterial access to macrophages, which are key targets of *Listeria* during systemic infection [69].

Although life in the cytosol provides access to a wide-range of different nutrients, intracytosolic bacteria have also to face specific defense mechanisms such as autophagy [70,71]. Autophagy is a degradation process by which cytosolic components are delivered to lysosomal compartments. This process involves the formation of a double-membrane phagophore that closes to form an autophagosome, which then fuses to lysosomes leading to the degradation of enclosed material. Selective autophagy permits the targeting of specific components such as intracellular bacteria to autophagosomes via their detection by specific receptors. Bacterial autophagy was first described as an important host response degrading intracellular bacteria replicating in the cytosol. Of note, this process may also target intravacuolar bacteria. Consistently, several pathogens have evolved mechanisms to avoid autophagy-mediated degradation. This is the case for *Listeria*, which possesses at least two virulence factors, ActA and InlK, that disguise the bacteria from recognition by the autophagy machinery [72,73]. In addition, autophagy was shown to favor bacterial replication in some instances, revealing a much more complex interplay between autophagy and pathogens than previously expected [70,71].

Among the different advantages conferred by intracellular lifestyles, the internalization of pathogens in specific cell types, such as cells migrating through host tissues, facilitates the dissemination of bacteria in their host. In parallel, several pathogens have evolved specific mechanisms to cross epithelial and endothelial barriers and to get access to a wide range of host tissues.

4. Crossing of host barriers

Several types of sentinel cells, such as M cells, luminal macrophages or dendritic cells (DCs) are continuously sensing the presence of pathogenic bacteria in the mucosal environment. Although these cells are playing a key role in coordinating the innate and adaptative immune response to limit the colonization of pathogens in the host, they also constitute entry portals for pathogens.

M cells are specialized cells found in the intestinal epithelium and other epithelia in humans. These cells have a function different from that of their neighboring epithelial cells. In the intestine, they continuously sample the lumen and transport luminal antigens across the epithelial barrier to the underlying lymphoid tissue thereby contributing to intestinal immunity [74] (Fig. 1). M cells are exploited by many different pathogens as a route of entry to deeper tissues of the host. Intestinal ligated loop infection models have established that *S.* Typhimurium can cross the intestinal barrier via these M cells [75]. Indeed, targeting of M cells by *S.* Typhimurium leads to M cells destruction, thereby introducing breaches in the intestinal barrier. Bacteria are then able to spread rapidly to organs before the establishment of an immune response [75]. In a similar fashion, *S. flexneri* was shown to target and enter into M cells allowing translocation of bacteria across the epithelial barrier, without being toxic for these cells [76]. *S. flexneri* then reinvade epithelial cells basolaterally and triggers an inflammatory response that disrupts the epithelium, thereby facilitating the translocation of additional bacteria [76] (Fig. 1).

DCs constitute another cell type that sense antigens of the mucosal environment and which play a central role in the adaptative immunity. These cells, present in mucosal tissues, may migrate to mesenteric lymph nodes, where they

interact with lymphocytes. The phagocytic activity of these cells and their ability to migrate from periphery to circulation has been exploited by different bacterial pathogens [77]. *S.* Typhimurium, for example, may be taken up by DCs located in intestinal Peyer's patches, but also by inter-epithelial DCs that send dendrites between absorptive cells without altering the epithelial permeability [78] (Fig. 1). This mechanism was proposed to also participate to the rapid crossing of the host epithelium by *Salmonella* and to facilitate its dissemination in the host via reaching of the bloodstream [78].

Translocation through non phagocytic cells of the intestinal epithelium is another key mechanism used by pathogens to reach the lamina propria and to cause systemic infections. In the case of *Listeria*, interaction between the bacterial InlA surface protein and the host receptor E-cadherin is essential for crossing of the intestinal barrier [54,79]. E-cadherin is a key component of adherens junctions, and was first considered as being inaccessible for bacteria located in the intestinal lumen. However, accessible E-cadherin has been reported to be present at sites of cell extrusion at the tip of intestinal villi and at junctions between mucus-secreting goblet cells and adjacent enterocytes. These two locations have been shown to be sites of invasion for *Listeria* [80,81], (Fig. 1). In addition to the well-established intracellular lifestyle of *Listeria*, involving bacterial escape from the internalization vacuole, entry of bacteria through accessible E-cadherin can also lead to its rapid translocation, via transcytosis, across enterocytes, without bacterial escape from the internalization vacuole [81]. Of note, this mechanism of epithelium traversal has also been reported for *S.* Typhimurium which can traffic to the basolateral side of epithelial cells after invasion and is then released in the underlying lamina propria [82]. InlB, another *Listeria* internalin mediating its internalization in host cells, is not required for crossing of the intestinal barrier. However, it has been shown that this internalin is crucial, in addition to InlA, to cross the placental barrier [83]. The proposed mechanism is that InlB, via the activation of PI3 kinase, potentiates InlA-mediated downstream signaling, thereby increasing internalization efficiency at the level of the placenta [84].

Increasing epithelial or endothelial permeability is another strategy widely used by bacterial pathogens to cross host barriers [29,36,54]. Many pathogens are targeting cell–cell junctions to increase barrier permeability, thereby enhancing bacterial dissemination in the host (Fig. 3). For example, some T3SS effectors secreted by EPEC and EHEC destabilize tight junctions and induce a loss of trans-epithelial resistance [29,54]. *Vibrio cholerae* secretes a metalloprotease, called HA/P (hemagglutinin/protease), which cleaves the extracellular domain of host occludin, a key component of the tight junctions [85]. Another pathogenic species of *Vibrio*, *V. parahaemolyticus*, delivers in the host cytoplasm a virulence factor that AMPylates Rho GTPases (i.e. catalyzes the covalent addition of AMP to Rho GTPases), leading to the disruption of the actin cytoskeleton integrity and the rounding of targeted cells [86]. *N. meningitidis* is able to cross the host blood barrier by altering endothelial permeability. This bacterium is thought to gain access to the cerebrospinal fluid via the blood by crossing the vessel endothelium barrier. One proposed mechanism is that upon binding to endothelial cells, *N. meningitidis* recruits host cell proteins involved in the formation and the stabilization of adherens and tight junctions [87,88]. Several junction proteins are thereby depleted from cellular junctions and relocated beneath *Neisseria* microcolonies. This process may lead to a destabilization of endothelial junctions and increase the permeability of the vessels, facilitating bacterial escape from the blood vessel and colonization of the cerebrospinal fluid.

Finally, triggering of inflammation, in addition to its role on the microbiota discussed above, was also proposed as a tool used by pathogens for host barriers disruption [89]. Some inflammatory cytokines, such as TNF-α, indeed disrupt tight junctions and impair gut barrier integrity, and thereby may facilitate access to deeper tissues for bacterial intruders [90]. Interestingly, it was shown that during the course of infection by *S.* Typhimurium, a major portion of bacteria that invade epithelial cells is actually killed, but this fraction triggers the inflammation response of the host that benefits to the surviving bacteria [91]. This example illustrates the concept of phenotypic heterogeneity and cooperation for pathogenic bacteria, where some bacteria from a given population may fulfill specialized functions for the benefits of the overall community.

5. Conclusion

The diversity of niches that may be colonized by pathogenic bacteria in the human body is huge. Bacteria have evolved various mechanisms to adhere to the surface of organs in contact with the external milieu, such as the intestine. In addition, some bacteria can adopt an intracellular lifestyle and get internalized inside various host cells types to replicate away from the humoral host immune defenses. In this case, there is again a wide-range of strategies adopted by pathogenic bacteria, which can be illustrated by the different cellular locations they are able to use for replication. Finally, pathogenic bacteria can get access to deeper tissues using various mechanisms to cross mucosal barriers, and access the bloodstream, which is an entry portal for potentially all host organs, and is often associated to severe clinical symptoms.

In addition to mucosal surfaces, the skin also corresponds to a preferential site of contact with pathogens. As for mucosal barriers, the production of antimicrobial molecules and the presence of specific immune cells play important roles in cutaneous defenses [3]. Whereas most pathogens are unable to cross the skin barrier, they can however access the underlying tissues via ruptures in the skin, such as cuts, microlesions or bites (in particular for pathogens transmitted via arthropod vectors).

With regard to the diversity of niches used by pathogenic bacteria for replication, scientists have classified bacteria as extra- or intra-cellular or, for intracellular bacteria, as intravacuolar or intracytosolic. However, increasing evidence now shows that bacteria initially thought to remain strictly extracellular can indeed be found inside host cells as

exemplified by the case of *Staphylococcus aureus* [92]. In addition, some intracellular bacteria can be observed both in vacuoles or free in the cytoplasm. This situation is well accepted for some pathogens such as *Salmonella* but is more controversial for others such as *Mycobacterium or Legionella*. Further work is therefore needed to clearly define the different compartments where a given bacterium can be found, and more particularly during infection *in vivo*. Deciphering the respective role of these compartments in the establishment of the associated disease is also critical as some of them may only represent dead-ends during the course of infection.

The frontier between commensals and pathogens is also not as straightforward as expected. Indeed, some bacteria normally considered as commensals, can become pathogenic when they escape their original niche and start to colonize deeper tissues. Bacteria belonging to the microbiota, and therefore considered as commensals, can also become pathogenic if their growth rate raises and if they outcompete other members of the intestinal flora. For *bona fide* pathogens, variability in the expression of virulence factors has also been observed. Indeed, virulence factors are not constitutively expressed and their production tightly depends on the environmental conditions faced by the bacterium. As a given bacterium can be found, depending on the stage of infection, in the intestinal lumen, inside epithelial cells or professional phagocytes, or in the bloodstream, the set of virulence factors expressed in these different conditions has to vary accordingly in order to face the different host defense mechanism encountered. *Shigella flexneri*, for example, has the capacity to sense the gradient of oxygen that is present between the anaerobic intestinal lumen and the oxygenized intestinal tissues. Activation of its T3SS is effective only at its precise site of action, in relatively oxygenized area, nearby intestinal epithelial cells, thereby allowing enhanced invasion and virulence [93]. Similarly, intracellular *Salmonella* Typhimurium is sensing environmental pH to coordinate the secretion of T3SS effectors. Assembly of one of its T3SS is indeed done after internalization into host cells, in response to the acidification of the *Salmonella*-containing vacuoles (SCVs) [94]. The corresponding T3SS effectors are further secreted only after the tip of T3SS needle gets in contact with the neutral pH lying outside of the SCVs, allowing a tightly regulated secretion of these bacterial molecules in the cytoplasm of the infected cells [95].

It is also important to mention that the result of a bacterial infection is tightly dependent on host susceptibility. Genetic polymorphism in the host population accounts for a great variability in the type or intensity of responses triggered against the encountered pathogen. The same bacterium can thus cause a large spectrum of clinical manifestations from asymptomatic infection to fatal disease, depending on host genetic variability.

Finally, in addition to genetic-driven host susceptibility, it is now well-established that the microbiota is playing a critical role to limit colonization and invasion by enteric pathogens. Many studies are now highlighting that the composition of this microbiota may be altered by various external parameters including overuse of antibiotics, changes in diet and elimination of constitutive partners such as nematodes [96,97]. For example, after antibiotic treatment, the composition of the microbiota was demonstrated to be different from the original one, and this modified microbiota may be more prone to colonization by specific bacterial pathogens [98]. Characterization of the composition of microbiota from patients by high throughput sequencing techniques will open new avenues for the development of personalized diagnosis, the potential manipulation and modification of this microbiota, and the development of new treatments to prevent and limit infections by enteric bacterial pathogens.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

Work in the Pascale COSSART laboratory is supported by the Institut Pasteur, INSERM, INRA, ANR (ERANET Pathogenomics LISTRESS ANR-2010-PATH-001-01, ERANET Infect-ERA PROANTILIS ANR-2013-IFEC-0004-02 and BACNET 10-BINF-02-01), the French Government's Investissements d'Avenir program, Laboratoire d'Excellence “Integrative Biology of Emerging Infectious Diseases” (grant n° ANR-10-LABX-62-IBEID), ERC (Advanced Grant #233348 MODELIST), Human Frontier Science Program (RGP0011/2013), the Fondation le Roch les Mousquetaires and the Fondation Louis-Jeantet. D.R. is a Research Associate from INSERM and P.C. is a senior international research scholar of the Howard Hughes Medical Institute.

References

[1] Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science 2009;326:1694–7.
[2] Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207–14.
[3] Schommer NN, Gallo RL. Structure and function of the human skin microbiome. Trends Microbiol 2013;21:660–8.
[4] Lemon KP, Klepac-Ceraj V, Schiffer HK, Brodie EL, Lynch SV, Kolter R. Comparative analyses of the bacterial microbiota of the human nostril and oropharynx. MBio 2010;1.
[5] Wade WG. The oral microbiome in health and disease. Pharmacol Res 2013;69:137–43.
[6] Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. Annu Rev Microbiol 2012;66:371–89.
[7] McGuckin MA, Linden SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. Nat Rev Microbiol 2011;9:265–78.
[8] Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci U S A 2008;105:15064–9.
[9] Putsep K, Axelsson LG, Boman A, Midtvedt T, Normark S, Boman HG, et al. Germ-free and colonized mice generate the same products from enteric prodefensins. J Biol Chem 2000;275:40478–82.
[10] Brandl K, Plitas G, Schnabl B, DeMatteo RP, Pamer EG. MyD88-mediated signals induce the bactericidal lectin RegIII gamma and

protect mice against intestinal Listeria monocytogenes infection. J Exp Med 2007;204:1891−900.
[11] Vaishnava S, Behrendt CL, Ismail AS, Eckmann L, Hooper LV. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc Natl Acad Sci U S A 2008;105:20858−63.
[12] Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2005;307:731−4.
[13] Suzuki K, Meek B, Doi Y, Muramatsu M, Chiba T, Honjo T, et al. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. Proc Natl Acad Sci U S A 2004;101:1981−6.
[14] Macpherson AJ, Gatto D, Sainsbury E, Harriman GR, Hengartner H, Zinkernagel RM. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 2000;288:2222−6.
[15] Linden SK, Sheng YH, Every AL, Miles KM, Skoog EC, Florin TH, et al. MUC1 limits Helicobacter pylori infection both by steric hindrance and by acting as a releasable decoy. PLoS Pathog 2009;5:e1000617.
[16] Celli JP, Turner BS, Afdhal NH, Keates S, Ghiran I, Kelly CP, et al. Helicobacter pylori moves through mucus by reducing mucin viscoelasticity. Proc Natl Acad Sci U S A 2009;106:14321−6.
[17] Guerry P. Campylobacter flagella: not just for motility. Trends Microbiol 2007;15:456−61.
[18] Ottemann KM, Lowenthal AC. Helicobacter pylori uses motility for initial colonization and to attain robust infection. Infect Immun 2002;70:1984−90.
[19] Lidell ME, Moncada DM, Chadee K, Hansson GC. Entamoeba histolytica cysteine proteases cleave the MUC2 mucin in its C-terminal domain and dissolve the protective colonic mucus gel. Proc Natl Acad Sci U S A 2006;103:9298−303.
[20] Boneca IG, Dussurget O, Cabanes D, Nahori MA, Sousa S, Lecuit M, et al. A critical role for peptidoglycan N-deacetylation in Listeria evasion from the host innate immune system. Proc Natl Acad Sci U S A 2007;104:997−1002.
[21] Raffatellu M, George MD, Akiyama Y, Hornsby MJ, Nuccio SP, Paixao TA, et al. Lipocalin-2 resistance confers an advantage to Salmonella enterica serotype Typhimurium for growth and survival in the inflamed intestine. Cell Host Microbe 2009;5:476−86.
[22] Stecher B, Hardt WD. Mechanisms controlling pathogen colonization of the gut. Curr Opin Microbiol 2011;14:82−91.
[23] Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol 2010;10:159−69.
[24] Shroff KE, Meslin K, Cebra JJ. Commensal enteric bacteria engender a self-limiting humoral mucosal immune response while permanently colonizing the gut. Infect Immun 1995;63:3904−13.
[25] Benveniste J, Lespinats G, Salomon J. Serum and secretory IgA in axenic and holoxenic mice. J Immunol 1971;107:1656−62.
[26] Pedron T, Sansonetti P. Commensals, bacterial pathogens and intestinal inflammation: an intriguing menage a trois. Cell Host Microbe 2008;3:344−7.
[27] Stecher B, Barthel M, Schlumberger MC, Haberli L, Rabsch W, Kremer M, et al. Motility allows S. Typhimurium to benefit from the mucosal defence. Cell Microbiol 2008;10:1166−80.
[28] Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, et al. Gut inflammation provides a respiratory electron acceptor for Salmonella. Nature 2010;467:426−9.
[29] Kim M, Ashida H, Ogawa M, Yoshikawa Y, Mimuro H, Sasakawa C. Bacterial interactions with the host epithelium. Cell Host Microbe 2010;8:20−35.
[30] Pizarro-Cerda J, Cossart P. Bacterial adhesion and entry into host cells. Cell 2006;124:715−27.
[31] Kline KA, Falker S, Dahlberg S, Normark S, Henriques-Normark B. Bacterial adhesins in host-microbe interactions. Cell Host Microbe 2009;5:580−92.
[32] Roberts JA, Marklund BI, Ilver D, Haslam D, Kaack MB, Baskin G, et al. The Gal(alpha 1-4)Gal-specific tip adhesin of Escherichia coli P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract. Proc Natl Acad Sci U S A 1994;91:11889−93.
[33] Lillington J, Geibel S, Waksman G. Biogenesis and adhesion of type 1 and P pili. Biochim Biophys Acta 2014;1840:2783−93.
[34] Melville S, Craig L. Type IV pili in Gram-positive bacteria. Microbiol Mol Biol Rev 2013;77:323−41.
[35] Mattick JS. Type IV pili and twitching motility. Annu Rev Microbiol 2002;56:289−314.
[36] Melican K, Dumenil G. Vascular colonization by Neisseria meningitidis. Curr Opin Microbiol 2012;15:50−6.
[37] Mairey E, Genovesio A, Donnadieu E, Bernard C, Jaubert F, Pinard E, et al. Cerebral microcirculation shear stress levels determine Neisseria meningitidis attachment sites along the blood-brain barrier. J Exp Med 2006;203:1939−50.
[38] Mikaty G, Soyer M, Mairey E, Henry N, Dyer D, Forest KT, et al. Extracellular bacterial pathogen induces host cell surface reorganization to resist shear stress. PLoS Pathog 2009;5:e1000314.
[39] Chamot-Rooke J, Mikaty G, Malosse C, Soyer M, Dumont A, Gault J, et al. Posttranslational modification of pili upon cell contact triggers N. meningitidis dissemination. Science 2011;331:778−82.
[40] Kang HJ, Baker EN. Structure and assembly of Gram-positive bacterial pili: unique covalent polymers. Curr Opin Struct Biol 2012;22:200−7.
[41] Cossart P, Roy CR. Manipulation of host membrane machinery by bacterial pathogens. Curr Opin Cell Biol 2010;22:547−54.
[42] Chagnot C, Listrat A, Astruc T, Desvaux M. Bacterial adhesion to animal tissues: protein determinants for recognition of extracellular matrix components. Cell Microbiol 2012;14:1687−96.
[43] Sokurenko EV, Vogel V, Thomas WE. Catch-bond mechanism of force-enhanced adhesion: counterintuitive, elusive, but... widespread? Cell Host Microbe 2008;4:314−23.
[44] Lai Y, Rosenshine I, Leong JM, Frankel G. Intimate host attachment: enteropathogenic and enterohaemorrhagic Escherichia coli. Cell Microbiol 2013;15:1796−808.
[45] Kenny B, DeVinney R, Stein M, Reinscheid DJ, Frey EA, Finlay BB. Enteropathogenic E. coli (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell 1997;91:511−20.
[46] Crepin VF, Girard F, Schuller S, Phillips AD, Mousnier A, Frankel G. Dissecting the role of the Tir:Nck and Tir:IRTKS/IRSp53 signalling pathways in vivo. Mol Microbiol 2010;75:308−23.
[47] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2004;2:95−108.
[48] Bjarnsholt T, Alhede M, Alhede M, Eickhardt-Sorensen SR, Moser C, Kuhl M, et al. The in vivo biofilm. Trends Microbiol 2013;21:466−74.
[49] Pizarro-Cerda J, Cossart P. Listeria monocytogenes membrane trafficking and lifestyle: the exception or the rule? Annu Rev Cell Dev Biol 2009;25:649−70.
[50] Pizarro-Cerda J, Kuhbacher A, Cossart P. Entry of Listeria monocytogenes in mammalian epithelial cells: an updated view. Cold Spring Harb Perspect Med 2012;2.
[51] Cossart P, Helenius A. Endocytosis of viruses and bacteria. Cold Spring Harb Perspect Biol 2014;6.
[52] Mengaud J, Ohayon H, Gounon P, Mege RM, Cossart P. E-cadherin is the receptor for internalin, a surface protein required for entry of L. monocytogenes into epithelial cells. Cell 1996;84:923−32.
[53] Shen Y, Naujokas M, Park M, Ireton K. InIB-dependent internalization of Listeria is mediated by the Met receptor tyrosine kinase. Cell 2000;103:501−10.
[54] Bonazzi M, Cossart P. Impenetrable barriers or entry portals? The role of cell-cell adhesion during infection. J Cell Biol 2011;195:349−58.
[55] Veiga E, Cossart P. Listeria hijacks the clathrin-dependent endocytic machinery to invade mammalian cells. Nat Cell Biol 2005;7:894−900.
[56] Bonazzi M, Vasudevan L, Mallet A, Sachse M, Sartori A, Prevost MC, et al. Clathrin phosphorylation is required for actin recruitment at sites of bacterial adhesion and internalization. J Cell Biol 2011;195:525−36.
[57] Mostowy S, Danckaert A, Tham TN, Machu C, Guadagnini S, Pizarro-Cerda J, et al. Septin 11 restricts InlB-mediated invasion by Listeria. J Biol Chem 2009;284:11613−21.

[58] Mostowy S, Nam Tham T, Danckaert A, Guadagnini S, Boisson-Dupuis S, Pizarro-Cerda J, et al. Septins regulate bacterial entry into host cells. PLoS One 2009;4:e4196.
[59] Schlumberger MC, Hardt WD. Salmonella type III secretion effectors: pulling the host cell's strings. Curr Opin Microbiol 2006;9:46–54.
[60] Misselwitz B, Barrett N, Kreibich S, Vonaesch P, Andritschke D, Rout S, et al. Near surface swimming of Salmonella Typhimurium explains target-site selection and cooperative invasion. PLoS Pathog 2012;8:e1002810.
[61] Romero S, Grompone G, Carayol N, Mounier J, Guadagnini S, Prevost MC, et al. ATP-mediated Erk1/2 activation stimulates bacterial capture by filopodia, which precedes Shigella invasion of epithelial cells. Cell Host Microbe 2011;9:508–19.
[62] van Schaik EJ, Chen C, Mertens K, Weber MM, Samuel JE. Molecular pathogenesis of the obligate intracellular bacterium Coxiella burnetii. Nat Rev Microbiol 2013;11:561–73.
[63] Agbor TA, McCormick BA. Salmonella effectors: important players modulating host cell function during infection. Cell Microbiol 2011;13:1858–69.
[64] Poh J, Odendall C, Spanos A, Boyle C, Liu M, Freemont P, et al. SteC is a Salmonella kinase required for SPI-2-dependent F-actin remodelling. Cell Microbiol 2008;10:20–30.
[65] Odendall C, Rolhion N, Forster A, Poh J, Lamont DJ, Liu M, et al. The Salmonella kinase SteC targets the MAP kinase MEK to regulate the host actin cytoskeleton. Cell Host Microbe 2012;12:657–68.
[66] Vazquez-Torres A, Xu Y, Jones-Carson J, Holden DW, Lucia SM, Dinauer MC, et al. Salmonella pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. Science 2000;287:1655–8.
[67] Cossart P. Illuminating the landscape of host-pathogen interactions with the bacterium Listeria monocytogenes. Proc Natl Acad Sci U S A 2011;108:19484–91.
[68] Hamon MA, Ribet D, Stavru F, Cossart P. Listeriolysin O: the Swiss army knife of Listeria. Trends Microbiol 2012;20:360–8.
[69] Czuczman MA, Fattouh R, van Rijn JM, Canadien V, Osborne S, Muise AM, et al. Listeria monocytogenes exploits efferocytosis to promote cell-to-cell spread. Nature 2014;509:230–4.
[70] Mostowy S, Cossart P. Bacterial autophagy: restriction or promotion of bacterial replication? Trends Cell Biol 2012;22:283–91.
[71] Gomes LC, Dikic I. Autophagy in antimicrobial immunity. Mol Cell 2014;54:224–33.
[72] Yoshikawa Y, Ogawa M, Hain T, Yoshida M, Fukumatsu M, Kim M, et al. Listeria monocytogenes ActA-mediated escape from autophagic recognition. Nat Cell Biol 2009;11:1233–40.
[73] Dortet L, Mostowy S, Samba-Louaka A, Gouin E, Nahori MA, Wiemer EA, et al. Recruitment of the major vault protein by InlK: a Listeria monocytogenes strategy to avoid autophagy. PLoS Pathog 2011;7:e1002168.
[74] Corr SC, Gahan CC, Hill C. M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis. FEMS Immunol Med Microbiol 2008;52:2–12.
[75] Jones BD, Ghori N, Falkow S. Salmonella typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. J Exp Med 1994;180:15–23.
[76] Perdomo OJ, Cavaillon JM, Huerre M, Ohayon H, Gounon P, Sansonetti PJ. Acute inflammation causes epithelial invasion and mucosal destruction in experimental shigellosis. J Exp Med 1994;180:1307–19.
[77] Niedergang F, Didierlaurent A, Kraehenbuhl JP, Sirard JC. Dendritic cells: the host Achille's heel for mucosal pathogens? Trends Microbiol 2004;12:79–88.
[78] Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2001;2:361–7.
[79] Lecuit M, Vandormael-Pournin S, Lefort J, Huerre M, Gounon P, Dupuy C, et al. A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. Science 2001;292:1722–5.
[80] Pentecost M, Otto G, Theriot JA, Amieva MR. Listeria monocytogenes invades the epithelial junctions at sites of cell extrusion. PLoS Pathog 2006;2:e3.
[81] Nikitas G, Deschamps C, Disson O, Niault T, Cossart P, Lecuit M. Transcytosis of Listeria monocytogenes across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. J Exp Med 2011;208:2263–77.
[82] Muller AJ, Kaiser P, Dittmar KE, Weber TC, Haueter S, Endt K, et al. Salmonella gut invasion involves TTSS-2-dependent epithelial traversal, basolateral exit, and uptake by epithelium-sampling lamina propria phagocytes. Cell Host Microbe 2012;11:19–32.
[83] Disson O, Grayo S, Huillet E, Nikitas G, Langa-Vives F, Dussurget O, et al. Conjugated action of two species-specific invasion proteins for fetoplacental listeriosis. Nature 2008;455:1114–8.
[84] Gessain G, Tsai YH, Travier L, Bonazzi M, Grayo S, Cossart P, et al. PI3-kinase activation is critical for host barriers permissiveness to Listeria monocytogenes. J Exp Med January 26, 2015 [Epub ahead of print].
[85] Wu Z, Nybom P, Magnusson KE. Distinct effects of Vibrio cholerae haemagglutinin/protease on the structure and localization of the tight junction-associated proteins occludin and ZO-1. Cell Microbiol 2000;2:11–7.
[86] Yarbrough ML, Li Y, Kinch LN, Grishin NV, Ball HL, Orth K. AMPylation of Rho GTPases by Vibrio VopS disrupts effector binding and downstream signaling. Science 2009;323:269–72.
[87] Coureuil M, Mikaty G, Miller F, Lecuyer H, Bernard C, Bourdoulous S, et al. Meningococcal type IV pili recruit the polarity complex to cross the brain endothelium. Science 2009;325:83–7.
[88] Coureuil M, Lecuyer H, Scott MG, Boularan C, Enslen H, Soyer M, et al. Meningococcus Hijacks a beta2-adrenoceptor/beta-Arrestin pathway to cross brain microvasculature endothelium. Cell 2010;143:1149–60.
[89] Berkes J, Viswanathan VK, Savkovic SD, Hecht G. Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. Gut 2003;52:439–51.
[90] Gitter AH, Bendfeldt K, Schmitz H, Schulzke JD, Bentzel CJ, Fromm M. Epithelial barrier defects in HT-29/B6 colonic cell monolayers induced by tumor necrosis factor-alpha. Ann N. Y Acad Sci 2000;915:193–203.
[91] Ackermann M, Stecher B, Freed NE, Songhet P, Hardt WD, Doebeli M. Self-destructive cooperation mediated by phenotypic noise. Nature 2008;454:987–90.
[92] Garzoni C, Kelley WL. Staphylococcus aureus: new evidence for intracellular persistence. Trends Microbiol 2009;17:59–65.
[93] Marteyn B, West NP, Browning DF, Cole JA, Shaw JG, Palm F, et al. Modulation of Shigella virulence in response to available oxygen in vivo. Nature 2010;465:355–8.
[94] Rappl C, Deiwick J, Hensel M. Acidic pH is required for the functional assembly of the type III secretion system encoded by Salmonella pathogenicity island 2. FEMS Microbiol Lett 2003;226:363–72.
[95] Yu XJ, McGourty K, Liu M, Unsworth KE, Holden DW. pH sensing by intracellular Salmonella induces effector translocation. Science 2010;328:1040–3.
[96] Willing BP, Russell SL, Finlay BB. Shifting the balance: antibiotic effects on host-microbiota mutualism. Nat Rev Microbiol 2011;9:233–43.
[97] Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell 2014;157:121–41.
[98] Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 2013;13:790–801.
[99] Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. Nat Rev Immunol 2010;10:131–44.