Chapter 13

Acting on Actin During Bacterial Infection

Elsa Anes

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66861

Abstract

Bacterial resistance to antibiotics is becoming a major threat to public health. It is imperative to find new therapeutic interventions to fight pathogens. Thus, deciphering host-pathogen interactions may allow defining targets for new strategies for effective treatments of infectious diseases. This chapter focuses on the bacterial manipulation of the host cell actin cytoskeleton. We discuss three infectious processes. The first is pathogen establishment of infection/invasion, explaining cellular uptake pathways that rely on actin, such as phagocytosis and macropinocytosis. The second process focus on the establishment of a replication niche, a process that subverts cytoskeletal functions associated with membrane trafficking namely phagosome maturation and cellular innate immune responses. Finally, pathogen dissemination is an emerging field that microfilaments have shown to participate: pathogen motility through the cytoplasm and from cell-to-cell or on the outer surface of the plasma membrane mimicking a receptor tyrosine kinase signaling pathway that helps the projection of pathogens to neighboring cells. It also establishes a connection with the innate immunity related with induction of cell signaling to inflammation, inflammasome activation, and programmed cell death. These studies revealed several potential targets related to actin cytoskeleton manipulation to design new therapeutic strategies for bacterial infections.

Keywords: actin, Rho GTPases, bacterial pathogens, phagocytosis, macropinocytosis, virulence mechanisms, innate immunity

1. Introduction

The cell cytoskeleton is composed of three distinct protein families each of which is assembled from monomers to form polymer networks namely from actin, tubulin, or intermediate-filament proteins. Host and pathogens have developed intrinsic interactions with the cytoskeletal system, playing a central role in several stages of their life cycles. Deciphering the complexity of these interactions is revealing new insights about the mechanisms of bacterial pathogenicity but also on defining new host targets for alternative therapies to available antibiotics.

Indeed, clarifying these bacterial mechanisms of host subversion has led to many discoveries about host cell biology, including the identification of new cytoskeletal proteins, regulatory pathways, and mechanisms of cytoskeletal function. Microorganisms exploit actin, microtubules, and intermediate filaments in diverse ways, however, it is mainly the actin cytoskeleton that appears to play a critical role in infection and is the topic of this chapter.

In host cells, actin is involved in the polymerization of stable filaments to assure the cell architecture; at the cell surface originates dynamic movements mediated via assembly and disassembly of microfilaments contributing to contour changes as well cellular locomotion,

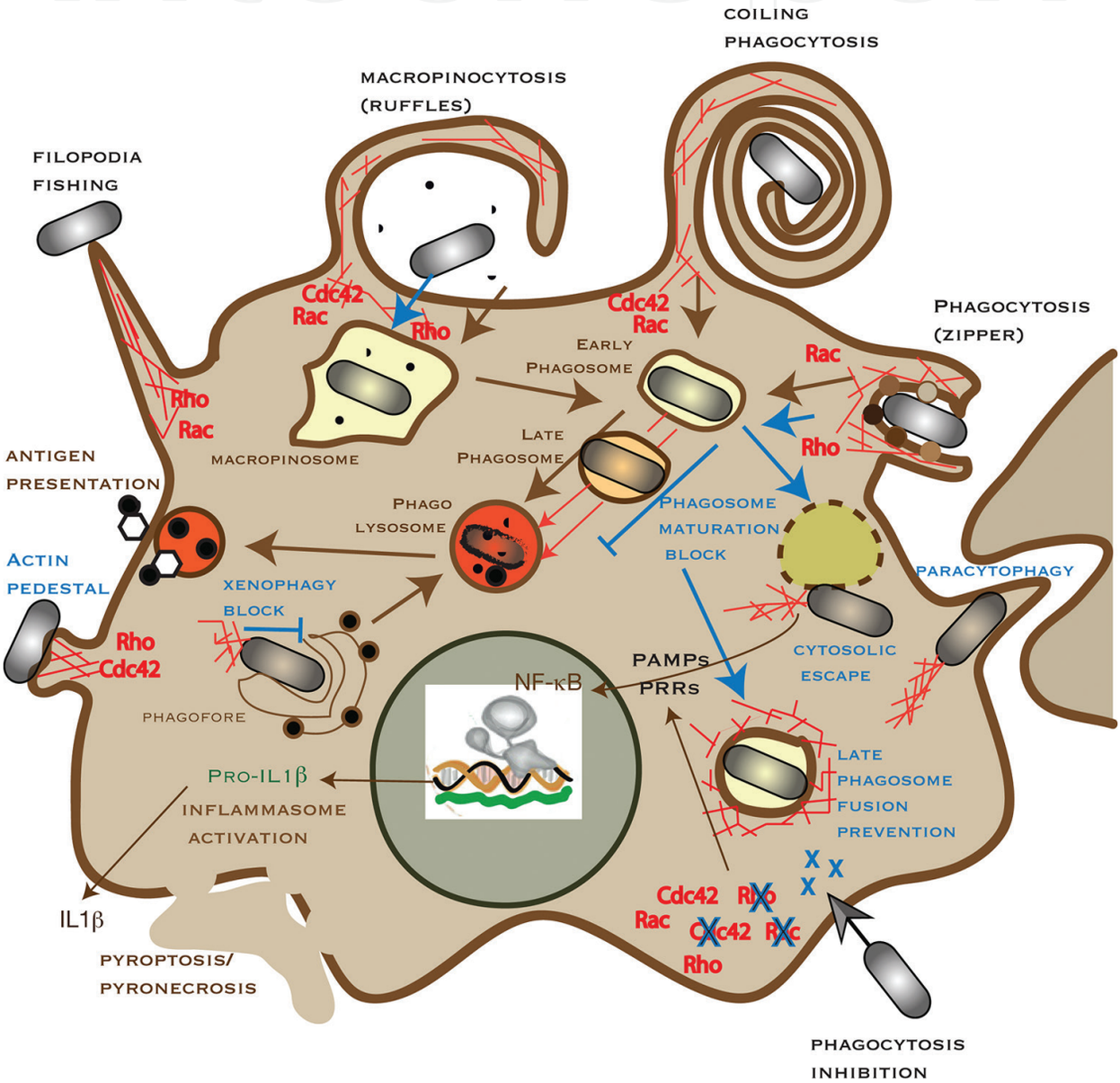

Figure 1. Schematic diagram of host cell actin rearrangements during bacterial infection. In red: actin filaments and actin polymerization promoting Rho GTPases. In brown: cell responses to bacterial infection. In blue: bacteria hijacking mechanisms of the host actin cytoskeleton.

http://dx.doi.org/10.5772/66861

cell-to-cell adhesion, and signaling. In the cytoplasm, the actin skeleton provides tracks and tails to direct vesicle trafficking. Thus, the importance of the actin cytoskeleton for eukaryotic host physiology from cell movement, cell-to-cell adherence, endocytosis, vesicle trafficking, and cell signaling, among others, has provided pathogenic bacteria with a plethora of opportunistic chances to be exploited.

The roles of the actin cytoskeleton in host-pathogen interactions can be summarized according to groups of pathogens and how they interact with this system. Some promote attachment to the plasma membrane, forming specialized actin structures (pedestals), allowing strong adherence to host epithelial surfaces. Others induce actin polymerization to enter into nonprofessional phagocytic cells; while others prevent polymerization to avoid uptake by professional phagocytic cells. A few pathogens use the actin cytoskeleton to allow other specialized internalization processes to occur in phagocytic cells as an alternative or in addition to phagocytosis. Intracellular pathogens manipulate the cytoskeleton to prevent membrane trafficking or fusion events leading to the establishment of a niche inside a vacuole often avoiding delivery into the degradative environment of the lysosome. Finally, some pathogens escape from the phagosome vacuole to the cytosol and use the actin machinery to move within cells and to spread directly from the cytoplasm of one cell into the cytoplasm of an adjacent cell. Recently, actin dynamics during infection was related to innate immune responses that rely on activation of cytosolic pattern recognition receptors (cytosolic PRRs) for inflammasome or autophagy assembly and programmed cell death.

This chapter provides a comprehensive summary of various strategies used by both extracellular and intracellular bacteria to hijack the host actin cytoskeleton (**Figure 1**).

2. Acting on actin during pathogen establishment of infection/invasion

Pathogens often have to overcome epithelial barriers to gain entry into the host cells. The first of which is the epithelial mucosae and a few pathogens, along their evolution, have developed strategies to overcome these barriers by means of active invasion mechanisms. Therefore some intracellular pathogens have evolved strategies to induce or modulate their uptake into these nonprofessional phagocytic cells. Alternatively, as a barrier circumventing mechanism, they may use the cells of the immune system (professional phagocytic cells such as macrophages, neutrophils, and dendritic cells) that patrols those epithelia. Here pathogens may or not play an active role in host cell internalization. Usually professional phagocytes recognize pattern signatures of pathogens (e.g., lipopolysaccharides: LPS), or opsonized bacteria (e.g., complement C3 or IgGs), by means of surface receptors. Likewise phagocytes play an active role in bacteria internalization. As part of the immune system these cells are equipped with a series of insult mechanisms designed to clear pathogens (as the proteolysis at low pH in the phagolysosome). Likewise, extracellular pathogens modulate the host cell plasma membrane for attachment and inhibition of phagocytosis in order to survive. In contrast, intracellular pathogens developed strategies to circumvent the bactericidal mechanisms of immune cells via establishing a protective vacuolar niche.

Several actin dependent mechanisms exist for allowing the establishment of infection: (1) Conventional phagocytosis meaning the entry into professional phagocytes by bilateral membrane pseudopodia formation that tightly encloses the bacteria. Phagocytosis always involves close contact between particle and plasma membrane by multivalence receptor-ligand interactions following morphological changes assembling a zipper mechanism. The host plays a central role for the internalization event while no action is required from the pathogen; (2) induced phagocytosis, a process of active induction of internalization into nonprofessional phagocytes such as epithelial cells, by pathogen manipulation of the host cell contractile system; both the host and the pathogen have active roles in the event. Mechanistically the process occurs by strong interactions between bacterial ligands with cell receptors as in conventional phagocytosis; (3) macropinocytosis: here there may be no direct contact between ligand-pathogen and cell-receptors. Literally, macropinocytosis means—cell drinking—and always involves extensive signaling (e.g., via EGF receptor, a type of tyrosine kinase receptor) that induces pseudopodia unilateral formation surrounding large amount of extracellular volume. So particles including bacteria go in passively along with extracellular fluid. Conventional macropinocytosis may occurs in several types of cells including professional and nonprofessional phagocytes leading to the formation of a large vacuole, the macropinosome; (4) induced macropinocytosis involves pathogen manipulation of the host cell cytoskeleton through growth factor induced signaling or directly using secretion systems that injects virulence factors into the cytosol. While referred classically as trigger phagocytosis, according to the type of morphological changes (with multiple ruffles at the cell surface), there is no direct connection between pathogen and plasma membrane. Finally, (5) an unconventional form of phagocytosis may be used for the establishment of infection via actin cytoskeleton. This is termed as coiling phagocytosis and involves single folds of the phagocyte plasma membrane wrapping around microbes in multiple turns **(Figure 1)**.

2.1. Phagocytosis of bacteria and inhibition of phagocytosis by pathogens

Phagocytosis is a universal phenomenon involving the recognition and binding of a particle (over 0.5 μm in diameter), in a multivalence receptor-dependent manner, to its internalization and degradation within the phagocytic cell [1]. Mechanistically the process of particle internalization from the plasma membrane is clathrin independent and requires actin polymerization [2]. Phagocytosis of one particle does not signal or permit the indiscriminate phagocytosis of other particles bound to the cell surface. In fact particle ingestion is not automatically triggered by initial particle binding, but requires the sequential recruitment of cell surface receptors into interactions with the remainder of the particle surface. The forming phagosome conforms to the shape of the particle as a close-fitting sleeve of plasma membrane, held in place by interactions between surface receptors and the particle surface, much as teeth hold a zipper together [3]. Phagocytosis can be broadly categorized into three steps: particle binding (along with receptor-cell signaling), internalization (i.e., phagosome formation and invagination) and phagosome maturation (i.e., biogenesis of the degradative compartment: the phagolysosome).

The phases prior to the establishment of interactions between bacterial ligands and phagocytic receptors may involve pathogen fishing by cell structures—this process is also dependent of filamentous actin (F-actin), filopodia extensions **(Figure 1)**. Filopodia serves differently in

pathogens and immune cells: pathogens will use it to approach cell membranes for invasion while macrophages will take advantage of these structures for fishing surrounding molecules in order to patrol the environment for possible invaders [4].

Phagocytosis was first discovered in the lower eukaryote amoebae that use it for feeding. In higher organisms, phagocytosis is fundamental for host defence against invading pathogens and contributes to the immune and inflammatory responses [5] including turnover and remodeling of tissues and disposal of dead cells. All cells may to some extent perform phagocytosis [6]. However in mammals, phagocytosis is the hallmark of specialized cells including macrophages, dendritic cells, and polymorphonuclear neutrophils—these cells are collectively referred to as professional phagocytes [6]. In certain circumstances, other cell types, such as fibroblasts engulfing apoptotic cells and bladder epithelial cells consuming erythrocytes, are able to perform conventional phagocytosis as efficiently as professional phagocytes [6].

Professional phagocytes express a series of cell surface receptors which recognize a variety of microbial ligands. Receptors on the surface of the phagocytic cell orchestrate a set of signaling events that are required for particle internalization. However, most pathogens possess many different ligands on their surface. Their phagocytic uptake occurs via multiligand interactions, which induce the engagement of many receptors at the same time.

Two major categories of receptors involved in pathogen recognition are opsonic receptors and nonopsonic receptors (pattern-recognition receptors: PRRs) [1]. Receptors for opsonins such as IgG antibodies and the complement fragment C3bi engage FcγRs and complement receptors (CR), respectively. PRRs include toll-like receptors (TLRs) and other receptor families as C-type lectins receptors that recognize sugar residues as mannose or fucose and lipopolysaccharides (LPS). TLRs often function as coreceptors in phagocytosis by their discrimination of a broad range of microbial products, including LPS and peptidoglycan. The role of TLRs in accelerating and modulating phagosome maturation is still a matter of debate [7].

Bacteria opsonized by complement C3b, by IgG or having lipoarabinomannans at the cell wall surface will be recognized by complement receptors such as CR1 and CR3/4, Fc receptors or Man-6P receptors respectively, each triggering phagocytosis without stimulating a strong superoxide burst. The entry via these phagocytic receptors leads to the maturation of the forming phagosome into a very degradative lysosomal compartment that will destroy microbes [8]. All these receptors will be downregulated during phagocyte activation either through bacterial proinflammatory components as in the case of LPS or cytokines as IFNγ [8].

Activated macrophages will in turn reprogram their expression profile in order to increase the ability to kill pathogens via oxidative bursts and decrease protein digestion extension from amino-acids to small peptides, for antigen presentation [9].

Phagocytosis uses the actin cytoskeleton to construct a cup and close the cup by contractile activities [10]. Latter along phagosome maturation the actin cytoskeleton is also utilized for vesicle trafficking and fusion along the endocytic pathway [11]. The induced polymerization of filamentous actin (F-actin) from globular actin (G-actin) beneath the site of attachment of the particle is the driving force behind ingestion and proceeds from signal transduction downstream of the phagocytic receptors [1]. The precise signaling cascades linking activated

receptors to actin polymerization are not fully understood yet it is well known that Rho GTPase family plays critical roles in controlling these cytoskeletal rearrangements [1]. These, RhoA, Rac1, and cell division cycle 42 (Cdc42) act as molecular switches in controlling actin dynamics by regulating the actin-related protein 2/3 (Arp2/3) complex [12]. Arp2/3 requires activation by nucleation-promoting factors, such as the Wiskott-Aldrich syndrome protein (WASP) family. Nucleation-promoting factors exist in an autoinhibited conformation until activated by Cdc42 and Rac1, as well as by phosphoinositide (PI) signaling (discussed latter in this chapter). Effectors such as Cdc42 and the phosphoinositide 4,5-bisphosphate PI(4,5)P2 (PIP2) synergize to activate WASP homolog N-WASP which triggers actin polymerization via Arp2/3 [13]. As the newly formed actin branch grows, the plasma membrane is forced out, extending the membrane as pseudopodia **(Figure 1)**.

Various extracellular and intracellular cues including those from pathogens stimulate Rho GTPases, leading to actin-mediated membrane manipulation. RhoA, Rac1, and Cdc42 have all been shown to accumulate at the nascent phagosome cup. These proteins are preferred targets for bacterial toxins that in turn modulate the organization of the actin skeleton allowing invasion into nonprofessional phagocytic cells and preventing phagocytosis into professional phagocytes. These toxins modify the activity of Rho GTPases through covalent modification or regulation of the nucleotide state. Toxins such as *Clostridium difficile* toxin A and B modify Rho leading to inactivation of its function. This bacterium and the toxin it produces are a global health problem especially affecting the elderly who need to be prescribed prolonged doses of antibiotics. In fact extracellular bacteria, such as *Clostridium* spp., release toxins that glycosylate Rho GTPases in order to disorganize actin to reduce immune cell migration and phagocytosis and also to break down epithelial cell barriers [14].

Another group of toxins regulates the nucleotide state and thus the function of various Rho GTPases by acting as GTPase-activating proteins (GAPs). *Yersinia* spp. an enteropathogenic group of bacteria have secretion systems that inject a type of these Rho GAP toxins, Yop virulence factors leading to actin filamentation blocking and consequently to inhibition of phagocytosis in all host cells to where a contact is established with either professional or non-professional phagocytic cells [15].

Pseudomonas has the capacity to inactivate all Rho GTPases [16]. *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen that causes life-threatening infections in cystic fibrosis patients, individuals with burn wounds, and the immuno-compromised. *P. aeruginosa* pathogenicity involves cell-associated and secreted virulence factors as ExoS one of four type III cytotoxins injected into the cytosol. *In vivo* the Rho GAP activity of ExoS stimulates the reorganization of the actin cytoskeleton by inhibition of Rac and Cdc42 and stimulates actin stress fiber formation by inhibiting of Rho [16]. The consequences are the prevention of phagocytosis. Moreover, the perturbation of F-to G-actin content together with cytosolic stress is sensed by the PRR pyrin triggering caspase 1 and inflammasome assembly leading to inflammation and cell death by pyroptosis.

Many intracellular bacterial pathogens have evolved to survive and even proliferate within immune phagocytic cells. Depending on the route of entry, the fate of intracellular bacteria varies significantly. Some opsonized bacteria as *Brucella*, the agent of brucellosis, for example, are destroyed efficiently within macrophages while the nonopsonised survive [17]. An essential

feature of the pathogenicity of *Salmonella* is its capacity to cross a number of barriers requiring invasion of a large variety of phagocytic and nonphagocytic cells (reviewed in Ref. [18]). Virulent *Salmonella enterica* serovar Thyphimurium infection of macrophages triggers cell lysis while opsonized noninvasive mutants do not thus reinforce the idea that distinct overcomes depend on the internalization route [19]. The cytotoxicity of serovar Typhimurium is related to the capacity of this organism to invade cells. Mutants lacking invasion proteins encoded by the salmonella pathogenicity island 1 genome region (SPI-1) failed to induce cell lysis in murine macrophages [20]. This is an important step of salmonella infection allowing the pathogen escaping to macrophages to reach the basolateral membrane of the gut cells for invasion.

The uptake of *Mycobacterium* spp. by phagocytes has been intensively studied since these cell types, especially macrophages, are the preferred targets of this successful pathogen. An important class of *Mycobacterium* pathogens includes tuberculosis bacilli. This intracellular facultative pathogen controls the bacterial load during macrophage internalization by interfering with actin polymerization at the phagocytic cup [21]. This is a necessary step in virulence for preventing apoptosis and therefore to prevent pathogen intracellular killing [22]. For this, during early phases of *Mycobacterium* infection, the microRNA 142-3p is overexpressed in response to phagocytosis and interferes with the expression of N-WASP and consequently with the Arp2/3 complex required for actin nucleation at the cell membrane [21]. Therefore, a low bacterial load is accomplished intracellularly, preventing the apoptosis of the infected cells. In addition, recently, miR-142-3p was shown to directly regulate protein kinase Cα (PKCα), a key gene involved in phagocytosis [23].

The heterodimeric host surface receptor complement-receptor 3 (CR-3), mediates uptake of opsonized and nonopsonized mycobacteria. Interestingly, CR-3 is targeted by other intracellular pathogens, such as *Coxiella burnetii*, the Q-fever agent, in order to avoid phagocytosis. This strategy is based on ensuring a spatial location of CR-3 outside the pseudopod extensions [24].

Lipid modification by receptor signaling creates the potential for radiating signals that can affect large areas of the plasma membrane. Phospholipid kinases, lipid phosphatases, and hydrolases are activated during phagocytosis. Classes of phospholipids typically found on the inner face of biomembranes include phosphatidylinositol (PI). The generation of phosphoinositides derived from PI via phosphorylation events will generate classes of important lipids enrolled in cell signaling and phagocytosis as example of phosphatidylinositol (4)-phosphate (PI(4)P=PIP), PI(5)P, PI(4,5)P2 (PIP2), PI (3,4)P2, and PI(3,4,5)P3 (PIP3). As mentioned previously in this chapter, these phosphoinositides, especially PIP2 and PIP3, are capable of binding and increasing the activity of proteins that modify membrane chemistry and the actin cytoskeleton. As an example, PIP2 increases the activity of WASP, a protein that stimulates actin polymerization via Arp2/3.

This class of PIs in addition to their relevance in particle internalization is important during the phase of phagosome maturation into a degradative compartment, the phagolysosome. In phagosomal membranes PIP2 activates the actin nucleators of the Ezrin, Moesin, and Radixin family inducing polymerization of F-actin and therefore phagosome maturation [11]. This will be addressed later in this chapter in the context of the manipulation of the actin cytoskeleton by pathogens in order to establish an intracellular niche.

2.2. Induced phagocytosis by invasive pathogens

Classically, the manipulation of the actin cytoskeleton by invasive pathogens was classified into two general mechanisms according to the type of morphological changes that occur in the host cell—the zipper and trigger phagocytosis [3]. Entry of uropathogenic *Escherichia coli*, *Yersinia*, *Helicobacter*, *Listeria*, and *Neisseria* into epithelial cells is reminiscent of the classical model of zipper phagocytosis. The trigger model will be addressed as macropinocytosis in the next section of this chapter as it is not in fact a phagocytosis event. Moreover, the zipper mechanism may also be triggered actively by pathogens.

Adherence to nonprofessional phagocytic cells, epithelium by a pathogen is necessary to avoid mechanical clearance and is the first step of colonization by for example enteropathogens. Thus bacterial pathogens exhibit a large variety of cell surface adhesins, including fimbriae (pili) and afimbrial adhesins some of which participate in the internalization step. Likewise, in this type of entry, a bacterial adhesin binds to a host cell surface receptor involved in cell-to-cell adhesion and/or activates regulatory proteins that modulate cytoskeleton dynamics. Moreover, adherence and internalization into epithelial cells looks to be a strategy used by pathogens to escape destruction by immune cells as described below.

Most type I pili expressed by pathogenic *E. coli* bind to host mannose-containing glycoproteins some expressed in gut epithelial cells including M cells (microfold cells of Payer's Patches) [25]. Others such as FimH from uropathogenic *E. coli* can bind to β1 and α3 integrins and thereby promote bacterial internalization following a process that to date has only been described in urinary bladder epithelial cells. Uropathogenic *E. coli* (UPEC) cause the majority of community-onset urinary tract infections (UTI). Early in acute cystitis, UPEC gains access to an intracellular niche that protects a population of replicating bacteria from arriving phagocytes [26]. Transition bacillary forms of UPEC (1–2 μm in length) are readily engulfed, while filamentous UPEC resist phagocytosis, even when in direct contact with neutrophils and macrophages. Despite these strong host defenses, a subpopulation of UPEC is able to persist for months in a quiescent reservoir state which may serve as a seed for recurrent infections [27].

Yersinia spp. such as *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* invades gut mucosae at the ileum terminal end and multiplies in the underlying lymphoid tissue. Invasin and YadA (Yersinia adhesion A) are crucial for yersinia adherence via β1 integrins and matrix components, respectively. β1 integrins exist on the basolateral face of enterocytes and on the apical surface of the epithelia derived M cells. The coalescence of integrins following bacteria invasin linkage will lead to yersinia internalization by a "zipper mechanism". Binding of invasin to β1 integrin activates focal adhesion tyrosine kinase and triggers a complex cascade implicating Rac1-Arp2/3 pathways but also phosphoinositide-3-kinase (PI3K) leading to the closure of the phagocytic cup. In contrast, YadA binds diverse extracellular matrix components, such as collagen, laminin, and fibronectin, thus indirectly mediating integrin binding [28]. Yersinia species also hijack host cell phosphoinositide metabolism for their uptake. Rac-1 recruits, and Arf6 activates the type I phosphatidylinositol-4-phosphate-5-kinase (PtdIns(4) P(5)Ka), which forms PIP2 at the entry site, and this lipid may regulate phagocytic cup formation by coordinating membrane traffic and controlling F-actin polymerization [29].

Helicobacter pylori is another example of pathogen that adheres to mucosa via β1integrins and invades nonphagocytic cells. Efficient infection of cultured epithelial cells seems to be restricted to certain *H. pylori* strains. This pathogen uses a type IV secretion system (T4SS) targeting β1 integrins to translocate the virulence factor CagA into the cytosol. The adhesin CagL present in the T4SS pilus surface bridge activates the integrin on the basolateral membrane of gastric epithelial cells. In all cases, however, invasion of *H. pylori* seems to involve a typical zipper-like entry process. Both PI3-K and PKC are required for bacterial uptake and induction of cytoskeletal rearrangements [30]. Curiously preinfection of cultured gastric cells with yersinia expressing Yop virulence factors that interfere with the same signaling events impaired phagocytosis of *H. pylori* [30]. Internalized *H. pylori* was shown to be located in tight phagosomes and in close association with condensed actin filaments and localized tyrosine phosphorylation signals. Similar to UPEC in bladder epithelial cells, invasion of epithelial cells by *H. pylori* may constitute one of the evasion strategies used by this pathogen to circumvent the host immune response and persist in stomach.

Curiously the vaccinal strain for tuberculosis *Mycobacterium bovis* BCG has been used as the more effective treatment for bladder cancer [31]. The bacillus induces phagocytosis in tumor cells via their surface fibronectin attachment protein (FAP) to β1integrins. After phagocytosis a strong cytotoxic effect is displayed via T-helper CD8 stimulation leading to antitumor activity.

Listeria monocytogenes is a food-borne Gram-positive bacterium that makes use of two surface proteins, Internalin A (InlA) and B (InlB), to engage, in a species-specific manner, to host adhesion molecules E-cadherin and hepatocyte growth factor receptor Met respectively, to induce its internalization [32]. Only InlA is critical for invasion of the gut epithelial cells. The specific engagement of E-cadherin initiates activation of the adherens junction machinery inducing the recruitment of β-catenin, Rho GAP protein ARHGAP10, α-catenins to the site of the entry. Internalization is then further mediated by Rac- and Arp2/3-dependent actin polymerization. In contrast to this, InlB is essential for *Listeria* uptake by most nonphagocytic cell types, such as hepatocytes, endothelial cells, fibroblasts, and certain epithelial cell lines. Additionally, it is known that ActA, a *Listeria* protein required for actin-tail formation and intracellular cytosolic movement, can also mediate *Listeria* uptake by epithelial cells [32]. Recently a new phagocytic process was characterized that allows human endothelial cells to internalize listeria independent of all known pathogenic bacterial surface proteins. Here bacteria adhesion is mediated by Rho kinase and the control of the internalization step is coordinated by formins (as FHOD1 and FMNL3) a class of actin nucleation proteins. The overall control of the event is mediated by cytoskeletal proteins usually enrolled in cell shape and locomotion including Rho, focal adhesions, and PI kinases [33].

Neisseria gonorrhoeae, is an exclusive human pathogen that primarily infects the urogenital epithelia, causing the sexually transmitted disease gonorrhoea. Entry of *N. gonorrhoeae* into human epithelial cells is multifactorial. Initial attachment is mediated by pili (a T4SS), followed by tight adherence via the phase-variable colony opacity (Opa) proteins. These are a family of 11 outer membrane proteins variably expressed at the surface of the bacterium. However, only OpaA confers invasion into epithelia [34]. This entry is mediated by heparan sulfate proteoglycan (HSPG) receptors of the syndecan family expressed on the target cell

surface. Pilus engagement has also been demonstrated to play a role in host cell cytoskeletal rearrangements inducing microvilli formation at the cell surface to surround the bacteria for a zipper mechanism of internalization [35].

In endothelial cells, the T4SS-pilus-mediated adhesion of *Neisseria meningitidis* induces the formation of membrane protrusions similar to microvilli leading to bacterial uptake. These protrusions result from a Rho- and Cdc42-dependent cortical actin polymerization, and from the activation of the ErbB2 tyrosine-kinase receptor and the Src kinase, leading to tyrosine phosphorylation of cortactin, an activator of Arp2/3 [36]. Adhesion of *N. meningitidis* to endothelial cells promotes the local formation of membrane protrusions reminiscent of epithelial microvilli structures that surround bacteria and provoke their internalization within intracellular vacuoles.

2.3. Macropinocytosis, induced macropinocytosis, and coiling phagocytosis

Unique molecular properties associated with the process of macropinocytosis are beginning to be elucidated. Because of their size and the fact that they may be formed without activation by ligands, the large vacuoles (macropinosomes) formed during this pinocytosis event can contain extracellular fluid and pathogens. At the mechanistic level, phagocytosis and macropinocytosis present many similarities including the involvement of phosphoinositol phosphate signaling and actin cytoskeleton reorganization. During macropinocytosis it is not observed a direct connection between bacteria/cargo and multiple receptors but it was demonstrated the relevance of tyrosine kinase receptors involved in responses to growth factors as the epidermal growth factor and platelet-derived growth factor. The consequence of intensive actin remodeling results in ruffling protrusions at the cell surface, or in unilateral large pseudopodia formation leading to the formation of large macropinosomes. Activated receptor tyrosine kinases, as well as the Src family kinases, are clearly observed on newly formed macropinosomes. Therefore in concert with the morphological definition provided by Lewis in 1931 based on ruffling formation, and elevation in response to growth factor stimulation can be used to define macropinocytosis [37].

Macropinocytosis has been observed in professional phagocytes as well in epithelial cells. Immature dendritic cells and activated macrophages display high levels of constitutive macropinocytosis [38]. The consequent internalization of large volumes of extracellular solute that accompanies macropinocytosis facilitates their capacity to continuously survey the extracellular space for foreign material. In fact, this increased levels of macropinocytosis upon encounter with the antigen/pathogen enhances both antigen capture and antigen presentation by dendritic cells as well as the complete clearance of pathogens after macrophage activation by inflammatory stimulus [38].

In epithelial cells, an induced form of macropinocytosis was observed after infection with pathogens such as *Shigella*, *Salmonella*, enterophatogenic *E.coli* (EPEC), and *Mycobacterium tuberculosis*. Therefore, individual pathogens have developed a range of strategies to modulate the host's normal macropinocytic pathways both to invade the host cells and to manipulate the lipid and protein composition of the encapsulating macropinosome to promote cell uptake and then survival. A few virulence factors secreted by pathogens are able to induce

http://dx.doi.org/10.5772/66861

ruffling similar to the growth factors named above. The closure of ruffles back to themselves will entrap pathogens into a large vacuole (micropinosome) incorrectly named in distinct publications as "spacious phagosome".

Invasive enteropathogens, such as *Shigella flexneri* and *S. enterica* serovar Typhimurium, use the trigger mechanism of invasion in epithelial cells to induce membrane ruffles and macropinocytosis. This is a phenomenon dependent on a type III secretion system encoded by both bacteria. The T3SS effectors activate host Cdc42 and Rac1 albeit via distinct cellular relays. In *Salmonella*, SopE acts as a guanyl-nucleotide-exchange factor for Rho [39]. This induced Rho GTPase perturbation is recognized in the cytosol by PRRs (NOD1 sensor) inducing a proinflammatory response and innate immune responses. SigD/SopB is another protein secreted by the SPI-1 T3SS of *Salmonella* to invade nonphagocytic cells. The phosphatidyl-inositol phosphatase activity of SigD/SopB induces rapid disappearance of PIP2 from invaginating regions of the cytoplasmic membrane leading indirectly to Rho activation and macropinocytosis. Once inside the host cell, *Salmonella* induces the recovery of normal cytoskeleton dynamics via SptP, a SPI-1 effector with Cdc42 and Rac1 GAP activity that returns these proteins to their nonactivated state.

In comparison, the effectors IpaC, IpgB1, and VirA of *Shigella* bind to initiate a focal adhesion structure required for internalization via a process that recruit Rho isoforms [40]. Consequently, the injection of the effectors IpaC, IpgB1, and VirA by *S. flexneri* induces Rac1/Cdc42-dependent actin polymerization. Finally, the translocated effector IpaA binds vinculin and enhances its association to actin filaments, thus mediating the localized depolymerization of actin, which is required to close the phagocytic cup [40].

S. flexneri invasion has been classically described as a macropinocytosis-like process, however the role of macropinosomes in intracellular bacterial survival remains elusive. There is evidence that bacterial entry and membrane ruffling are associated with different bacterial effectors and host responses during *S. flexneri* invasion. Rho isoforms are recruited differentially to either entering bacteria or membrane ruffles, and entry has been proposed to occur initially via effector mediated contact of *S. flexneri* to specific receptors suggesting entry is akin to receptor mediated phagocytosis. In fact, the host surface molecules β1-integrins and CD44 (hyaluronic acid receptor) are needed for *Shigella* entry [40].

Recently, the mechanism of *Shigella* invasion of epithelial cells was observed using advanced large volume correlative light electron microscopy (CLEM) indicating a combination of induced phagocytosis and macropinocytosis [41]. Here, the macropinocytic event instead of being the major effector for internalization was in fact shown to be required for release of the bacteria from the phagosome and cytosolic escape later in phagocytosis. Macropinocytic vesicles formed at the invasion site are functionally involved in vacuolar rupture. This unique and surprising pathogenic strategy stands in stark contrast to other invasive pathogens that induce direct lysis of their surrounding vacuole via the action of destabilizing bacterial proteins.

S. enterica is an invasive, T3SS-employing pathogen and shares many common host entry characteristics with *S. flexneri*. It was hypothesized that salmonella containing vacuole and macropinosomes may be distinct, as they are sorted into different intracellular routes [42].

These evidence suggest that pathogen induced enhanced uptake of extracellular fluid in *S. enterica serovar* Typhimurium-infected epithelial cells is an event related to the invasion mechanisms used by this pathogen but not the major mechanism for bacteria internalization as referred in most published data.

Surface-adherent pathogens, such as enteropathogenic or enterohaemorrhagic *E. coli* (EPEC or EHEC, respectively), use their T3SS to secrete a transmembrane receptor into the host membrane to stimulate actin polymerization and generate cellular extensions called pedestals. EPEC uses the T3SS apparatus to inject the intimin receptor (Tir). Tir acts as a cell receptor of host kinases activating N-WASP and the actin nucleator Arp2/3 resulting in actin polymerization and pedestal formation at the site of the attachment. While stabilizing bacteria connection to epithelial cells the actin pedestal formation promotes T3SS mediated injection of additional effector proteins able to subvert other host pathways. Where bacteria are attached, microvilli are lost; the epithelial cells form cup-like pedestals upon which the bacteria rest. The underlying cytoskeleton of the epithelial cell is disorganized, with a proliferation of filamentous actin. Although EPEC have traditionally been considered to be noninvasive, accumulating evidence casts doubt on this assumption. From the earliest published electron micrographs of EPEC infection, bacteria have been observed within epithelial cells at the sites of attaching [43]. The virulence factor dependent on Tir signaling EspG contributes to the ability of EPEC pathogens to establish infection through a modulation of the host cytoskeleton involving transient microtubule destruction and actin polymerization in a manner akin to the *S. flexneri* VirA protein [28, 44].

Patients with inflammatory bowel disease exhibited an increased number of mucosae-associated *E. coli* with invasive properties. The adherent-invasive *E. coli* (AIEC) uses M cells to reach macrophages of Payer's Patches where they survive and replicate inside large macropinosomes that share features of phagolysosomes. To survive, these bacteria, inside the vacuoles, adapted to the harsh acidic environment that is the key signal to activate virulence genes. In fact infected macrophages with AIEC secrete large amounts of tumor necrosis factor alpha leading to local granuloma formation. Those macrophages will subsequently aggregate and fuse releasing bacteria that then will reach the basolateral domain of gut epithelial cells for invasion. Epithelial cell invasion is a key virulence factor only for EIEC, which may lead to a dysentery-like illness similar to that caused by *S. flexneri* [45].

Alveolar macrophages constitute the main defense against *M. tuberculosis* infection. However, tuberculosis bacilli resist phagocytic cell bactericidal mechanisms and replicate within them. Although *M. tuberculosis* survives within phagocytic cells, this bacterium may also bind and invade alveolar epithelial cells [46] and endothelial lymphatic cells [47]. Infection of epithelial cells was concomitant with large lamellipodia projections (ruffles) similar to macropinocytosis. Likewise, *Mycobacterium* can induce formation of macropinosomes however; this does not depend on a bacterial secretion system, as the culture media in the absence of pathogen was sufficient to induce this process. Since nonviable bacteria fail to induce macropinocytosis in opposition to live bacteria, the most prominent candidate to induce ruffling is pointed as being secretory products actively produced by life bacilli. There are no requirements for bacteria to attach directly to the plasma membrane. In endothelial cells, scanning electron

microscopy (SEM) micrographs show that mycobacteria were internalized by characteristic phagocytosis-like and macropinocytosis events [47]. However the mycobacterial determinants leading to actin reorganization and pathogen active internalization are not clarified. It is very likely that the invasion and survival in epithelial and endothelial cells contributes to the one-third of the human population latently infected with this microorganism.

Coiling phagocytosis is an actin dependent endocytic event, morphologically accompanied by a typical pseudopodia that looks like whorls or wrapps around the bacteria in several turns (**Figure 1**). A definition of the phenomena is complex as it presents similarities to macropinocytosis and conventional phagocytosis: for the first due to the large pseudopodia; for the second due to cargo specific entrapment. In coiling phagocytosis, the single pseudopodia do not trap fluid droplets but enclose microbes; however, the multiple pseudopod whorls have largely self-apposed surfaces instead of those that are microbe-apposed surfaces. *Legionella pneumophila* and *Borrelia burgdorferi* the agents of Legionellosis and Lyme disease, respectively, use this form of endocytosis for establishment of the infection within macrophages. It was demonstrated that coiling phagocytosis is an active and selective process of the phagocytes, initially triggered by heat- and aldehyde-insensitive moieties of the microbial surface [48], suggesting that coiling and conventional phagocytosis are very closely related, most likely starting from the same phagocytosis-promoting receptor(s). The lack of difference between viable and killed microbes indicates that coiling phagocytosis is actively driven by the phagocytes and not by the microbes. This distinguishes coiling phagocytosis from nonclassical uptake mechanisms such as the induced phagocytosis or macropinocytosis. In this respect, the identification of granulocyte macrophage colony-stimulating factor (GM-CSF) and phorbol esters such as PMA as coiling-promoting substances may be a clue as to the regulatory mechanisms involved in coiling phagocytosis [48]. On the side of the phagocytes, coiling phagocytosis obviously is clearly a regulated mechanism, because the monocytes used it selectively for certain spirochetes, which is inconsistent with simply an accidental trapping of pericellular microbes.

In summary, deciphering the players that induce or prevent phagocytosis in one infection context may be used as strategies to clear pathogens in other context. It is an interesting observation that preinfection of cultured gastric cells with yersinia expressing Yop virulence factors that interfere with the same signaling events, impaired phagocytosis of *H. pylori*. This may be a potential starting strategy to fight gastric cancer due to this pathogen.

Define what receptors stimulate to induce a more bactericidal response of infected cells, how to control bacterial load that is internalized to induce apoptosis, as is the case of microRNAs that control WASP in tuberculosis context; how to neutralize factors that prevent Rho family of GTPases to modify actin in order to induce phagocytosis of extracellular pathogens, these are a few targets to explore deeply. Other relevant area to act is how to neutralize bacterial adhesins, secretion systems or their access to surface receptors as integrins to prevent epithelia invasion. It is imperative to decipher what are the virulence factors that mimics or induce growth factors that leads to induced macropinocytosis. In addition, it is important to find how to neutralize secretion systems that reorganize the actin cytoskeleton for macropinosome formation and therefore for pathogen invasion of epithelial and endothelial cells, important reservoirs of latent infections.

3. Acting on actin for the establishment of an intracellular niche

In addition to particle binding and internalization, phagocytosis includes the process of phagosome maturation leading to pathogen destruction in the acidic hydrolytic environment of the phagolysosome. These events are important innate immune mechanisms. Indeed a consequence of phagosome maturation is the activation of the antigen presentation machinery. Macropinocytosis culminates in the appearance of a large vacuole that, indeed follows the fate of the phagosome. Some pathogens have evolved to establish sustained infection in professional phagocytes preventing phagosome maturation as is the case of *M. tuberculosis* and *S. enterica*. Other's diverts the endocytic pathway into a distinct vacuole more similar to the secretory pathway (e.g., *Legionella pneumophila* associates with the endoplasmic reticulum). By doing this, pathogens establish an intracellular niche were they survive, escape the immune bactericidal responses and have access to nutrients. Finally, a group of pathogens are able to escape the endocytic pathway by lysing the vacuole and move to the cytosol (e.g., *Mycobacterium marinum* within macrophages; *M. tuberculosis* within endothelial cells; *Shigella*, listeria within epithelial cells) **(Figure 1)**.

The material in endosomes or phagosomes that is destined for lysosome degradation by endocytosis or phagocytosis reaches this compartment by fusing with the organelle. Critical for this is the membrane composition of the correct repertoire of lipids, membrane-bound proteins, and also proteins that shuttle on and off membranes. The manipulation of the phagosomal membrane by pathogens may block the ability of fusion with lysosomes leading to a vacuole that may be trafficked apart from the endocytic route. In alternative, the vacuole may be arrested from maturation along the endocytic pathway by pathogen membrane manipulation leading to continuous transient fusion events with upper compartments.

Phagosome maturation is known to be influenced by the lipid species present on the outer and most likely inner membrane, and published studies have focused mostly on kinases that generates PIP, and PIP2, which binds actin nucleation proteins [49]. Additionally, the ability to nucleate actin leading to F-actin polymerization from phagosomal membranes was associated to the formation and availability of actin tracks for organelles to move towards the actin-nucleating source, increasing vesicle trafficking, fusion events, and phagolysosome biogenesis **(Figure 1)** [50]. Identifying key roles for PIP and PIP2 opened the door for the analysis of several other lipids that interconnected with these phosphoinositides in the actin assembly process, as well as sphingolipids and fatty acids favouring phagosome maturation [11, 51]. Examples of F-Actin stimulatory factors includes the eicosanoide omega 6 arachadonic acid, ceramide and sphingosine-1-phosphate.

Several groups have explored the role of actin cytoskeleton during *Mycobacterium* late phases of phagocytosis. Pioneering work by de Chastellier and co-workers shows that *Mycobacterium* avium a pathogen common in AIDS patients, disrupt the macrophage actin filament network highlighting here the target for the bacterium that allows sustained intracellular survival. It was demonstrated that in contrast to nonpathogenic mycobacteria, pathogenic *M. tuberculosis* prevents actin polymerization on phagosomal membranes [11, 52]. Therefore, the enrichment of *M. tuberculosis* phagosomal membranes with classes of lipids that leads to PIP2 was shown to induce F-actin tracks from the vacuole membrane. This is concomitant with an increase of

fusion events, phagolysosome biogenesis and, consequently *M. tuberculosis* intracellular killing [11]. Drug-induced manipulation of the pathogen actin nucleation-induced blockade represents interesting alternative therapies for tuberculosis.

Another pathogen that blocks phagosome maturation is *Salmonella*. Several hours after bacterial uptake into different host cell types, *Salmonella* induces the formation of an F-actin meshwork around the *Salmonella*-containing vacuole (SCV), which is a modified phagocytic compartment. SCV integrity is closely linked to a surrounding meshwork of actin that in contrast to what happens during mycobacteria infection, acts as a barrier that prevents membrane contact and, therefore vacuole fusion with other endocytic organelles [53]. This process does not require the Inv/Spa type III secretion system or cognate effector proteins, which induce actin polymerization during bacterial invasion. A second T3SS, the salmonella pathogenicity island 2 (SPI2), translocate effectors from the phagosomal membrane to the cytosol. The consequence of this event is the induced polymerization of actin around the SCV that will allow salmonella intravacuolar survival. The spv virulence locus will express the SpvB protein and ADP-ribosyl transferase that will promote actin depolymerisation in latter stages of infection. Treatment with actin-depolymerizing agents significantly inhibited intramacrophage replication of salmonella. Furthermore, after this treatment, bacteria were released into the host cell cytosol, whereas SPI-2 mutant bacteria remained within vacuoles [53]. In conclusion, while during *M. tuberculosis* infection actin assembly is prevented or F-actin is disrupted to allow the establishment of an intracellular niche, in the case of salmonella infection the generation of an F-actin induced mesh is required to maintain and position a vacuole that sustains bacterial growth.

4. Acting on actin for pathogen dissemination: actin-based motility of pathogens and innate immunity

Early after host invasion some pathogens escape lysosomal destruction and antigen presentation by escaping into the cytosol. Thereafter, actin polymerization is manipulated by several cytosolic pathogens such as *L. monocytogenes*, *S. flexneri*, *Burkholderia pseudomallei*, *Rickettsia* spp., and *M. marinum*. These generate and use actin tails to move within and between cells.

When intracellular moving bacteria reaches the plasma membrane, they push out long protrusions that are taken up by neighboring cells, facilitating the infection to spread from epithelial cell to cell in the absence of immune surveillance. At the cell-to-cell cytoplasmic membranes sites, the cytosolic actin-based moving pathogens induce the formation of surface protrusions that force the internalization from the infected cell into noninfected neighbor cells. The process of engulfment is called paracytophagy and involves internalization of a double membrane containing pathogen: the inner from the donor cell and the outer from the recipient cell (**Figure 1**) [54, 55]. At this point the pathogen may escape again to cytosol to start a new infection process.

In the case of enterophatogenic *E. coli* EPEC it was found that some actin pedestal of the attached EPECs also translocate along the cell surface, reaching speeds of 0.007 μm/s allowing bacteria to spread between attached cells [34] (**Figure 1**). While this model shares similarities with the *Listeria* or *Shigella* systems, the main difference is the presence of a membrane between the pathogen and the cell cytoskeleton (**Figure 1**: as in the case of filopodia fishing

compared to paracytophagy). The actin polymerization system Arp2/3 complex has been manipulated by several pathogens differently. Some mimics the Wiskott-Aldrich syndrome protein (WASP) family [56], while other's recruit WASP directly to activate Arp2/3 [57]. Examples of the first include the actA protein of listeria and RickA of riquetsia. For the second examples exist as is the case of IcsA of *S. flexneri* and nondetermined factors of *M. marinum* but dependent on the ESAT-6 secretion system 1 [57]. *M. marinum* is a water-borne bacterium that naturally infects fish and amphibians and is an opportunistic pathogen for humans causing tuberculosis while *Rickettsia conorii* belongs to the spotted fever group of *Rickettsia* species transmitted by ticks [55].

The actin-based motility of *B. pseudomallei* the causative agent of melioidosis occurs by a mechanism distinct to that used by other intracytoplasmic pathogens. In fact, the actin tails induced by this pathogen contains Arp2/3 components but it is not clear in the enrollment of the intracellular motility of *B. pseudomallei* [58]. The overexpression of Scar1 a cellular actin nucleating promoting factor that in the context of *S. flexneri*, *L. monocytogenes* and *R. conorii*, blocks actin tail formation and motility, during *B. pseudomallei* infection as no effect on actin-based motility [58].

The predominance of a membrane surrounding vacuole during the infection of most intracellular pathogens looks to be related to immune protection from the defensive mechanisms that exist in the cytosol. The arrival of a pathogen or their PAMPs to the cytosol could "wake up" several patrol mechanisms that include cytosolic PRRs. The sensing by cytosolic innate receptors leads to an inflammatory response by secretion of proinflammatory cytokines and chemokines or a interferon type I response that overall leads to antimicrobial response; the stress in the cytosol induce inflammasome assembly [59].

Therefore, the arrival of the pathogens in the cytosol establishes a bridge to the innate immune response by contact of the pathogen-associated molecular patterns (PAMPS) with PRRs, such as NLRPs (Nod like, similar to Toll like receptors- TLRs on cell membranes). Additionally, and by causing cytosol stress, PAMPS will activate (via PRRs) the inflammasome, a complex structure of proteins similar to the apoptosome [60]. Inflammasome assembly will lead to pro-Interleukin1β (pro-IL-1β) and pro-IL-18 inflammatory cytokine activation via caspase 1 and to the programmed cell death dependent on caspase 1, as it is pyroptosis and pyronecrosis [22]. This is a natural immune response in gut and respiratory epithelial cells but not in endothelial vascular and lymphatic cells that lakes these cytosolic receptors and constitutes important host niches for intracellular pathogen survival [33, 47].

Rickettsiae possess a tropism to endothelial cells, a tissue that usually serves as barrier to intravascuolar blood from surrounding tissues. This tropism leads to the endothelial cell injury associated with complications of the disease. RickA (mentioned previously in this chapter) is a protein present in the pathogenic species *R. conorii*, but absent in *Rickettsia thyphi* [56]. This absence is responsible for an erratic actin-based motility of *R. thyphi* leading to the hypothesis of existence of multiple actin-polymerization mechanisms in pathogenic rickettsia. A consequence of this erratic movement may be the delayed spread from cell to cell and continuous replication of thyphi species leading to bacterial overload and necrotic cell lysis [56]. For *R. conorii* paracytophagy cell-to-cell-spread is the common mechanism for pathogen dissemination [55].

Macrophages, in contrast to endothelial cells, possess NLRs and other PRRs families. During *M. tuberculosis* as well as for *M. marinum* infection phagolysosomal rupture and bacteria escape to the cytosol usually leads to necrotic cell death [61, 62]. The existence of a functional RD1 region expressing ESAT-6 is relevant for the activation of the inflammasome, the necrotic cell death and the secretion of proinflammatory cytokines IL-1β [21]. In endothelial cells, however, the tubercle bacilli survives [47].

The detection of cytosolic LPS, as a consequence of disruption of replication vacuoles harboring Gram-negative bacteria was shown to trigger the activation of murine caspase-11 that leads to the assembly of a noncanonical inflammasome [63]. Caspase-11 (Casp-4 in humans) is also crucial for clearance of bacteria that escape the vacuole, such as *Burkholderia*. In addition, detection of *sdhA* mutants of *Legionella* and *sifA* mutants of *Salmonella* activate caspase-11-dependent pyroptosis [63]. Detection of cytosolic pathogens thus leads to caspase-1- or caspase-11-mediated pyroptosis and restricts bacterial growth.

Another potent host defense mechanism that restricts intracellular pathogens is autophagy. Some intracellular bacteria cause the formation of ubiquitinated aggregates around either bacterial structures or replication vacuoles, and the autophagic machinery can recognize these. The process of bacterial clearance by selective autophagy is called xenophagy. *Listeria* moves within the host cytoplasm through actin-based motility, promoted by the bacterial ActA protein, which is important for avoiding recognition by autophagy [64]. In contrast to the ActA protein, the *Shigella* IcsA protein that also promotes actin-based motility from one pole of the bacterium binds to the autophagy protein Atg5 thus targeting the bacterium to a phagophore. *Shigella* uses two different mechanisms to escape the host autophagic response: first, it secretes IcsB, a protein that competitively binds to IcsA and prevents its recognition by Atg5 thus preventing LC3 recruitment and the process of autophagy [65].

All together these findings let us to postulate that important strategies to fight pathogens will pass by control their life cycle in the cytosol. Either addressing the linkage of actin tails to Arp2/3 or WASP proteins or neutralizing the bacteria actin nucleators to prevent motility and spread to neighbor cells; either to induce death of the infected cell by apoptosis, pyroptosis, or necrotic lysis; either by exposition of pathogen signatures that leads to xenophagy; altogether these are a few potential strategies to address in the future.

5. Concluding remarks

During evolution, higher eukaryotic organisms have developed epithelial barriers and phagocytic immune cells to resist and fight infections. The discovery of antibiotics in the early part of the last century led to predictions that bacterial infections would be kept under tight control via natural systems and treatment with drugs. But the capacity of bacteria to evade natural protective systems and rapidly develop resistance to antibiotics had led to the current situation of bacteria posing major health problems in both the developed and underdeveloped world. There is now a major requirement to find alternative treatments to fight bacterial pathogens. Over the years, various studies have elucidated the mechanisms by which bacterial PAMPs,

adhesins, and secretion systems together with their translocated effectors target and alter the host actin dynamics. Targeting the host actin machinery is important for the survival and pathogenesis of several extracellular, vacuolar, and cytosolic bacteria. Studying the manipulation of host actin by pathogens has vastly improved our understanding of various basic cell biological processes in host cells while giving key insights into both bacterial pathogenesis and host innate immunity. Together this opens a new and exciting field of research with the objective of discovering new classes of antibiotics that directly or indirectly interfere with this actin-modulating mechanism.

Author details

Elsa Anes

Address all correspondence to: eanes@ff.ulisboa.pt

Research Institute for Medicines iMed-ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

References

[1] Niedergang F, Chavrier P. Regulation of phagocytosis by Rho GTPases. Current Topics in Microbiology and Immunology 2005;291:43–60.

[2] Cannon GJ, Swanson JA. The macrophage capacity for phagocytosis. Journal of Cell Science 1992;101:907–13.

[3] Swanson JA, Baer SC. Phagocytosis by zippers and triggers. Trends in Cell Biology 1995;5:89–93.

[4] Bornschlögl T. How filopodia pull: what we know about the mechanics and dynamics of filopodia. Cytoskeleton 2013;70:590–603.

[5] Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. Annual Review of Immunology 1999;17:593–623.

[6] Rabinovitch M. Professional and non-professional phagocytes: an introduction. Trends in Cell Biology 1995;5:85–7.

[7] Yates RM, Russell DG. Phagosome maturation proceeds independently of stimulation of toll-like receptors 2 and 4. Immunity 2005;23:409–17.

[8] Yates RM, Hermetter A, Taylor GA, Russell DG. Macrophage activation downregulates the degradative capacity of the phagosome. Traffic 2007;8:241–50.

[9] Russell DG, VanderVen BC, Glennie S, Mwandumba H, Heyderman RS. The macrophage marches on its phagosome: dynamic assays of phagosome function. Nature Reviews Immunology 2009;9:594–600.

[10] Mercanti V, Charette SJ, Bennett N, Ryckewaert J-J, Letourneur F, Cosson P. Selective membrane exclusion in phagocytic and macropinocytic cups. Journal of Cell Science 2006;119:4079–87.

[11] Anes E, Kuhnel M, Boss E, Moniz-Pereira J, Habermann A, Griffiths G. Selected lipids activate phagosome actin assembly and maturation resulting in killing of pathogenic mycobacteria. Nature Cell Biology 2003;5:793–802.

[12] Pollard TD, Cooper JA. Actin, a central player in cell shape and movement. Science 2009;326:1208–12.

[13] Welch MD. The world according to Arp: regulation of actin nucleation by the Arp2/3 complex. Trends in Cell Biology 1999;9:423–7.

[14] Jank T, Giesemann T, Aktories K. Rho-glucosylating Clostridium difficile toxins A and B: new insights into structure and function. Glycobiology 2007;17:15R–22R.

[15] Cornelis GR, Wolf-Watz H. The yersinia yop virulon. Molecular Microbiology 1997;23:861–7.

[16] Krall R, Sun J, Pederson KJ, Barbieri JT. In vivo rho GTPase-activating protein activity of Pseudomonas aeruginosa cytotoxin ExoS. Infection and Immunity 2002;70:360–7.

[17] Alonso A, Portillo FG-D. Hijacking of eukaryotic functions by intracellular bacterial pathogens. International Microbiology 2004;7:181–91.

[18] Darwin KH, Miller VL. Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clinical Microbiology Reviews 1999;12:405–28.

[19] Santos RL, Tsolis RM, Bäumler AJ, Smith R III, Adams LG. Salmonella enterica Serovar typhimurium induces cell death in bovine monocyte-derived macrophages by early sipb-dependent and delayedsipb-independent mechanisms. Infection and Immunity 2001;69:2293–301.

[20] Chen LM, Kaniga K, Galán JE. Salmonella spp. are cytotoxic for cultured macrophages. Molecular Microbiology 1996;21:1101–15.

[21] Bettencourt P, Marion S, Pires D, Santos LF, Lastrucci C, Carmo N, et al. Actin-binding protein regulation by microRNAs as a novel microbial strategy to modulate phagocytosis by host cells: the case of N-Wasp and miR-142-3p. Frontiers in Cellular and Infection Microbiology 2013;3:19.

[22] Welin A, Eklund D, Stendahl O, Lerm M. Human macrophages infected with a high burden of ESAT-6-expressing M. tuberculosis undergo caspase-1-and cathepsin B-independent necrosis. PloS One 2011;6:e20302.

[23] Naqvi AR, Fordham JB, Nares S. miR-24, miR-30b, and miR-142-3p regulate phagocytosis in myeloid inflammatory cells. The Journal of Immunology 2015;194:1916–27.

[24] Meconi S, Jacomo V, Boquet P, Raoult D, Mege JL, Capo C. Coxiella burnetii induces reorganization of the actin cytoskeleton in human monocytes. Infection and Immunity 1998;66:5527–33.

[25] Miller H, Zhang J, Kuolee R, Patel GB, Chen W. Intestinal M cells: the fallible sentinels? World Journal of Gastroenterology 2007;13:1477–86.

[26] Olson PD, Hunstad DA. Subversion of host innate immunity by uropathogenic *Escherichia coli*. Pathogens 2016;5(1):2.

[27] Justice SS, Hung C, Theriot JA, Fletcher DA, Anderson GG, Footer MJ, et al. Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis. Proceedings of the National Academy of Sciences of the United States of America 2004;101:1333–8.

[28] Reis RSD, Horn F. Enteropathogenic *Escherichia coli*, *Samonella*, *Shigella* and *Yersinia*: cellular aspects of host-bacteria interactions in enteric diseases. Gut Pathogens 2010;2:8.

[29] Wong K-W, Isberg RR. Arf6 and phosphoinositol-4-phosphate-5-kinase activities permit bypass of the Rac1 requirement for beta1 integrin-mediated bacterial uptake. The Journal of Experimental Medicine 2003;198:603–14.

[30] Kwok T, Backert S, Schwarz H, Berger J, Meyer TF. Specific entry of *Helicobacter pylori* into cultured gastric epithelial cells via a zipper-like mechanism. Infection and Immunity 2002;70:2108–20.

[31] Sinn HW, Elzey BD, Jensen RJ, Zhao X, Zhao W, Ratliff TL. The fibronectin attachment protein of bacillus Calmette-Guerin (BCG) mediates antitumor activity. Cancer Immunology, Immunotherapy 2008;57:573–9.

[32] Cossart P, Pizarro-Cerda J, Lecuit M. Invasion of mammalian cells by Listeria monocytogenes: functional mimicry to subvert cellular functions. Trends in Cell Biology 2003;13:23–31.

[33] Rengarajan M, Hayer A, Theriot JA. Endothelial cells use a formin-dependent phagocytosis-like process to internalize the bacterium listeria monocytogenes. PLoS Pathogens 2016;12:e1005603.

[34] Dramsi S, Cossart P. Intracellular pathogens and the actin cytoskeleton. Annual Review of Cell and Developmental 1998;14:137–66.

[35] Griffiss JM, Lammel CJ, Wang J, Dekker NP, Brooks GF. *Neisseria gonorrhoeae* coordinately uses pili and opa to activate hec-1-b cell microvilli, which causes engulfment of the gonococci. Infection and Immunity 1999;67:3469–80.

[36] Lambotin M, Hoffmann I, Laran-Chich M-P, Nassif X, Couraud PO, Bourdoulous S. Invasion of endothelial cells by Neisseria meningitidis requires cortactin recruitment by a phosphoinositide-3-kinase/Rac1 signalling pathway triggered by the lipo-oligosaccharide. Journal of Cell Science 2005;118:3805–16.

[37] Kerr MC, Teasdale RD. Defining macropinocytosis. Traffic 2009;10:364–71.

[38] BoseDasgupta S, Pieters J. Inflammatory stimuli reprogram macrophage phagocytosis to macropinocytosis for the rapid elimination of pathogens. PLoS Pathogens 2014;10:e1003879.

[39] Guiney DG, Lesnick M. Targeting of the actin cytoskeleton during infection by Salmonella strains. Clinical Immunology 2005;114:248–55.

[40] Schroeder GN, Hilbi H. Molecular pathogenesis of *Shigella* spp.: controlling host cell signaling, invasion, and death by type III secretion. Clinical Microbiology Reviews 2008;21:134–56.

[41] Weiner A, Mellouk N, Lopez-Montero N, Chang Y-Y, Souque C, Schmitt C, et al. Macropinosomes are key players in early *Shigella* invasion and vacuolar escape in epithelial cells. PLoS Pathogens 2016;12:e1005602.

[42] Garcia-del Portillo F, Finlay BB. Salmonella invasion of nonphagocytic cells induces formation of macropinosomes in the host cell. Infection and Immunity 1994;62:4641–5.

[43] Donnenberg MS, Calderwood SB, Donohue-Rolfe A, Keusch GT, Kaper JB. Construction and analysis of tnphoa mutants of enteropathogenic escherichia coli unable to invade HEp-2 cells. Infection and Immunity 1990;58:1565–71.

[44] Hardwidge PR, Deng W, Vallance BA, Rodriguez-Escudero I, Cid VJ, Molina M, et al. Modulation of host cytoskeleton function by the enteropathogenic *Escherichia coli* and *Citrobacter rodentium* effector protein EspG. Infection and Immunity 2005;73:2586–94.

[45] Boudeau J, Glasser A-L, Masseret E, Joly B, Darfeuille-Michaud A. Invasive ability of an *Escherichia coli* strain isolated from the ileal mucosa of a patient with Crohn's disease. Infection and Immunity 1999;67:4499–509.

[46] García-Pérez BE, Mondragón-Flores R. Internalization of mycobacterium tuberculosis by macropinocytosis in non-phagocytic cells. Microbial Pathogens 2003;35:49–55.

[47] Lerner TR, de Souza Carvalho-Wodarz C, Repnik U, Russell MRG, Borel S, Diedrich CR, et al. Lymphatic endothelial cells are a replicative niche for Mycobacterium tuberculosis. The Journal of Clinical Investigation 2016;126:1093–108.

[48] Rittig MG, Jagoda JC, Wilske B, Murgia R, Cinco M, Repp R, et al. Coiling phagocytosis discriminates between different spirochetes and is enhanced by phorbol myristate acetate and granulocyte-macrophage colony-stimulating factor. Infection and Immunity 1998;66:627–35.

[49] Griffiths G. On phagosome individuality and membrane signalling networks. Trends in Cell Biology 2004;14:343–51.

[50] Kjeken R, Egeberg M, Habermann A, Kuehnel M, Peyron P, Floetenmeyer M, et al. Fusion between phagosomes, early and late endosomes: a role for actin in fusion between late, but not early endocytic organelles. Molecular Biology of the Cell 2004;15:345–58.

[51] Kuhnel M, Mayorga LS, Dandekar T, Thakar J, Schwarz R, Anes E, et al. Modelling phagosomal lipid networks that regulate actin assembly. BMC Systems Biology 2008;2:107.

[52] Anes E, Peyron P, Staali L, Jordao L, Gutierrez MG, Kress H, et al. Dynamic life and death interactions between Mycobacterium smegmatis and J774 macrophages. Cellular Microbiology 2006;8:939–60.

[53] Méresse S, Unsworth KE, Habermann A, Griffiths G, Fang F, Martínez Lorenzo MJ, et al. Remodelling of the actin cytoskeleton is essential for replication of intravacuolar Salmonella. Cellular Microbiology 2001;3:567–77.

[54] Robbins JR, Barth AI, Marquis H, de Hostos EL, Nelson WJ, Theriot JA. Listeria monocytogenes exploits normal host cell processes to spread from cell to cell. Journal of Cell Biology 1999;146:1333–50.

[55] Schaible UE, Haas A. Intracellular Niches of Microbes: A Pathogens Guide through the Host Cell. Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim; 2009. p. 738 DOI: 10.1002/9783527629176.

[56] Jeng RL, Goley ED, D'Alessio JA, Chaga OY, Svitkina TM, Borisy GG, et al. A Rickettsia WASP-like protein activates the Arp2/3 complex and mediates actin-based motility. Cellular Microbiology 2004;6:761–9.

[57] Stamm LM, Pak MA, Morisaki JH, Snapper SB, Rottner K, Lommel S, et al. Role of the WASP family proteins for Mycobacterium marinum actin tail formation. Proceedings of the National Academy of Sciences of the United States of America 2005;102:14837–42.

[58] Breitbach K, Rottner K, Klocke S, Rohde M, Jenzora A, Wehland J, et al. Actin-based motility of Burkholderia pseudomallei involves the Arp 2/3 complex, but not N-WASP and Ena/VASP proteins. Cellular Microbiology 2003;5:385–93.

[59] Asrat S, de Jesús DA, Hempstead AD, Ramabhadran V, Isberg RR. Bacterial pathogen manipulation of host membrane trafficking. Annual Review of Cell and Developmental Biology 2014;30:79–109.

[60] Stutz A, Golenbock DT, Latz E. Inflammasomes: too big to miss. The Journal of Clinical Investigation 2009;119:3502–11.

[61] Simeone R, Bobard A, Lippmann J, Bitter W, Majlessi L, Brosch R, et al. Phagosomal rupture by mycobacterium tuberculosis results in toxicity and host cell death. PLoS Pathogens 2012;8:e1002507.

[62] van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, Pierson J, et al. *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. Cell 2007;129:1287–98.

[63] Aachoui Y, Leaf IA, Hagar JA, Fontana MF, Campos CG, Zak DE, et al. Caspase-11 protects against bacteria that escape the vacuole. Science 2013;339:975–8.

[64] Yoshikawa Y, Ogawa M, Hain T, Yoshida M, Fukumatsu M, Kim M, et al. Listeria monocytogenes ActA-mediated escape from autophagic recognition. Nature Cell Biology 2009;11:1233–40.

[65] Ogawa M, Yoshikawa Y, Kobayashi T, Mimuro H, Fukumatsu M, Kiga K, et al. A Tecpr1-dependent selective autophagy pathway targets bacterial pathogens. Cell Host & Microbe 2011;9:376–89.