Review

Cell PRESS

Cytoplasmic access by intracellular bacterial pathogens

Jennifer Fredlund and Jost Enninga

Unité 'Dynamique des interactions hôte–pathogène', Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris, France

Entry into host cells is a strategy widely used by bacterial pathogens, after which they either remain within membrane-bound compartments or rupture the endocytic vacuole to reach the cytoplasm. During recent years, cytoplasmic access has been documented for an increasing number of pathogens. Here we review how classical cytoplasmic bacterial pathogens rupture their endocytic vacuoles as well as the mechanisms used to accomplish this task by bacterial species for which host cytoplasmic localization has only recently been identified. We also discuss the consequences for pathogenesis resulting from this change in intracellular localization, with a particular focus on the role of the host. What emerges is that cytoplasmic access plays an important role in the pathophysiology of an increasing number of intracellular bacterial pathogens.

Intracellular localization of bacterial pathogens

Bacterial pathogens reside in two major niches during host infection: they either remain extracellular or are internalized via active or passive pathways [1]. When internalized, they are surrounded by host cell membranes. Subsequently, pathogens can remain vacuole-bound through the entire course of infection by blocking delivery of the lysosome or by modulating the physiological conditions of the vacuolar niche. An alternative strategy is rupture of the vacuole, or phagolysosome, giving the bacterium access to the host cell cytoplasm. The decision to remain within an endomembrane compartment or to escape into the cytoplasm has important consequences for both the invader and the infected cell. The physiological environment of each niche differs drastically with regard to nutritional access, differential pathogen recognition, and spread to neighboring cells. Consequently, induced innate and adaptive immune responses occur in different ways depending on the bacterial localization.

Until recently, intracellular pathogens were neatly separated into two groups, either cytoplasmic or vacuolar. This rigid definition has been challenged by a series of scientific reports (Figure 1) suggesting that access to the host cytoplasm is more frequent than previously anticipated.

Corresponding author: Enninga, J. (jostenn@pasteur.fr).

Keywords: intracellular bacteria; vacuolar rupture; membrane trafficking; host cell death.

0966-842X/$ – see front matter

 http://dx.doi.org/10.1016/j.tim.2014.01.003

Bacterial pathogens that reach the host cytoplasm: the classics

For a number of bacteria, the mechanisms of cytosolic access have been studied in detail. We discuss known bacterial and host factors important for this process and present a brief overview of how they function during vacuolar rupture. The proteins involved that are discussed in this and in the following section are highlighted in Table 1.

Shigella *triggers its entry to rapidly escape from the intracellular vacuole*

Host cellular entry into both epithelial cells and macrophages by *Shigella* species has been studied for decades, mostly using *Shigella flexneri* [2,3]. *S. flexneri* invades cells via a trigger mechanism that involves injecting approximately 25 effector proteins directly into the host cytoplasm through the *mxi-spa* type III secretion system (T3SS). Cytoplasmic localization of *Shigella* within different cell types was first observed by transmission electron microscopy (Box 1, Figure 1A) [2]. Furthermore, *Shigella* induces 'comet tails' composed of host actin via the bacterial factor IcsA, and these have been commonly used as markers for bacterial cytoplasmic localization.

Initially it was proposed that bacterial effectors play a major role in cytoplasmic access and vacuolar rupture by *Shigella* [4]. In particular, the T3SS translocator proteins IpaB and IpaC have been shown to destabilize eukaryotic cell membranes using red blood cell lysis assays at very high concentrations of the bacterial proteins [5]. Recent *in vitro* studies on IpaB have suggested that its assembly into multiprotein complexes [6,7] allows an influx of potassium ions through endolysosomal membranes, suggesting pore formation and an involvement in vacuolar rupture [6]. Underlining the possibility that other factors may be involved in the vacuolar rupture process, the T3SS effector $IpaH_{7.8}$ has also been associated with cytoplasmic access; however, its function is not yet established [8]. Furthermore, a non-characterized region of the *Shigella* virulence plasmid allowed uncoupling of bacterial entry and vacuolar rupture, hinting at potential involvement of additional factors in the rupture process [9]. On vacuolar rupture, a pool of smaller vesicles formed from the vacuolar membrane remnants suggests that the autophagy machinery is recruited to the site of ruptured membranes [10,11]. Taken together, current data suggest that the rupture process represents a signaling platform with important roles for

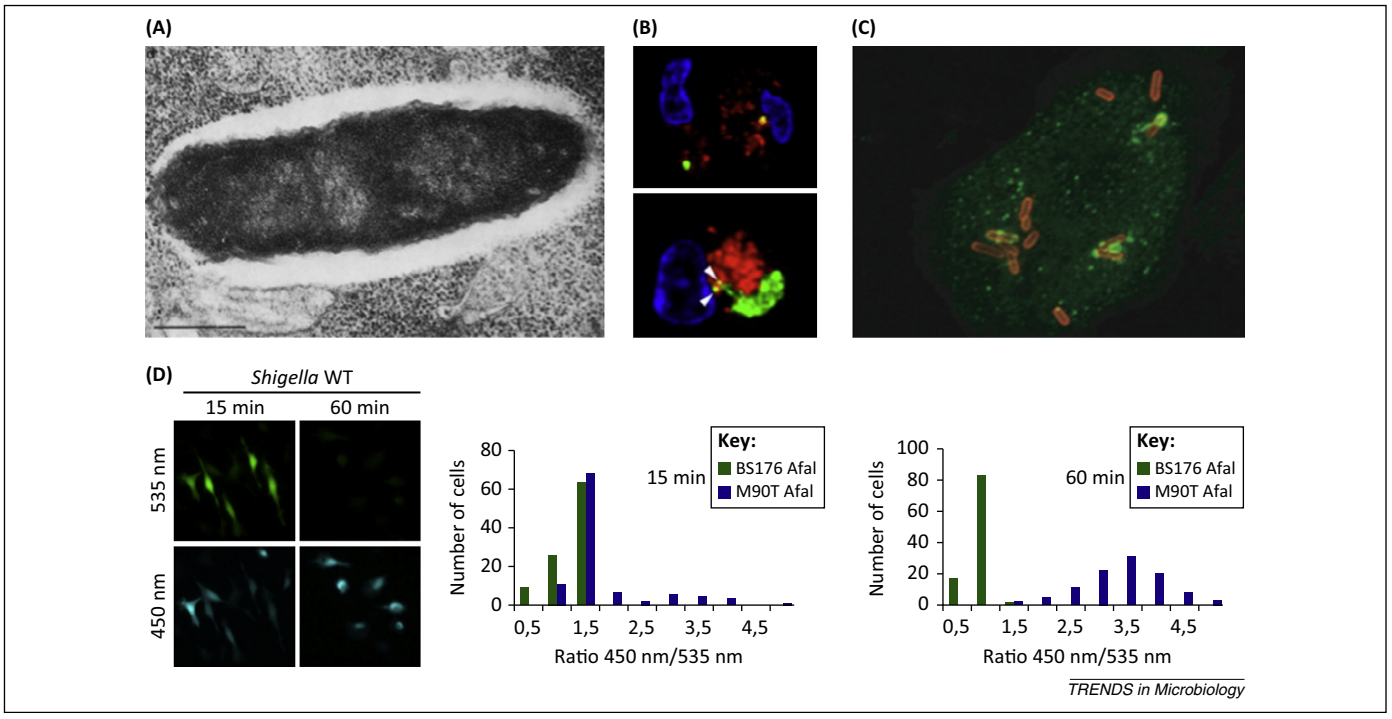

Figure 1. Measuring the cytoplasmic access by bacterial pathogens. **(A)** Transmission electron microscopy has been extensively used as evidence of cytoplasmic access by bacteria, for example for *Shigella* (scale bar 500 nm; reprinted with permission from [3]). **(B)** The absence of markers for endosomal or lysosomal compartments hints at cytoplasmic localization. Here, dendritic cells (blue nuclei) were infected with *Mycobacterium tuberculosis* (green) for 4 h (upper panel) or 96 h (lower panel). At early time points, the bacteria colocalize with lysosomal associated protein 1 (LAMP1, red); later, they spread throughout the cytoplasm [38]. **(C)** Galectin-3 (green) flags damaged vacuolar membrane structures after their rupture by *Shigella* (red) [83]. **(D)** A fluorescence resonance energy transfer (FRET)-based approach measures access to the cytoplasm in HeLa cells infected with wild type (WT) *Shigella*. The cephalosporin-derived FRET probe is cleaved by β-lactamase on the surface of the invading bacteria, resulting in a signal switch from green to blue (left panel). The fluorescent ratio (450 nm/535 nm) can be plotted against the number of infected cells to highlight vacuolar rupture after 60 min of infection. *Shigella* BS176 AfaI cannot enter host cells and M90T AfaI is the invasive strain [84].

both bacterial and host proteins, and with effectors of the *Shigella* T3SS orchestrating the process.

Listeria*: zippering into host cells and forming pores in the vacuole*

Invasion by the human pathogen *Listeria monocytogenes* is achieved via bacterial surface proteins such as internalin A (InlA) and InlB that respectively bind to human E-cadherin and have affinity for host glycosaminoglycans, the receptor for the human globular part of the complement component gC1q-1 (gC1q-R), and the Met receptor (the main receptor for InlB) [12]. This interaction triggers a complex signaling cascade involving a battery of host factors, including kinases, GTPases, and even clathrin, leading to vacuolar formation that has been described in detail [12].

The secreted protein listeriolysin-O (LLO) is the key bacterial factor leading to vacuolar rupture15 min after *Listeria* internalization [12]. It is a member of the cholesterol-dependent cytolysin family, which also includes streptolysin-O and perfringolysin-O. Even though a link has been made between LLO activity and vacuolar pH that results in membrane permeation, it is still not clear whether this effect is direct or requires additional factors [13]. This is because the bacterial phospholipases PI-PLC and PC-PLC are also necessary to achieve efficient cytoplasmic access [14]. One explanation for this could be that initial membrane damage via LLO allows better cytoplasmic access for the mature phospholipases.

Similar to *Shigella* infection, it is clear that *Listeria* proteins function in concert with host factors to grant the bacterium cytoplasmic access. For example, host gamma-interferon-inducible lysosomalthiol reductase (GILT) chemically reduces LLO, thereby increasing LLO activity [15], and the cystic fibrosis transmembrane conductance regulator (CFTCR) increases intravacuolar chloride concentrations to potentiate LLO activity [16]. In addition, subversion of the *Listeria*-containing vacuole from the endosomal pathway requires Ca^{2+} flux through the LLO pores [13]. These fluxes inhibit the normal vacuolar maturation that would eventually result in lysosomal fusion and bacterial destruction. Finally, an inducible increase in membrane resistance to pressure, termed renitence, has been identified. This mechanism may limit vacuolar membrane damage and appears to involve the host heat shock protein HSP70 [17]. Vacuolar escape within activated macrophages is reduced by the production of reactive oxygen and nitrogen intermediates (ROIs and RNIs) [18]; however, their mode of action in this process needs to be further studied.

Rickettsia*: concerted action of hemolysins and phospholipases*

Rickettsiales represent an order of important obligate human intracellular pathogens targeting mainly endothelial cells and macrophages [19]. Owing to the complex handling requirements for this pathogen, mostly genomic comparison has been used to delineate its infection