Role of *Listeria monocytogenes* Exotoxins in Virulence

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Abstract

Listeria monocytogenes is an opportunistic intracellular bacterium responsible for the disease listeriosis. This review will update the knowledge on the four exotoxins secreted by this intracellular pathogen: the cholesterol-dependent cytolysin listeriolysin O (LLO), phosphatidylinositol-specific phospholipase C (PlcA), broad-range phospholipase C (PlcB), and hemolysin listeriolysin S (LLS). Each one of these exotoxins has evolved to perform specific and important functions in the extracellular or intracellular environment during the life cycle of *L. monocytogenes*. LLO, PlcA, and PlcB were discovered decades ago; however, recent studies are revisiting their functions and revealing new, previously unexpected insights. In the same line, LLS was discovered almost a decade ago, but it was recently deciphered that it is not only a toxin for eukaryotic cells but also a bacteriocin targeting bacteria closely related to *L. monocytogenes*. These latest findings, together with the knowledge generated during the history of listeriology, will be discussed in the light of their impact on the infectious process.

Keywords

Listeria monocytogenes • Exotoxins • Listeriolysin O (LLO) • Phosphatidylinositol-specific phospholipase C (PlcA) • Broad-range phospholipase C (PlcB) • Listeriolysin S (LLS)

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Introduction

Listeria monocytogenes was discovered by E.G.D. Murray in 1926 during the investigation of an epidemic disease of rabbits which induced an extreme monocytosis on the blood, receiving firstly the name *Bacterium monocytogenes* (Murray et al. 1926). J.H.H. Pirie identified the same microorganism in 1927 from the liver of gerbils in South Africa, and the name *Listerella hepatolytica* was adopted in honor to the surgeon J. Lister. Finally, the bacterium was renamed *Listeria monocytogenes*. This Gram-positive facultative intracellular bacterium infects animals and humans and is able to cross the intestinal barrier, the blood–brain barrier, and the fetoplacental barrier. As a consequence, *L. monocytogenes* can cause self-limiting gastroenteritis in healthy individuals as well as meningitis and meningoencephalitis in immunocompromised individuals, and abortions in pregnant women (Cossart 2011). Listeriosis cases are sporadic, although epidemics can occur with a high mortality rate (20–30%) and neurological sequelae in at-risk populations (Cossart 2011). *L. monocytogenes* can be found in nature, including vegetation, water, and soil, and can adopt a planktonic life or form biofilms (Cossart 2011). This bacterium can grow at 4 °C, at extreme pH, and in high salt concentration, ready-to-eat food products being usually involved in listeriosis outbreaks reported worldwide (Cossart 2011).

After ingestion of highly contaminated food, *L. monocytogenes* reaches the intestine where it competes with the intestinal microbiota to survive. From the intestinal lumen, this bacterium is able to cross the intestine invading phagocytic and non-phagocytic cells and then pass from primarily infected cells to neighboring cells, disseminating within tissues without being exposed to antimicrobial molecules and phagocytes of the immune system in the extracellular environment (Stavru et al. 2011).

The intracellular life cycle is critical for *L. monocytogenes* pathogenesis since strains that are incapable of infecting host cells are not able to cause disease (Seveau

2014). *L. monocytogenes* has developed an elaborated arsenal of toxins to successfully colonize the intestine, invade eukaryotic cells, escape from the internalization vacuole, resist autophagic killing, and multiply and spread from cell to cell (Cossart 2011; Stavru et al. 2011). Many *L. monocytogenes* virulence factors are regulated by PrfA and SigB, although small RNAs, antisense RNAs, and riboswitches play an important role during the intracellular lifestyle (Cossart 2011; Quereda et al. 2014). Epidemic *L. monocytogenes* strains possess three pathogenicity islands, LIPI-1, LIPI-3, and LIPI-4. LIPI-1 is regulated by the transcriptional factor PrfA and encodes for three toxins: the cholesterol-dependent cytolysin listeriolysin O (LLO), a phosphatidylinositol-specific phospholipase C (PlcA), and a broad-range phospholipase C (PlcB). LIPI-1 also codes for ActA which plays an important role in actin polymerization, for Mpl which is a metalloprotease associated with PlcB activation, and for PrfA itself (Cossart 2011). The internalin A/internalin B locus encodes for two surface molecules, which respectively bind E-cadherin and the receptor tyrosine kinase Met in the eukaryotic cell to mediate entry (Cossart 2011). LIPI-3 contains listeriolysin S (LLS), a posttranslational modified peptide that exhibits properties of both bacteriocins and hemolytic–cytotoxic factors (Cotter et al. 2008; Quereda et al. 2016). LIPI-4 encodes for a cellobiose PTS system necessary for central nervous system infection (Maury et al. 2016). This book chapter reviews the literature on the exotoxins (LLO, PlcA, PlcB, and LLS) secreted by *L. monocytogenes* and their contribution to the infectious process.

Listeriolysin O

Listeriolysin O is a pore-forming toxin encoded by the *hly* gene (1590 bp) that is transcriptionally regulated by PrfA, the master virulence transcriptional regulator of *L. monocytogenes* (Fig. 1a). This transcriptional factor belongs to the cAMP receptor protein (Crp)/fumarate nitrate reductase regulator (Fnr) family of bacterial transcription factors, and positively controls the transcription of the LIPI-1 and the internalin A/internalin B locus virulence genes of *L. monocytogenes* (Cossart 2011; Seveau 2014). PrfA is under the control of a 5′-UTR thermosensor which allows increased translation at 37 °C when *L. monocytogenes* is inside animal hosts (Cossart 2011; Seveau 2014). Additionally, host cell-derived glutathione contributes towards activating PrfA, which finally leads to higher transcription of virulence genes expression when this bacterium is inside eukaryotic cells (Reniere et al. 2015). *hly* codes for a preprotein of 529 residues with an amino-terminal secretion signal sequence, that after secretion and cleavage releases a mature protein of 504 residues (56kd) (Seveau 2014). LLO is secreted by the general secretory pathway. *hly* is also present in the animal pathogen *Listeria ivanovii*, while it is absent in nonpathogenic *Listeria* species (with the exception of *L. seeligeri*). An epidemiological and comparative genomics study showed that *L. monocytogenes* clonal complexes CC1, CC2, CC4, and CC6 are strongly associated with human clinical cases, and interestingly all of them have a complete *hly* gene, highlighting the importance of LLO for infection (Maury et al. 2016).

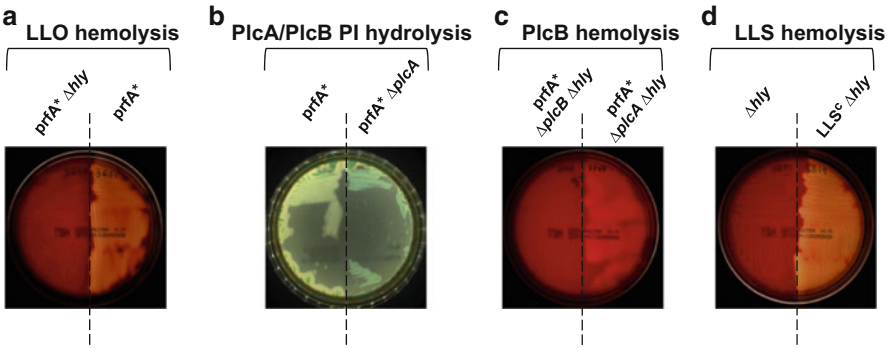

Fig. 1 Lytic activities of *L. monocytogenes* toxins. Hemolytic activities in horse blood agar of LLO (**a**), PlcB (**c**), and LLS (**d**), as well as phosphatidylinositol (*PI*) hydrolysis in ALOA agar of PlcA and PlcB (**b**). Note the powerful hemolysis produced by LLO and LLS (**a** and **d**). Since LLS is not produced in vitro, a synthetic strain that constitutively produces LLS (under the control of the pHELP promoter) was generated in a Δ*hly* background (LLS^c Δ*hly*) (**d**). PlcB is less hemolytic than LLO or LLS (**c**) while PlcA PI hydrolytic activity is higher than that of PlcB (**b**). *L. monocytogenes* strains in plates **a**, **b,** and **d** were streaked and cultured at 37 °C for ≈30 h. *L. monocytogenes* strains in plate **c** were streaked and cultured in anaerobiosis at 37 °C for ≈30 h

LLO belongs to the cholesterol-dependent cytolysin (CDC) family which includes other toxins, such as streptolysin O from *Streptococcus pyogenes*, pneumolysin from *Streptococcus pneumoniae*, suilysin produced by *Streptococcus suis*, intermedilysin from *Streptococcus intermedius*, lectinolysin produced by *Streptococcus mitis*, perfringolysin O from *C. perfringens*, and anthrolysin O synthesized by *B. anthracis* (Schnupf and Portnoy 2007). LLO monomers are secreted as water-soluble monomers that bind to cholesterol-rich regions of eukaryotic membranes, oligomerize, and form large pores of up to 35 nm in diameter consisting of 30–50 monomers (Peraro and van der Goot 2016). Remarkably, pore-dependent membrane damage is reversible. Bacterial membranes lack sterols and are therefore protected from the cytolytic activity of CDCs (Schnupf and Portnoy 2007). Although no CDC has been crystallized in the pore configuration, oligomerization of CDCs seems to follow a sequential accumulation of monomers or multimers. Nevertheless, oligomerization can fail to occur entirely, resulting in the formation of arc-shaped oligomers, faced on the opposite side by a free edge of the lipid membrane that can nevertheless be active pores as confirmed for suilysin (Peraro and van der Goot 2016). A recent study showed by electron microscopy that LLO full-circled pores on the erythrocyte ghost membranes differ in shape, finding occasionally ring arcs, incomplete rings, and slit-shaped structures (Koster et al. 2014). Other studies also showed that LLO can create pores of different size, confirming that diverse-sized pores exist upon infection (Hamon et al. 2012).

LLO activity is regulated by pH due to an acidic triad that triggers a premature unfolding of LLO at neutral pH and pore formation at acidic pH (Hamon et al. 2012). A functional difference between LLO and the rest of CDC toxins is that LLO is

produced by an intracellular pathogen. Thus, LLO activity is increased in acidic environments, such as the phagosomes containing *L. monocytogenes*. Neutral pH causes LLO aggregation and degradation, although it is protected from denaturation and continues to be active if bound to a lipid bilayer. LLO denaturation is slow since several minutes at 37 °C and pH 7.4 in physiological buffer are necessary for its inactivation. As a consequence, LLO secreted by extracellular bacteria can create pores in membranes and affect cellular processes of distant eukaryotic cells in physiological conditions (Seveau 2014). CDCs trigger hemolysis in their reduced state, whereas they are inactive when oxidized. Accordingly, the cellular enzyme gamma-interferon-inducible lysosomal thiol reductase (GILT) present on lysosomes of macrophages contributes to activate LLO in cells (Seveau 2014; Singh et al. 2008) (see below).

Effects of LLO in the Host Organism

Organism Level

Early LLO toxicity studies determined that ≈0.8 μg per mouse was the LD_{50} of LLO administered by intravenous injection in ICR female Swiss mice (Geoffroy et al. 1987). Mice died with convulsions and opisthotonos within 1–2 min after toxin administration. When injected through the intraperitoneal route, the LD_{50} was ≈1.7 μg, and mice died several hours later. LLO is essential for virulence of *L. monocytogenes* in intravenous and oral mouse infection models, as revealed by several logs of reduction of bacterial growth in the spleen and liver of infected animals (Cossart et al. 1989; Lecuit et al. 2007). No mortality was observed after intradermal inoculation of up to 5 μg of LLO (Geoffroy et al. 1987), although it induced a rapid inflammatory response (30 min) mediated by polymorphonuclear cells (Geoffroy et al. 1987). LLO produced during infections induces the production of anti-listeriolysin O antibodies, which have been used for serodiagnosis of human listeriosis (Berche et al. 1990).

Intestine Level

The natural route of *L. monocytogenes* infection is ingestion of contaminated food. Once this bacterium reaches the intestine, it crosses the intestinal barrier through two mechanisms: (1) across ileal Peyer's patches via M cells where InlB plays an important role and (2) upon interaction between *L. monocytogenes* surface protein InlA and E-cadherin, luminally accessible around goblet cells and extruding enterocytes (Nikitas et al. 2011). *L. monocytogenes* is rapidly transcytosed in a vacuole across enterocytes and egresses from them to the lamina propria by exocytosis in a LLO-independent manner (Nikitas et al. 2011). LLO induces chloride secretion and perturbs epithelial barrier function as assessed by transepithelial resistance in HT-29/B6 human colon cells (Richter et al. 2009). Furthermore, LLO is the main determinant of the intestinal transcriptional response to lineage II *L. monocytogenes* infection via the oral route. Histological analyses showed that *L. monocytogenes* Δ*hly* mutants are able to invade ileum enterocytes positioned at

the tips of intestinal villi and accumulate at the lamina propria, although no leukocytic infiltrates are detected in it while present in wild-type infected mice (Lecuit et al. 2007).

Liver, Spleen, and Immune System Levels

After crossing the intestinal barrier, *L. monocytogenes* reaches the liver, spleen, brain, and placenta via the lymph nodes and the blood. *L. monocytogenes* distributes among the liver parenchymal cell and non-parenchymal cell populations in the mouse liver (Cossart and Toledo-Arana 2008). It has been demonstrated that secreted LLO perforates the host cell plasma membrane as a strategy to enhance the internalization of *L. monocytogenes* into human HepG2 hepatocytes. LLO-induced bacterial entry into HepG2 cells occurs upon LLO pore formation in a dynamin-/F-actin-dependent and microtubule-/clathrin-independent way (Seveau 2014; Vadia et al. 2011). Hepatic apoptosis occurs 24 h postinfection as shown by the detection of TUNEL-positive infected hepatocytes with pyknotic nuclei (Carrero et al. 2004).

Splenic white pulp apoptosis is also observed after *L. monocytogenes* infection, and although apoptotic splenic lymphocytes are not infected with *L. monocytogenes*, extracellular bacteria are detected near dying cells (Carrero et al. 2004). Purified LLO produces caspase activation and DNA fragmentation, accompanied by phosphatidylserine exposure and loss of mitochondrial membrane potential 1 hour after treatment of in vitro-cultured T cells and lymph node lymphocytes (Carrero et al. 2004). Apoptosis of lymphocytes decreases host immunity and facilitates *L. monocytogenes* proliferation. Besides apoptosis, LLO influences the inflammatory and immune response of the host through different mechanisms. Firstly, LLO activates Toll-like receptor 4, which subsequently controls the inflammatory response. Secondly, LLO-mediated vacuolar rupture releases *L. monocytogenes* into the host cytosol, activating NOD-like receptors and the inflammasome. Thirdly, LLO perforation of epithelial cell plasma membranes induces NF-kB activation, surface expression of adhesion molecules, and the production of pro-inflammatory cytokines (Seveau 2014). Finally, LLO is the principal source of T cell epitopes during *L. monocytogenes* infection (containing two CD4+ and one CD8+ immunodominant epitopes) (Seveau 2014), but LLO also triggers the expression of negative regulators of TCR signaling which finally affects the proliferation of CD4+ T cells and silences the host adaptive immune responses (Hamon et al. 2012; Gekara et al. 2010).

Intracellular LLO Activities

Vacuolar Escape

In the intracellular environment, LLO function is tightly restricted to the endocytic vacuoles since its activity outside these compartments can damage host organelles and the plasma membrane, leading to cell death and bacterial exposure to the extracellular milieu and immune system effectors. *L. monocytogenes* mutants for which LLO activity is not compartmentalized to vacuoles are cytotoxic and less

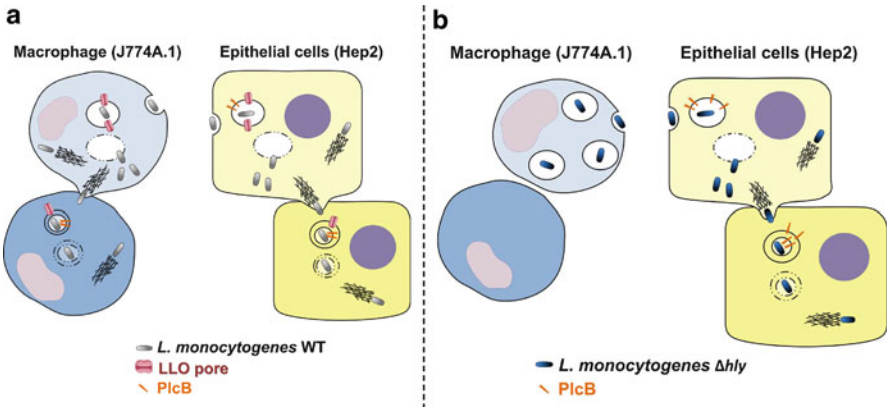

Fig. 2 Graphic representation of the *L. monocytogenes* toxin activities that mediate escape from the intracellular vacuoles (**a**: wild-type *L. monocytogenes*; **b**: Δ*hly L. monocytogenes*). *L. monocytogenes* can be passively internalized by phagocytosis in macrophages or induce its own uptake by receptor-mediated phagocytosis in non-phagocytic epithelial cells. Secreted LLO and PlcB disrupt the phagosomal membrane to allow bacterial access to the cytosol. Once in the cytosol, *L. monocytogenes* replicates and recruits the host actin-polymerization machinery to propel the bacterium in the cytosol and form intercellular protrusions which allow spreading to neighboring cells. In the recipient adjacent cells, *L. monocytogenes* is entrapped in a double-membrane vacuole where PlcA or PlcB cause the dissolution of the inner membrane and LLO disrupts the outer vacuolar membrane (originated from the recipient host cell). Note that LLO is absolutely necessary to escape from vacuoles in macrophages (*right panel*) and that in epithelial cells, PlcB is sufficient for vacuolar escape from the primary phagosome and from secondary phagosomes containing a double membrane generated from cell-to-cell spread. We thank Servier Medical Art (http://www.servier.com/Powerpoint-image-bank) for providing drawings

virulent because they lyse their replicative niche in eukaryotic host cells and cannot avoid extracellular defenses of the host (Schnupf and Portnoy 2007). Ubiquitination and proteasomal degradation, as well as pH sensitivity restricts the activity of LLO in the host cytoplasm (Hamon et al. 2012) Intriguingly, an LLO L461T mutant with hemolytic activity at neutral pH needs acidification of the vacuole for *L. monocytogenes* cytoplasmic escape, suggesting that the acidification requirement for phagosomal rupture does not seem to be caused by the higher hemolytic potential of LLO at an acidic pH (Glomski et al. 2002). Listerial escape from primary and secondary host vacuoles is a multistep process characterized by perforation (mainly dependent on LLO) followed by rupture (Fig. 2). The exact mechanism by which LLO disrupts vacuoles remains unclear since the osmotic pressure inside of the vacuole and in the host cytoplasm is similar, excluding a "lysis"-like mechanism for vacuolar escape (Schnupf and Portnoy 2007; Hamon et al. 2012).

LLO is crucial for *L. monocytogenes* escape from the primary endocytic vacuole in phagocytic and non-phagocytic cells, i.e., human epithelial Caco-2 cells, murine macrophages (BMDM and J774), and fibroblasts (CL.7) (Fig. 2). Furthermore, LLO is sufficient to allow escape of *Bacillus subtilis*, a nonpathogen soil bacterium, from J774 macrophage phagosomes (Schnupf and Portnoy 2007). However, *L. monocytogenes*

LLO deletion mutants are able to escape from internalization vacuoles in non-phagocytic human cell lines such as HeLa, HepG2, Henle 407, HEp-2, HCT116, HEK-293, and also dendritic cells, where PlcB plays a fundamental role in vacuolar rupture (see below) (Fig. 2). Lastly, activated macrophages are less susceptible to *L. monocytogenes*-induced phagosomal degradation. Vacuolar rupture and access to the host cytosol occurs 15–30 min after infection of epithelial cells and macrophages. LLO produced by *L. monocytogenes* slows down the maturation of the vacuole as shown by the delayed recruitment of the late endosomal and lysosomal marker (LAMP)-1 (Henry et al. 2006). LLO also allows *L. monocytogenes* replication in macrophage LAMP-1 (+) vacuoles (termed spacious *Listeria*-containing phagosomes) during persistent infection of severe combined immunodeficient (SCID) mice (Birmingham et al. 2008). Furthermore, extracellular LLO modulates the composition of the host cell endosomal network that will fuse with the *Listeria* phagosome. Particularly, extracellular LLO and PI-PLC regulate the translocation of PKC beta II on J774 early endosomes which finally controls *L. monocytogenes* escape from the phagosome (Seveau 2014).

Although the precise molecular mechanisms that control *L. monocytogenes* vacuolar escape in different species and cell types remain unknown, it is clear that vacuolar rupture is not only dependent on bacterial exotoxins but also on eukaryotic cell factors, i.e., calpain, gamma-interferon-inducible lysosomal thiol reductase (GILT), and cystic fibrosis transmembrane conductance regulator (CFTR) (Seveau 2014; Hamon et al. 2012). Calpain is a cytosolic cysteine protease co-opted to facilitate *L. monocytogenes* escape from the phagosome. As mentioned above, GILT is a thiol reductase enriched in the phagosomes of macrophages which activates LLO by maintaining it in a reduced state. As a consequence, GILT-activated LLO facilitates vacuolar rupture in in vitro and in vivo models of infection (Singh et al. 2008). Another cell factor that controls the activity of LLO is the CFTR. CFTR increases phagosomal chloride concentration, potentiating LLO oligomerization, pore formation, and vacuole escape. CFTR inhibition suppresses *L. monocytogenes* vacuolar rupture in culture and decreases systemic infection in mice (Radtke et al. 2011). Calpain, GILT, and CFTR are highly expressed in macrophages, but their contribution to virulence needs to be assessed in other cell types. Interestingly, human α-defensin HNP-1 blocks LLO-dependent perforation of macrophage membranes and the release of LLO from the bacteria, enabling macrophage control of *L. monocytogenes* phagosomal escape and intracellular growth (Arnett et al. 2011).

Once *L. monocytogenes* has escaped from the internalization vacuole, it replicates and moves intracellularly using a cellular actin-related machinery. The *L. monocytogenes* surface protein ActA mimics the eukaryotic WASP family proteins by recruiting the Arp 2/3 complex and promoting actin polymerization, which finally propels the bacteria inside the cytosol and facilitates cell-to-cell spread (Cossart 2011). Bacteria found in infected neighboring cells are located in a two-membrane vacuole originated from the donor and recipient cells, from which *L. monocytogenes* needs to escape to continue its infectious cycle (Fig. 2). In primary murine macrophages, the bacterial phospholipases (either PlcA or PlcB) cause

rupture of the inner membrane of the spreading vacuole, although they are not sufficient for disruption of the outer membrane (see below). In this scenario, LLO is critical for cell-to-cell spread where it is mainly involved in the disruption of the vacuolar outer membrane originated from the recipient host cell (Alberti-Segui et al. 2007). Furthermore, LLO is unnecessary for cell-to-cell spread between U937 human macrophages and human HEp-2 epithelial cells (a cell line permissive for vacuolar escape of LLO-negative bacteria), where PlcA and PlcB mediate escape from double-membrane spreading vacuoles as described below (Alberti-Segui et al. 2007) (Fig. 2).

Activation of Autophagy

Autophagy is a cytosolic process in eukaryotic cells that degrades and recycles (through a regulated mechanism) intracellular cargos, such as organelles or multiprotein complexes. Moreover, autophagy is an innate defense system for the elimination of microbial invaders. During this process, cellular or foreign material is targeted to double-membrane vacuoles that will fuse with lysosomes. Some intracellular pathogens like *Shigella flexneri* and *Salmonella enterica* induce autophagy, triggering protective innate immune and stress responses. LLO membrane damage activates autophagy both from the extracellular milieu and from the intracellular environment after vacuolar escape (Meyer-Morse et al. 2010; Birmingham et al. 2007) (Fig. 3). *L. monocytogenes* infection of macrophages activates the autophagy system in an LLO-dependent manner at 1 h post infection (p.i.) when bacteria are within vacuoles. At 4 h p.i., only 10% of intracellular bacteria colocalize with the autophagy marker LC3. The role of autophagy has variable effects depending on the cell type: it decreases infection of fibroblasts, but has no effect on infected bone marrow-derived macrophages. Autophagy markers and polyubiquitinated proteins are recruited on the *L. monocytogenes* surface and/or phagosome membranes after disruption, but actin-based motility as well as InlK expression and PlcA secretion counteract the effect of LLO on autophagy and facilitate colonization and replication in the eukaryotic niche (Hamon et al. 2012) (Fig. 3).

Control of the Microbicidal Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase 2 (NOX2) of Host Cells

The phagocyte NOX2, an isoform of NADPH oxidase, produces reactive oxygen species (ROS) which play a fundamental role in immune responses. *L. monocytogenes* phospholipases C (PlcA and PlcB) activate the NADPH oxidase during infection of phagocytes (Lam et al. 2011). However, LLO suppresses ROS produced by NADPH oxidase by preventing its localization to phagosomes (Fig. 3). This LLO-related activity can be also performed by the cytolysin perfringolysin O, suggesting that other bacteria have evolved similar mechanisms to inhibit the NADPH oxidase and avoid killing (Lam et al. 2011). In vivo studies show that loss of NADPH oxidase activity in mice augments the replication of *L. monocytogenes* compared to wild-type animals (Lam et al. 2011). In vitro research indicates that NOX2 NADPH oxidase limits vacuolar escape of *L. monocytogenes* in macrophages (Hamon et al. 2012; Lam et al. 2011).

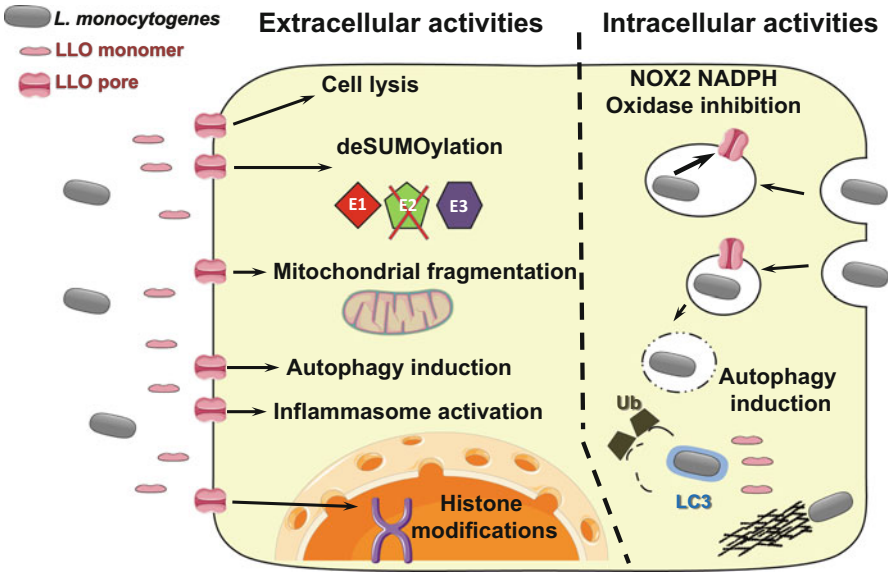

Fig. 3 Role of extracellular and intracellular LLO during infection. Extracellular LLO monomers bind cholesterol and assemble to form the transmembrane pore. Major roles associated with extracellular LLO include global deSUMOylation of host proteins by induced cleavage of the E2 enzyme Ubc9, mitochondrial fragmentation, and histone modifications, among others. Note that all the extracellular effects of LLO depicted require pore formation. Once *L. monocytogenes* is internalized in membrane-bound compartment, the secreted LLO prevents the NOX2 NADPH oxidase localization to phagosomes avoiding bacterial killing by reactive oxygen species during the respiratory burst. As shown in Fig. 2, LLO is critical for phagosomal disruption and *L. monocytogenes* translocation to the host cytoplasm. Additionally, intracytoplasmic LLO induces autophagy, leading to the recruitment of ubiquitin (Ub) to membrane remnants and of the marker LC3 around *L. monocytogenes* autophagosomes. We thank Servier Medical Art (http://www.servier.com/Powerpoint-image-bank) for providing drawings used

Extracellular LLO Activities

SUMOylation

SUMOylation is a reversible posttranslational modification in which proteins of the small ubiquitin-related modifier (SUMO) family are conjugated to proteins to regulate transcription, DNA repair, chromosome segregation, nuclear transport, intracellular transport, stress responses, protein stability, and defense against microbial pathogens among others (Hamon et al. 2012). *L. monocytogenes* infection of human epithelial cells decreases the levels of cellular SUMO-conjugated proteins in a LLO-induced pore-dependent manner (Fig. 3). Interestingly, the *L. monocytogenes* Δ*inlB* mutant (which is impaired in entry into HeLa cells) or noncytotoxic concentrations of LLO decrease the level of SUMO-conjugated proteins, indicating that this decrease is triggered by extracellular LLO. This effect is also shared by the cytolysins perfringolysin O and pneumolysin, and is based on the proteasome-independent degradation of Ubc9, the human E2 SUMO enzyme of the SUMOylation machinery.

Since no other E2 SUMO enzyme exists in the human genome, Ubc9 degradation leads to a general deSUMOylation of host proteins. Moreover, LLO triggers not only a global deSUMOylation event but also degradation of some SUMO-conjugated proteins promoting the infection capacity of *L. monocytogenes*. These in vitro effects of LLO are also observed in vivo since Ubc9 levels in livers of mice infected with *L. monocytogenes* showed a significant reduction at 48 h and 72 h after infection (Ribet et al. 2010).

Mitochondria and Endoplasmic Reticulum

Mitochondria are essential organelles that provide cellular ATP and biosynthetic intermediates. *L. monocytogenes* infection alters mitochondrial dynamics by causing transient mitochondrial network fragmentation through extracellular LLO-mediated calcium influx across the host cell plasma membrane (Fig. 3). The molecular consequence of this LLO effect is a unique dynamin-like protein 1 (Drp1) and optic atrophy protein 1 (Opa1)-independent mitochondrial fission mechanism, where the endoplasmic reticulum marks fragmentation regions. This mitochondrial fragmentation shuts down cellular bioenergetics and could be a strategy used by this bacterium to impair the cell capability to control the onset of infection (Stavru et al. 2013).

LLO produced by intracellular *L. monocytogenes* or secreted by extracellular bacteria injures the endoplasmic reticulum, causing a rapid swelling and activation of the unfolded protein response (UPR). The UPR has a defensive function at the early stages of infection, although continued endoplasmic reticulum (ER) stress favors macrophage death at later time points of infection (Hamon et al. 2012; Gekara et al. 2007).

Histones, DNA Damage Response, and Telomerase Reverse Transcriptase Alterations

Histones contribute to the packaging of DNA in the eukaryotic cell nuclei while retaining the properties of DNA for replication. Nucleosomes are composed of DNA and a histone octamer which consist of two copies each of the histones H2A, H2B, H3, and H4. Histone modifications control DNA accessibility in chromatin. LLO secreted extracellularly mediates a dramatic dephosphorylation of histone H3 and deacetylation of histone H4 during early phases of infection (Fig. 3). Other CDC toxins from extracellular pathogens like perfringolysin and pneumolysin induce similar histone modifications. The reduction of the levels of histone modifications leads to decreased transcriptional activity of some host genes, including key immunity genes (such as *cxcl2*, *dusp4*, or *egr1*) (Hamon et al. 2007). Further research has shown that the efflux of potassium from the eukaryotic cytoplasm is the signal that triggers the dephosphorylation of H3 (Hamon et al. 2012).

The DNA damage response (DDR) is an essential signaling pathway that senses and responds to DNA lesions caused, for example, by UV radiation or bacterial infection, in order to maintain cellular genetic integrity. *L. monocytogenes* induces LLO-mediated degradation of the principal DNA damage sensor, Mre11, blocking downstream signaling and dampening the DNA damage response which finally promotes bacterial replication (Samba-Louaka et al. 2014).

Telomeres are nucleoprotein complexes that protect the ends of chromosomes from degradation. Human telomerase reverse transcriptase (hTERT) is the catalytic subunit of the human telomerase complex. hTERT is involved in telomere maintenance and cell physiology functions, independent of telomere elongation (i.e., mitochondrial functions). Calcium influx through the LLO pores causes a decrease in hTERT levels at early time points of infection of HeLa cells, which surprisingly is detrimental for *L. monocytogenes* replication (Samba-Louaka et al. 2012).

Phospholipases

L. monocytogenes expresses two phospholipases C, a phosphatidylinositol-specific phospholipase C (or PlcA encoded by *plcA*, 954 bp) and a broad-range phospholipase C (PlcB encoded by *plcB*, 870 bp). Both genes are transcriptionally regulated by the transcriptional activator PrfA. These phospholipases hydrolyze phospholipids and damage host membranes.

PlcA is a 33-kd secreted phospholipase that possesses a signal cleavage site between alanine 29 and tyrosine 30. This enzyme is specific for phosphatidylinositol (PI), although it is able to weakly hydrolyze glycosyl PI (GPI)-anchored eukaryotic membrane proteins, with a pH optimum range from 5.5 to 7.0 (Goldfine and Knob 1992; Mengaud et al. 1991) (Fig. 1b). From all known bacterial phosphatidylinositol-specific phospholipases C, PlcA is the only one that lacks a beta-strand, which in the case of *Bacillus cereus* forms contacts with the glycan linker of GPI anchors and presumably enhances its activity on GPI-anchored proteins. Interestingly, expression of *Bacillus cereus* phosphatidylinositol-specific phospholipase in *L. monocytogenes* decreases its virulence, suggesting that loss of the PlcA beta-strand and decrease in GPI cleavage evolved to increase virulence (Wei et al. 2005).

PlcB is a zinc-dependent metalloenzyme secreted as a 264-amino acid inactive proenzyme that is processed in the extracellular medium by proteolytic cleavage to produce the active 29–30-kDa PlcB form. PlcB is secreted as an inactive proenzyme to prevent degradation of the phospholipids contained in the bacterial membrane. The metalloprotease Mpl is encoded in the same operon as PlcB, and it is also a zinc-dependent metalloprotease. Mpl processes the PlcB proenzyme into its active form and controls its cell wall translocation (Vazquez-Boland et al. 2001). Additionally, there is a Mpl-independent activation pathway dependent on acidification of the

vacuolar compartment (Vazquez-Boland et al. 2001). PlcB has a broad-optimum pH range between 5.5 and 8.0. It hydrolyzes phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin. It weakly hydrolyzes phosphatidylinositol and possesses a weak calcium-independent hemolytic activity at 37 °C in guinea pig, horse, and human erythrocytes, but not in sheep red blood cells (Vazquez-Boland et al. 1992, 2001) (Fig. 1c).

plcA and *plcB* are present in pathogenic *Listeria* species (*L. monocytogenes* and *L. ivanovii*) and absent in nonpathogenic species (with the exception of *L. seeligeri*). A large-scale and systematic analysis integrating molecular epidemiological data, as well as comparative genomics, demonstrated that *plcA* and *plcB* are conserved in all the *L. monocytogenes* human clinical isolates (Maury et al. 2016).

Virulence at the Organism Level

Using the intravenous mouse model of infection, it has been shown that PlcA contributes minimally to *L. monocytogenes* virulence as reflected by a threefold increase in mouse LD_{50} for the Δ*PlcA* mutant, whereas PlcB impacts more in virulence since its deletion causes a 20-fold increase in LD50. However, the double mutant Δ*plcA*Δ*plcB* is severely impaired in virulence as demonstrated by a 500-fold increase in LD50. These results demonstrate an overlapping role between the two listerial phospholipases (Smith et al. 1995). PlcB plays an important role in the pathogenesis of meningoencephalitis in an intracerebral infection murine model. Mice infected via intracerebral route with the *L. monocytogenes* Δ*plcB* strain survive longer and have less intracerebral bacterial colony-forming unit (CFU) (≈1 log less), compared to mice infected with the wild-type bacteria. Furthermore, histopathology analyses reveal that the Δ*plcB* strain presents a significantly delayed intracerebral spread (Schluter et al. 1998).

The majority of in vivo experiments performed to evaluate the contribution of *L. monocytogenes* phospholipases to virulence have been performed by the intravenous route in the mouse model. Interestingly, removal of the N-terminal propeptide of PlcB produces a constitutively active PlcB that causes damage to host cell membranes and negatively reduces the virulence in an oral mouse model of infection (200-fold decrease compared with the wild type in a competitive infection assay) (Yeung et al. 2007). It is important to stress that listeriosis is a foodborne disease where the intestinal stage plays a critical role (Quereda et al. 2016), and it would be interesting to evaluate if these phospholipases have an important role during this stage.

Strikingly, purified PlcB is not toxic in mice by intravenous administration. Mice survived after receiving up to 25 μg i.v. (Vazquez-Boland et al. 2001; Geoffroy et al. 1991). No experiments have been performed to evaluate the toxicity of purified PlcA, although due to its low impact in virulence compared to PlcB, it could be speculated that its toxic effect could be negligible.

Activity at the Cellular Level

Vacuolar Escape

PlcB plays a key role in vacuolar escape and cell-to-cell spread, not only between cells of the same tissular origin, but also between macrophages and brain microvascular endothelial cells in vitro, as well as for spread in murine brain tissue as already mentioned (Alberti-Segui et al. 2007; Vazquez-Boland et al. 2001). PlcA slightly contributes to escape from primary phagosomes and secondary double-membrane vacuoles. As for virulence in mice experiments, PlcA and PlcB work synergistically to promote vacuolar dissolution in in vitro experiments (Alberti-Segui et al. 2007). In the absence of LLO, the role of phospholipases is more important, and it has been suggested that a slow vacuolar maturation facilitates phospholipases-mediated escape (Burrack et al. 2009). Importantly, in LLO-deficient strains of *L. monocytogenes*, PlcB is required for rupture of primary vacuoles in human epithelial cell lines (i.e., Henle 407, HEp-2, and HeLa) (Fig. 2). As mentioned above, the proposed model for cell-to-cell spread is that *L. monocytogenes* phospholipases participate in the dissolution of the inner membrane, while LLO is involved in the disruption of the outer membrane (Alberti-Segui et al. 2007) (Fig. 2). This model implies that during listeriosis, spreading of *L. monocytogenes* to distant organs could be dependent on the action of these two phospholipases and that cell-to-cell spread can still occur in cells in which LLO is not strictly necessary for vacuolar escape (Alberti-Segui et al. 2007).

Phospholipid Metabolism

Eukaryotic cell phospholipids are differently generated and distributed at various intracellular locations to regulate a variety of important cellular processes. Some signaling proteins are recruited specifically to membrane sites by direct interaction with phospholipids. Membrane-derived extracellular vesicles also possess phospholipids and regulate important physiological processes. Phospholipid hydrolysis by *L. monocytogenes* phospholipases generates products like diacylglycerol (DAG), ceramide, and inositol phosphates which have important roles in host signaling pathways (i.e., synthesis of cytokines, cell growth, and apoptosis). Both LLO and PI-PLC induce translocation of protein kinase C delta to the periphery of J774 cells, and translocation of PKC beta II to early endosomes (Seveau 2014). In this way, *L. monocytogenes* phospholipases could modulate the host response to facilitate its intracellular survival. LLO and PlcA exogenously added to HUVEC cells synergize to elicit phosphoinositide metabolism and diacylglycerol accumulation. It has been proposed that the pore created by LLO allows access of PlcA to the phosphatidylinositol in the inner monolayer of the plasma membrane. Similarly, this LLO–PlcA synergy was observed in neutrophils where these two *Listeria* toxins provoked phosphoinositide hydrolysis and respiratory burst (Sibelius et al. 1999).

Autophagy

PI3P is generated from PI by the Vps34/Beclin-1 complex and plays an important role in autophagic flux and autophagosome formation. Interestingly, PlcA cleaves PI

into diacylglycerol (DAG) and myo-inositol, which finally affects the PI available for PI3P synthesis. PlcA reduces the cellular levels of PI3P and LC3 autophagic flux causing pre-autophagosomal structure stalling and preventing efficient autophagic targeting of cytosolic *L. monocytogenes* (Birmingham et al. 2007; Tattoli et al. 2013). As mentioned above, *L. monocytogenes* possesses other mechanisms to subvert autophagy defense pathways apart from PlcA, including the bacterial surface proteins InlK and ActA (Cossart 2011).

Listeriolysin S

LLS is an hemolytic and cytotoxic factor encoded by the structural gene *llsA* (150 bp) (Cotter et al. 2008) (Figs. 1d and 4). This toxin was discovered in 2008 and belongs to a family of posttranslationally modified peptides, the streptolysin S-like peptides. In this family there are other toxins produced by Gram-positive pathogens such as S*treptococcus pyogenes* (streptolysin S), *Staphylococcus aureus* str. RF122 (stapholysin S), *and Clostridium botulinum* (clostridiolysin S) (Molloy et al. 2011). In silico analysis of this cluster demonstrated similarities with the clusters responsible for production of streptolysin S and of the bacteriocin microcin B17 (Clayton et al. 2011).

LLS knowledge is very limited due to its recent discovery (2008) and to its absence from the majority of the most frequently used *L. monocytogenes* laboratory

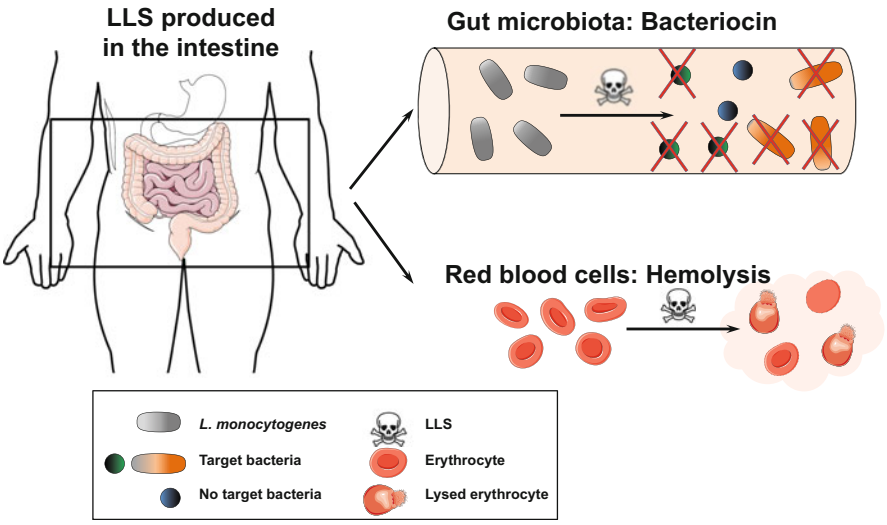

Fig. 4 LLS production is specifically triggered in the intestine of infected hosts. LLS is a bacteriocin that controls protective microorganisms from the gut microbiota and favors *L. monocytogenes* colonization of the intestine. LLS is additionally a potent hemolytic factor (see also Fig. 1). We thank Servier Medical Art (http://www.servier.com/Powerpoint-image-bank) for providing drawings used

strains (EGD-e, EGD, and 10403S) (Cotter et al. 2008). LLS cluster is only present in a subset of lineage I strains responsible for the majority of *L. monocytogenes* epidemics in humans and absent from lineage II and III *L. monocytogenes* strains (Cotter et al. 2008). It was recently shown that *L. monocytogenes* strains that contained LIPI-3 were epidemiologically associated with infections in humans (Maury et al. 2016).

llsA is located within a gene cluster-denominated LIPI-3 that also contains *llsX* (a gene specific of *L. monocytogenes* whose function remains elusive); *llsG* and *llsH* (encoding a putative ATP-binding cassette transport machinery); *llsB*, *llsY*, and *llsD* (these three genes are predicted to form a synthetase complex necessary for the posttranslationally modification of LLS); and *llsP*, a CAAX protease which is a putative immunity protein (Cotter et al. 2008). *llsG*, *llsH*, *llsX*, *llsB*, *llsY*, and *llsD* are essential for LLS hemolytic activity, while *llsP* has no effect on the toxin hemolytic phenotype (Clayton et al. 2011). LLS contains a putative Ala-Gly leader cleavage motif (amino acid 26) as well as a C-terminal core region with an extreme predominance of Cys, Ser and Thr residues which allow posttranslationally modifications resulting in the formation of a distinctive heterocyclic compound. Although the LLS posttranslational modifications are currently unknown, it is probable that like in other toxins of the same family, the Cys, Ser, and Thr residues are converted to thiazole, oxazole, and methyloxazole heterocycles, respectively (Molloy et al. 2011).

One study reported that the *llsA* promoter expression was negligible in *in vitro* laboratory conditions and only induced upon exposure to cumene hydrogen peroxide and hydrogen peroxide (Cotter et al. 2008). Another study also showed that *llsA* expression is negligible in *in vitro* classic laboratory conditions and that the promoter expression only can be observed in infected mice (Quereda et al. 2016). Strikingly, LLS expression was detected in the intestine while absent from the liver and spleen (despite higher CFU *Listeria* numbers can be detected in these two last organs) (Quereda et al. 2016). Hydrogen peroxide-mediated induction of the *llsA* promoter could not be reproduced in this latter study. The discrepancies between these two studies require further investigations.

LLS displays properties of both bacteriocins and hemolytic–cytotoxic factors. Bacteriocins are proteinaceus substances synthesized by bacteria that inhibit the growth of closely related bacteria, favoring the growth of the producing organism in various ecological niches. As a bacteriocin, LLS restricts the growth of other closely related Gram-positive bacteria like *Lactococcus lactis*, *Staphylococcus aureus*, and even *L. monocytogenes* from lineage II (EGD and 10403S) that lack the LLS operon. It has been proposed that in in vitro conditions, LLS hemolytic activity is only active in a bacteria-associated form but inactive in the culture supernatant. Addition of RNA core (ribonuclease-resistant fraction of yeast RNA) in an appropriate buffer containing ammonium acetate as a stabilizer in the culture medium results in hemolytic activity in the supernatant fraction. What remains unknown is if the RNA core acts as an inducer of LLS production or if it releases LLS from the cell wall of the bacteria (Cotter et al. 2008). Finally, liquid cocultures of *L. monocytogenes* constitutively producing LLS and target bacterial species showed

that secreted LLS could inhibit growth of the target species (Quereda et al. 2016). This implies that either LLS-bacteriocin activity is functional in the bacteria-free form against other target bacteria, or that LLS is transmitted upon contact between the LLS producer *L. monocytogenes* strain and the LLS-target strain.

When *L. monocytogenes* reaches the intestine after ingestion of contaminated food, LLS expression is triggered, and as a consequence, the host microbiota is altered (Fig. 4). In particular, LLS expression reduces the number of representatives of the genera *Allobaculum* and *Alloprevotella*. These two genera include species that produce butyric and acetic acid, respectively. These fatty acids negatively impact the ability of *L. monocytogenes* to grow and colonize the intestine. As a consequence, LLS expression in the intestine plays a critical role in *L. monocytogenes* survival within this organ, the first barrier before access to deeper host tissues. Importantly, *L. monocytogenes* lineage I strain F2365 (which contains LLS) colonizes better the intestine than lineage II strains EGD-e and 10403S (which lack LLS) in a mouse oral infection model (Quereda et al. 2016). The higher CFU numbers in the intestine allow the F2365 strain to better colonize deeper organs like the spleen or liver.

One study reported that LLS is also cytotoxic for human cell lines (like C2-Bbe), mouse cell lines (like J774 and CT26), as well as sheep erythrocytes (Cotter et al. 2008). The same study also showed that LLS improves the survival of *L. monocytogenes* in purified polymorphonuclear neutrophils, a crucial component of the immune system for the resolution of *Listeria* infections. Accordingly, LLS plays a role in the intraperitoneal mouse model of infection, where *L. monocytogenes* Δ*llsB* CFU numbers in the spleen and liver are approximately twofold lower compared with the wild-type strain (Cotter et al. 2008).

Conclusion and Future Directions

The exotoxin arsenal of *L. monocytogenes* plays a critical role in its adaptation to mammalian hosts and in particular to the intracellular life. The recent discovery of LLS and its bimodal activity on eukaryotic and prokaryotic membranes opens now new perspectives, not only on *L. monocytogenes* virulence but also on its environmental distribution: how the expression of these different exotoxins is regulated? Do these toxins, and in particular LLS, have a role in *L. monocytogenes* survival in nature? Does LLS cooperate with LLO, PlcA, and PlcB during the *L. monocytogenes* intracellular life cycle? How does LLS perforate red blood cells and what is its bactericidal mechanism in target bacterial species? Addressing all these questions will allow us to better understand the natural history of *L. monocytogenes* and in particular its molecular mechanisms to promote disease.

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References

Alberti-Segui C, Goeden KR, Higgins DE. Differential function of *Listeria monocytogenes* listeriolysin O and phospholipases C in vacuolar dissolution following cell-to-cell spread. Cell Microbiol. 2007;9(1):179–95.

Arnett E, Lehrer RI, Pratikhya P, Lu W, Seveau S. Defensins enable macrophages to inhibit the intracellular proliferation of *Listeria monocytogenes*. Cell Microbiol. 2011;13(4):635–51.

Berche P, Reich KA, Bonnichon M, Beretti JL, Geoffroy C, Raveneau J, et al. Detection of anti-listeriolysin O for serodiagnosis of human listeriosis. Lancet. 1990;335(8690):624–7.

Birmingham CL, Canadien V, Gouin E, Troy EB, Yoshimori T, Cossart P, et al. *Listeria monocytogenes* evades killing by autophagy during colonization of host cells. Autophagy. 2007;3(5):442–51.

Birmingham CL, Canadien V, Kaniuk NA, Steinberg BE, Higgins DE, Brumell JH. Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. Nature. 2008;451 (7176):350–4.

Burrack LS, Harper JW, Higgins DE. Perturbation of vacuolar maturation promotes listeriolysin O-independent vacuolar escape during *Listeria monocytogenes* infection of human cells. Cell Microbiol. 2009;11(9):1382–98.

Carrero JA, Calderon B, Unanue ER. Listeriolysin O from *Listeria monocytogenes* is a lymphocyte apoptogenic molecule. J Immunol. 2004;172(8):4866–74.

Clayton EM, Hill C, Cotter PD, Ross RP. Real-time PCR assay to differentiate Listeriolysin S-positive and -negative strains of *Listeria monocytogenes*. Appl Environ Microbiol. 2011;77 (1):163–71.

Cossart P. Illuminating the landscape of host-pathogen interactions with the bacterium *Listeria monocytogenes*. Proc Natl Acad Sci U S A. 2011;108(49):19484–91.

Cossart P, Toledo-Arana A. *Listeria monocytogenes*, a unique model in infection biology: an overview. Microbes Infect. 2008;10(9):1041–50.

Cossart P, Vicente MF, Mengaud J, Baquero F, Perez-Diaz JC, Berche P. Listeriolysin O is essential for virulence of *Listeria monocytogenes*: direct evidence obtained by gene complementation. Infect Immun. 1989;57(11):3629–36.

Cotter PD, Draper LA, Lawton EM, Daly KM, Groeger DS, Casey PG, et al. Listeriolysin S, a novel peptide haemolysin associated with a subset of lineage I *Listeria monocytogenes*. PLoS Pathog. 2008;4(9):e1000144.

Gekara NO, Westphal K, Ma B, Rohde M, Groebe L, Weiss S. The multiple mechanisms of Ca2+ signalling by listeriolysin O, the cholesterol-dependent cytolysin of *Listeria monocytogenes*. Cell Microbiol. 2007;9(8):2008–21.

Gekara NO, Zietara N, Geffers R, Weiss S. *Listeria monocytogenes* induces T cell receptor unresponsiveness through pore-forming toxin listeriolysin O. J Infect Dis. 2010;202 (11):1698–707.

Geoffroy C, Gaillard JL, Alouf JE, Berche P. Purification, characterization, and toxicity of the sulfhydryl-activated hemolysin listeriolysin O from *Listeria monocytogenes*. Infect Immun. 1987;55(7):1641–6.

Geoffroy C, Raveneau J, Beretti JL, Lecroisey A, Vazquez-Boland JA, Alouf JE, et al. Purification and characterization of an extracellular 29-kilodalton phospholipase C from *Listeria monocytogenes*. Infect Immun. 1991;59(7):2382–8.

Glomski IJ, Gedde MM, Tsang AW, Swanson JA, Portnoy DA. The *Listeria monocytogenes* hemolysin has an acidic pH optimum to compartmentalize activity and prevent damage to infected host cells. J Cell Biol. 2002;156(6):1029–38.

Goldfine H, Knob C. Purification and characterization of *Listeria monocytogenes* phosphatidylinositol-specific phospholipase C. Infect Immun. 1992;60(10):4059–67.

Hamon MA, Batsche E, Regnault B, Tham TN, Seveau S, Muchardt C, et al. Histone modifications induced by a family of bacterial toxins. Proc Natl Acad Sci U S A. 2007;104(33):13467–72.

Hamon MA, Ribet D, Stavru F, Cossart P. Listeriolysin O: the Swiss army knife of Listeria. Trends Microbiol. 2012;20(8):360–8.

Henry R, Shaughnessy L, Loessner MJ, Alberti-Segui C, Higgins DE, Swanson JA. Cytolysin-dependent delay of vacuole maturation in macrophages infected with *Listeria monocytogenes*. Cell Microbiol. 2006;8(1):107–19.

Koster S, van Pee K, Hudel M, Leustik M, Rhinow D, Kuhlbrandt W, et al. Crystal structure of listeriolysin O reveals molecular details of oligomerization and pore formation. Nat Commun. 2014;5:3690.

Lam GY, Fattouh R, Muise AM, Grinstein S, Higgins DE, Brumell JH. Listeriolysin O suppresses phospholipase C-mediated activation of the microbicidal NADPH oxidase to promote *Listeria monocytogenes* infection. Cell Host Microbe. 2011;10(6):627–34.

Lecuit M, Sonnenburg JL, Cossart P, Gordon JI. Functional genomic studies of the intestinal response to a foodborne enteropathogen in a humanized gnotobiotic mouse model. J Biol Chem. 2007;282(20):15065–72.

Maury MM, Tsai YH, Charlier C, Touchon M, Chenal-Francisque V, Leclercq A, et al. Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. Nat Genet. 2016;48 (3):308–13.

Mengaud J, Braun-Breton C, Cossart P. Identification of phosphatidylinositol-specific phospholipase C activity in *Listeria monocytogenes*: a novel type of virulence factor? Mol Microbiol. 1991;5(2):367–72.

Meyer-Morse N, Robbins JR, Rae CS, Mochegova SN, Swanson MS, Zhao Z, et al. Listeriolysin O is necessary and sufficient to induce autophagy during *Listeria monocytogenes* infection. PLoS One. 2010;5(1):e8610.

Molloy EM, Cotter PD, Hill C, Mitchell DA, Ross RP. Streptolysin S-like virulence factors: the continuing sagA. Nat Rev Microbiol. 2011;9(9):670–81.

Murray EGD, Webb R, Swann MBR. A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J Pathol Bacteriol. 1926;29(4):407–39.

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(11):2263–77.

Peraro MD, van der Goot FG. Pore-forming toxins: ancient, but never really out of fashion. Nat Rev Microbiol. 2016;14(2):77–92.

Quereda JJ, Ortega AD, Pucciarelli MG, Garcia-Del PF. The listeria small RNA Rli27 regulates a cell wall protein inside eukaryotic cells by targeting a long 5′-UTR variant. PLoS Genet. 2014;10(10):e1004765.

Quereda J, Dussurget O, Nahori M, Ghozlane A, Volante S, Dilliès M, et al. A bacteriocin from epidemic Listeria strains alters the host intestinal microbiota to favor infection. Proc Natl Acad Sci U S A. 2016;113(18):5706–11.

Radtke AL, Anderson KL, Davis MJ, DiMagno MJ, Swanson JA, O'Riordan MX. *Listeria monocytogenes* exploits cystic fibrosis transmembrane conductance regulator (CFTR) to escape the phagosome. Proc Natl Acad Sci U S A. 2011;108(4):1633–8.

Reniere ML, Whiteley AT, Hamilton KL, John SM, Lauer P, Brennan RG, et al. Glutathione activates virulence gene expression of an intracellular pathogen. Nature. 2015;517 (7533):170–3.

Ribet D, Hamon M, Gouin E, Nahori MA, Impens F, Neyret-Kahn H, et al. *Listeria monocytogenes* impairs SUMOylation for efficient infection. Nature. 2010;464(7292):1192–5.

Richter JF, Gitter AH, Gunzel D, Weiss S, Mohamed W, Chakraborty T, et al. Listeriolysin O affects barrier function and induces chloride secretion in HT-29/B6 colon epithelial cells. Am J Physiol Gastrointest Liver Physiol. 2009;296(6):G1350–9.

Samba-Louaka A, Stavru F, Cossart P. Role for telomerase in *Listeria monocytogenes* infection. Infect Immun. 2012;80(12):4257–63.

Samba-Louaka A, Pereira JM, Nahori MA, Villiers V, Deriano L, Hamon MA, et al. *Listeria monocytogenes* dampens the DNA damage response. PLoS Pathog. 2014;10(10):e1004470.

Schluter D, Domann E, Buck C, Hain T, Hof H, Chakraborty T, et al. Phosphatidylcholine-specific phospholipase C from *Listeria monocytogenes* is an important virulence factor in murine cerebral listeriosis. Infect Immun. 1998;66(12):5930–8.

Schnupf P, Portnoy DA. Listeriolysin O: a phagosome-specific lysin. Microbes Infect. 2007;9 (10):1176–87.

Seveau S. Multifaceted activity of listeriolysin O, the cholesterol-dependent cytolysin of *Listeria monocytogenes*. Subcell Biochem. 2014;80:161–95.

Sibelius U, Schulz EC, Rose F, Hattar K, Jacobs T, Weiss S, et al. Role of *Listeria monocytogenes* exotoxins listeriolysin and phosphatidylinositol-specific phospholipase C in activation of human neutrophils. Infect Immun. 1999;67(3):1125–30.

Singh R, Jamieson A, Cresswell P. GILT is a critical host factor for *Listeria monocytogenes* infection. Nature. 2008;455(7217):1244–7.

Smith GA, Marquis H, Jones S, Johnston NC, Portnoy DA, Goldfine H. The two distinct phospholipases C of *Listeria monocytogenes* have overlapping roles in escape from a vacuole and cell-to-cell spread. Infect Immun. 1995;63(11):4231–7.

Stavru F, Archambaud C, Cossart P. Cell biology and immunology of *Listeria monocytogenes* infections: novel insights. Immunol Rev. 2011;240(1):160–84.

Stavru F, Palmer AE, Wang C, Youle RJ, Cossart P. Atypical mitochondrial fission upon bacterial infection. Proc Natl Acad Sci U S A. 2013;110(40):16003–8.

Tattoli I, Sorbara MT, Yang C, Tooze SA, Philpott DJ, Girardin SE. Listeria phospholipases subvert host autophagic defenses by stalling pre-autophagosomal structures. EMBO J. 2013;32 (23):3066–78.

Vadia S, Arnett E, Haghighat AC, Wilson-Kubalek EM, Tweten RK, Seveau S. The pore-forming toxin listeriolysin O mediates a novel entry pathway of *L. monocytogenes* into human hepatocytes. PLoS Pathog. 2011;7(11):e1002356.

Vazquez-Boland JA, Kocks C, Dramsi S, Ohayon H, Geoffroy C, Mengaud J, et al. Nucleotide sequence of the lecithinase operon of *Listeria monocytogenes* and possible role of lecithinase in cell-to-cell spread. Infect Immun. 1992;60(1):219–30.

Vazquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Dominguez-Bernal G, Goebel W, et al. Listeria pathogenesis and molecular virulence determinants. Clin Microbiol Rev. 2001;14(3):584–640.

Wei Z, Zenewicz LA, Goldfine H. *Listeria monocytogenes* phosphatidylinositol-specific phospholipase C has evolved for virulence by greatly reduced activity on GPI anchors. Proc Natl Acad Sci U S A. 2005;102(36):12927–31.

Yeung PS, Na Y, Kreuder AJ, Marquis H. Compartmentalization of the broad-range phospholipase C activity to the spreading vacuole is critical for *Listeria monocytogenes* virulence. Infect Immun. 2007;75(1):44–51.