

A Ride with *Listeria monocytogenes*: A Trojan Horse

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Summary

Listeriosis, a disease caused by *Listeria monocytogenes*- a facultative, intracellular bacterium, spreads through contaminated food. It affects epithelial cells and macrophages and has a mortality rate of about 30%. The bacterium can cross the blood brain barrier, causing meningitis, and the placental barrier, causing abortion. Some mechanisms for entry into cells include the InIA- E-cadherin adhesion and InIB-Met pathway. *hly*, one of the many genes activated during infection, leads to the production of Listeriolysin O (LLO). LLO and two distinct phospholipases are indispensable to the spread of *Listeria*. Phosphatidylinositol-specific phospholipase C (PI-PLC) activates a host protein kinase C (PKC), which facilitates the escape of the bacterium from the primary vacuole, along with LLO. Once inside the cell's cytoplasm, *Listeria* replicates. At this point, both the original bacterium and the daughter cells use Act A protein to exploit the cell's machinery to polymerize actin. Actin-based motility propels the *Listeria* throughout the cell and facilitates its intercellular spread. Current curative methods include ampicillin, gentamicin, and chloramphenicol, reserved for life threatening infections. Treatment via plant extracts of *Pluchea quitoc* is in the experimental stage. This review focuses on tracking the progression of the *L. monocytogenes* bacterium from its entry to spread.

The story of the Trojan horse: Introduction

Listeria monocytogenes (LM) is a ubiquitous, facultative intracellular bacterium which can thrive in a variety of environments and hosts. It is a Gram-positive bacterium and spreads primarily through contaminated foods (Portnoy et al., 2002). Foods that are most commonly contaminated by *Listeria* are meats, milk, soft cheese, dairy products and industrially-produced refrigerated food products (Vázquez-Boland et al., 2001). LM can survive and proliferate in acidic environments, high salt concentrations and even at very low temperatures (www.textbookofbacteriology.net, Vázquez-Boland et al., 2001).

Listeria microorganisms were first discovered in 1924 by E.G.D. Murray, R.A. Webb, and M. B.R. Swann, as the microorganisms causing a septicemic disease in rabbits and guinea pigs in their laboratory in England. However, the first case of the disease was reported in humans in Denmark in 1929 (Vázquez-Boland et al., 2001). An outbreak of Listeriosis, the infection caused by *L. monocytogenes*, in California, in 1985, claimed the lives of 18 adults and 30 fetuses, outlining the high mortality of this disease (www.textbookofbacteriology.net). LM has been studied

extensively in murine macrophages in the past 40-50 years. The study of the life cycle of LM in this experimental model has contributed significantly to the understanding of the immune response to intracellular pathogens (Portnoy et al., 2002).

Listeriosis manifests itself through flu-like symptoms and can lead to diarrhea, meningitis, encephalitis, meningoencephalitis and stillbirths. In humans, it primarily infects immunocompromised individuals like pregnant women, neonates, and the elderly. (Portnoy et al., 2002, Vázquez-Boland et al., 2001, and Dyer et al., 2002). Listeriosis is more dominant in females because of differential production of the immunosuppressive cytokine IL-10 (Pasche et al., 2005). Immunocompetent individuals usually survive the infection, whereas those with debilitating diseases often die (mean mortality rate of 30-40%) (Vázquez-Boland et al., 2001).

LM can cross the placental and blood brain barriers; however, in order to do so, it needs to pass the intestinal barrier and survive the harsh environment of the stomach. The primary method of entry into endothelial cells is believed to be via a zipper-like mechanism (Alberts et al., 2003) Invasion proteins on the surface of the bacteria, like Internalin A, and Internalin B, and P60, help the bacterium bind to host surface receptors (Drevets et al., 2004). This binding induces phagocytosis of the bacteria into the host cell. Another mechanism by which LM is internalized is phagocytosis by macrophages. Once inside the host cells, the bacteria secrete a pore forming hemolysin, known as Listeriolysin O (LLO) and two distinct phospholipases, PI-PLC and PC-PLC. These hemolysins, along with the phospholipases mediate the degradation of the phagolysosome and the escape of the bacteria from the vacuole into the intracytoplasmic environment of the host cell. Once LM is in the cytosol, the ActA protein recruits host cell Arp2/3 complexes to enable efficient actin-based motility. Actin-based motility enables the bacteria to form filopods (pseudopod-like extensions) and propels it into neighboring cells, resulting in spread of the infection (Portnoy et al., 2002 and Vázquez-Boland et al., 2001).

The immune response to *L. monocytogenes* is entirely cell mediated. CD8+ T-cells recognize and lyse infected cells. The NF- κ B pathway is used to activate immune response genes and in the subsequent production of interleukins (IL's) (Portnoy et al., 2002 and Vázquez-Boland et al., 2001).

Current treatments of Listeriosis are intravenous administration of ampicillin and gentamicin. Treatment can last for 10 days, but depends on the body's ability to fight the infection (www.kidshealth.org). This paper will review the current understanding of mechanisms underlying the internalization of *Listeria monocytogenes*, its subsequent escape from the phagolysosome, replication in the host cytosol and its actin-based motility (Fig 1). Currently available treatments will be discussed along with future experiments which could lead to more effective therapies.

The horse looks like a present, but...: *Listeria monocytogenes* entry mechanisms

LM being an intracellular bacterium, it is very important for this pathogen to gain entry into a cell in order to

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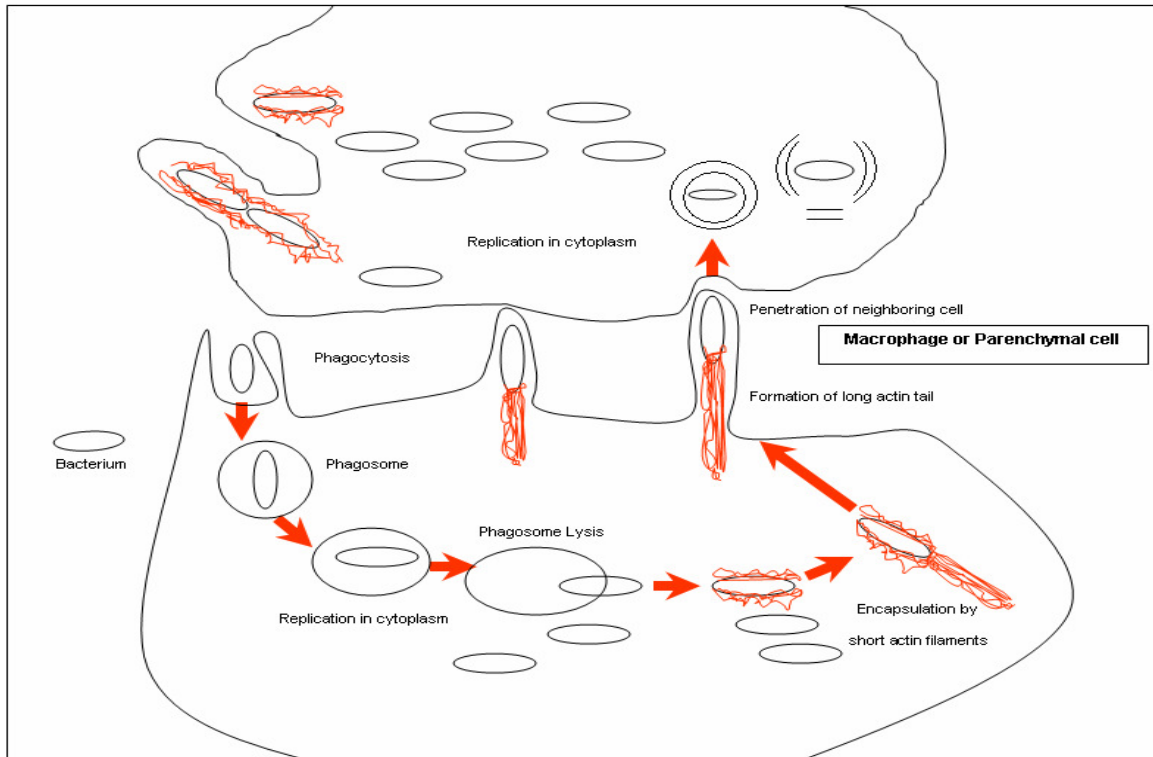


Figure 1: Model showing the entry and spread of *Listeria monocytogenes*

The bacterium is phagocytosed by the host cell, using cell adhesion proteins. It escapes the vacuole by secreting a pore-forming toxin, Listeriolysin, and the action of phospholipases. Subsequent to its escape from the vacuole, LM acquires actin based motility, which enables it to form filopods and spread from cell to cell (Reproduced from <http://textbookofbacteriology.net/Listeria.html>).

replicate and thus cause Listeriosis. LM has many mechanisms by which it can gain entry into a cell. A widely studied pathway involves proteins of the Internalin family. Internalin (InIA) and B (InIB) are involved in two distinct pathways by which they allow the bacterium to enter the cell.

Internalin (InIA) and Internalin B (InIB) dependent internalization of LM

The Internalin family of *Listeria* proteins is very large. It is composed of at least seven members including InIC, InIC2, InID, InIE, InIF, InIG, and InIH (Marrino et al., 2000). The two members of this family that are relevant to entry of LM into the host cell's are InIA and InIB. These proteins are found on the cell surface of LM and are involved in its internalization. Internalins bind specific receptors on the host cells surface and thus trigger phagocytosis of LM. Through these mechanisms, LM can induce phagocytosis in non-phagocytic cells *in vitro*. This phagocytosis is attributed to the reorganization of the actin cytoskeleton, which, in turn, leads to membrane folding (Marrino et al., 2000). These two proteins as well as other proteins that belong to this family have some structural features in common. Both proteins have a Leucine Rich Repeat region (LRR) and a β repeat region, as well as an Inter repeat region between the two (IR) that is extremely conserved (Lecuit et al., 1997). The LRR motif is involved in protein-protein interaction and is repeated in a highly regular fashion (Marrino et al., 2000). The LRR regions are flanked by highly-conserved sequences on either side that may play a role in the stability of the protein. At the N terminus there is a hydrophilic cap known as

the "N-terminal cap" and at the C terminus there is a conserved sequence known as the IR or Inter repeat region (Marrino et al., 2000). The LRR regions are structurally and functionally important to the internalization of LM (Dussurget et al., 2004).

Bacterial Internalin (InIA) is a ligand for E-Cadherin receptors

InIA is involved primarily in the infection of epithelial cells. InIA, specifically, is anchored to the cell wall of the bacteria through a LPXTG motif in its carboxyl terminal region (Lecuit et al., 1997). The receptor for InIA is E-Cadherin. E-cadherin is a part of the cadherin super-family of transmembrane glycoproteins that act as adhesion molecules. It is located at the adherens' junctions and allows for the Ca^{2+} dependent adhesion of two cells (Dussurget et al., 2004). In addition, not all E-cadherins are receptors for InIA. Rat and mouse E-cadherins cannot bind InIA. This may be due to the absence of a proline at position 16 of the rat and mouse E-cadherins that allows for such specificity (Lecuit et al., 2001). In species that do present a favorable E-cadherin receptor, InIA interacts with the first two ectodomains (protein domains outside the cell) of E-cadherin. Specifically, the amino terminal region of InIA that contains the LRR and IR regions interacts with E-cadherin and is necessary and sufficient to promote the internalization of LM. Furthermore, it is the LRR region that directly interacts with the E-cadherin ectodomains, whereas the IR region is important in the folding of the LRR region (Lecuit et al., 1997).

The carboxyl terminal of E-cadherin directly interacts with the intracellular β -catenin. α -catenin, in

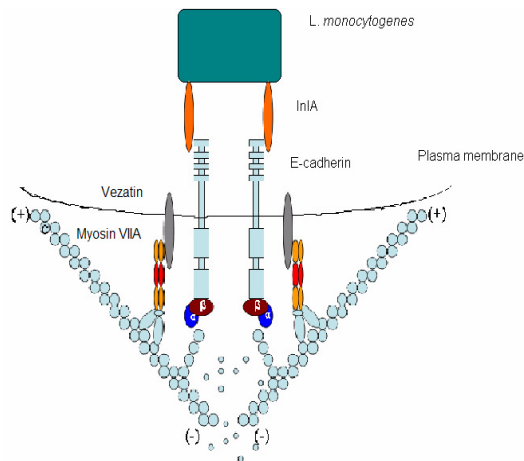


Fig 2: Model for InlA-dependent entry of LM into epithelial cells.

Proteins known to play a role in entry are indicated, including E-cadherin, α and β -catenins, vezatin, myosin VIIA, and actin. This model highlights how myosin VIIA could help the membrane rearrangements during LM entry. (Modified from Sousa et al., 2003)

turn, binds to β -catenin and interacts with actin (Lecuit et al., 2000). This interaction leads to the formation of a fusion molecule consisting of the ectodomains of the E-cadherin and the actin binding site of the α -catenin which eventually leads to LM entry (Dussurget et al., 2004). Furthermore, myosin VIIA and its ligand vezatin together function as the molecular motor in the internalization of *Listeria*. When myosin VIIA binds vezatin, coupled with an actin polymerization process, it provides the tension necessary for bacterial internalization (Fig. 2) (Sousa et al., 2003).

InlB as a virulence factor for hepatocytes and other non-epithelial cells during Listeriosis

InlB is a bacterial protein that is anchored to the cell wall of LM through a series of GW repeats (Lecuit et al., 1997). InlB has an elongated structure and its main receptor for LM invasion is the hepatocyte growth factor receptor, also known as Met (Dussurget et al., 2004). It is interesting to note that InlB does not strictly mimic the hepatocyte growth factor (HGF), in that HGF and InlB do not share sequence similarities. Once Met has been activated by InlB, it autophosphorylates two tyrosine kinase residues and recruits Gab 1, Sch and Cbl as well as PI 3-kinase. PI 3-kinase, which is known to be involved in control of the actin cytoskeleton, activates PLC- γ 1 (Vazquez-Boland et al., 2001). The activation of Met is enhanced in the presence of glycosaminoglycans (GAG's) (Bierne et al., 2002). These are normally involved in the oligomerization as well as storage and protection from extracellular proteases (Dussurget et al., 2004).

Another receptor for InlB is some form of the surface associated gC1q-R. However, the specific mechanism by which InlB binds and interacts with gC1q-R remains to be determined. Since this protein lacks a transmembrane domain, it may act as a signaling co-receptor (Vazquez-Boland et al., 2001). In addition, the partial inhibition of InlB-mediated signaling pathway due to gC1q-R antibodies supports this hypothesis. InlB-Met signaling leads to both

phagocytosis and membrane ruffling (Bierne et al., 2002).

Gene Expression in the Intracellular Life Cycle of *Listeria monocytogenes*

After the bacterium has been internalized, its gene expression changes with respect to its new environment. The virulence genes (*prfA*, *plcA*, *hly*, *mpl*, *actA*, and *plcB*) located in the major virulence gene cluster are strongly regulated during intracellular growth (Chatterjee, et al., 2006). The *PrfA* gene is an autoregulatory positive regulatory factor required for the regulation of the virulence genes, in addition to expression of other genes elsewhere on the chromosome, like *inlA*. *PrfA* is regulated by the general stress-response alternative sigma factor σ^B , which plays a crucial role in the invasion of cells, but not the systemic spread (Garner et al., 2004).

Genes important for the escape of bacteria from the phagolysosome to the intracytoplasmic environment are *hly* and *plcA*. *hly* encodes for the pore-forming toxin listeriolysin O (LLO), while the *plcA* gene is responsible for the production of phosphatidylinositol phospholipase C. Both of these proteins are essential for the escape of the pathogen from the primary vacuole (phagolysosome) into the cytoplasm of the host cell. In a study conducted using murine macrophages, it was shown that Δ *hly* and Δ *plcA* double-deletion mutant *Listeria* are rapidly killed (Chatterjee, et al., 2006). It was also shown that these genes are up-regulated during the intracellular phase of growth.

In an intracellular milieu, LM changes its normal sugar metabolism. Genes encoding enzymes in the second part of glycolysis were reduced during infection. Further, it was found that an operon encoding glycerol kinase and the glycerol uptake facilitator (lmo 1538 to lmo 1539) and glycerol-3-phosphate dehydrogenase (lmo 1293) were upregulated, indicating that glycerol was being used as the additional carbon source for intracellular growth. This could be a mechanism whereby the bacteria do not affect the host cell's energy source and are able to spread more rapidly, rather than killing the host cell. Glucose also inhibits the expression of *prfA*, and hence the expression of the major virulence gene cluster (Chatterjee et al., 2006).

Genes required for the normal replication of LM were downregulated. The *ftsZ* and *ftsA* genes, which are the major bacterial cell division determinants, were downregulated, suggesting lowered cell division. This lowered cell division activity is probably a result of the host cell's defense mechanism to keep bacterial multiplication in check.

Another important gene that is altered during the intracellular phase of LM's growth is lmo0593, which is a nitrite transporter gene. The increased transport of nitrite by the bacteria suggests that nitrite is used in place of oxygen as the final electron acceptor in the electron transport chain. This mechanism allows the bacteria to survive under oxygen deprived conditions in the host cell (Chatterjee et al., 2006).

There was no door, so how did they get out? : Escaping into the cell cytoplasm

Once inside the host cell, the bacterium is enclosed by the phagosomal membrane. LM must have a way to escape the vacuole because this is where the bacteria replicate. It is here that LM uses the host cell

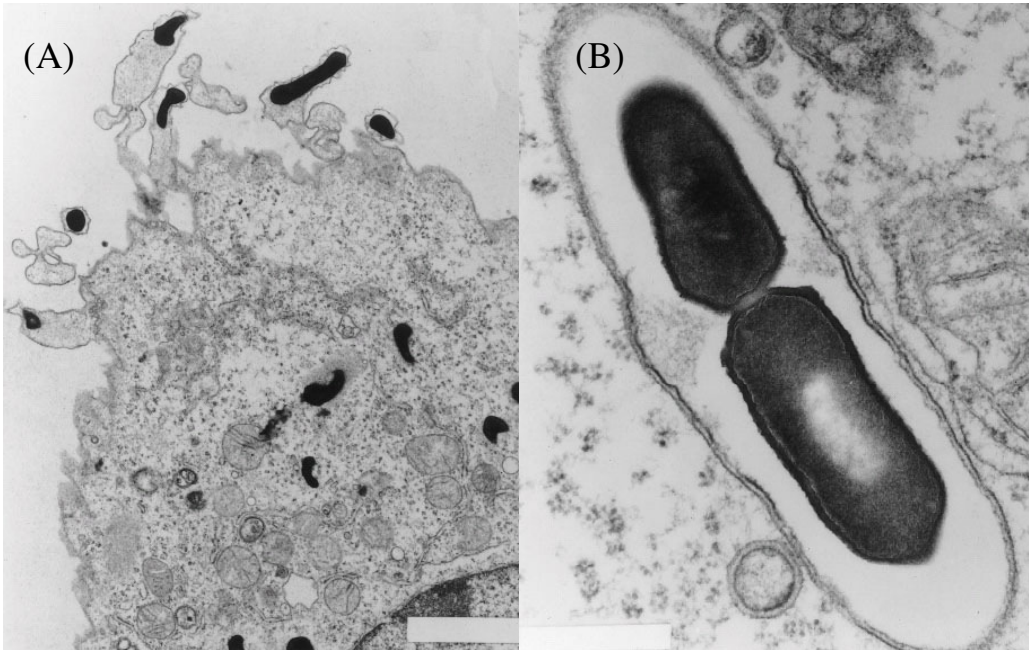


Figure 3: Transmission Electron Micrographs of *Listeria monocytogenes*

(A) Wild type *Listeria monocytogenes* free in the cytosol of a macrophage (size bar = 2 μ m). (B) A secondary macrophage with LM in a double membrane vacuole (size bar = 0.5 μ m) (Gedde et al, 1999).

machinery. In order for LM to enter the cytosol it first needs to escape the phagosome. Both LLO and two types of PLCs play a key role in mediating this escape.

Virulence Factors:

Listeriolysin O

LM is one of the many bacteria that produce hemolysins. *Listeria* secretes Listeriolysin O (LLO), which, along with a phospholipase, *PlcA*, plays a very important role in the escape of the pathogen from a vacuolar compartment (Portnoy et al., 1992). Listeriolysin is a member of the thiol-activated cytolytins, which include perfringolysin O (PFO), streptolysin O (SLO), and pneumolysin, among others. These hemolysins are inhibited by free cholesterol and cysteine; free cholesterol is the common hemolysin receptor, and cysteine oxidation causes reversible protein inactivation (Portnoy et al., 1992). It has been shown that the expression of listeriolysin in an extracellular non-pathogenic soil bacterium, *Bacillus subtilis*, enables the bacterium to grow in the cytoplasm of mammalian cells. In a subsequent study, it was shown that LLO is not the only cytolytin that allows bacteria to proliferate in the cytoplasm of host cells. When *B. subtilis* expresses PFO, it is also able to escape the phagolysosome and replicate in the cytoplasm. However, unlike LLO, PFO causes damage to host cells (Portnoy et al., 1992, 1994). It has also been shown that LM expressing PFO instead of LLO is much less virulent (Portnoy et al., 1994). This result is consistent with the observation that LLO works best in an acidic pH, whereas PFO functions in both acidic and neutral environments. The increased efficiency of LLO at a pH of 5.5 is a mechanism by which LM compartmentalizes the activity of LLO to escape the vacuole and subsequently replicates in the cytoplasm without causing damage to the host cell (Portnoy et al., 1992).

In a study by Lety et al, it was shown that a PEST-like motif in LLO is required for the escape of LM from the vacuole and for causing virulence. The removal of this PEST-like sequence results in a strain that is extremely toxic to host cells and is 10,000 times less virulent in mice macrophages (Portnoy et al., 2002).

In contrast to LLO and PFO, streptolysin O (SLO) was found to have a 10-fold lower activity. *B. subtilis* expressing SLO could not grow in the cytoplasm of host cells efficiently, presumably because they were unable to escape the phagolysosome. It is possible that SLO is less stable in an acidic environment and is not able to lyse the vacuole (Portnoy et al., 1992). Gedde et al used a genetic approach to investigate the role of LLO in intracellular growth and cell-to-cell spread. Six-His-tagged LLO (HisLLO), noncovalently bonded to the surface of nickel-treated LLO lacking LM, enabled some of these cells to escape the host vacuole and replicate in the cytoplasm. Both LLO lacking LM and wild type LM were able to replicate in the cytosol of the host cell (Fig 3A). LLO lacking LM could also spread to adjacent cells, however, these LM were trapped in double-membrane vacuoles (Fig. 3B). Surprisingly, phospholipase C (PC-PLC) and PI-PLC were also not required in the spread of LLO negative LM into secondary cells.

Phospholipases

LM also uses phospholipases C to aid in the escape from the vacuole. Two specific phospholipases (PLCS) are used. One is the phosphatidylinositol-specific PLC (PI-PLC), and the other is more general, phosphatidylinositol-specific (PC-PLC) (Portnoy, et al., 2002). LLO has been studied in great detail as the major factor for the permeation which leads to phagosomal escape. Studies have also attributed PC-PLC to the escape even in the absence of LLO (Sibeliuss et al., 1992).

The role of the PI-PLC secreted by LM is to catalyze the production of inositol phosphate and diacylglycerol (DAG) through cleavage of the membrane lipid PI. DAG then has the ability to activate protein kinase C (PKC). There are four types of PKCs, but the PKC β of the host is shown to be linked with the PI-PLC signaling cascade. The PKC β has been shown to facilitate the permeation of the phagosomal membrane before the bacteria escape (Poussin et al., 2005).

PI-PLC has another component which allows for the escape of the bacterium into the cytosol. It is known that LM has a weaker effect on the GPI-anchored proteins than most bacteria do. This has been found to be the result of the PI-PLCs of LM differing from those of other bacteria. There is a Vb β -strand which is found in other bacterial PI-PLCs which is absent from *L. monocytogenes*. The Vb β -strand is known to give a contact for the glycan linker of GPI-anchored protein. This contact enhances the ability of the cell to cleave the GPI anchors. When Vb β -strand was absent in LM, the cell's ability for the bacteria to escape the phagosome and be released into the cytosol of the host cell was increased. It is through these observations, as well previous knowledge of Vb β -strand, and PI-PLCs that it can be hypothesized that LM have evolved this absence or loss in order to promote growth inside the host cell (Wei et al., 2005).

PI-PLC and PC-PLC have also been shown to have an activating effect on immune response during LM infection. NF- κ B is a transcription factor which can be used by a number of genes. Some of these genes are activated during infection. One which is activated and is specific to LM is Listeriosis biphasic NF- κ B activation. This phase of NF- κ B is regulated by I κ B β . When I κ B β is degraded NF- κ B becomes active. This activation can be seen as a result of the degradation of I κ B β by lipoteichoic acid (LTA), but also through the listerial PI-PLCs and PC-PLCs which is in correlation with the I κ B β degradation. The NF- κ B transcription factor can then be used by LM to enter the host cell. LM can then use the host cell machinery for its own replication. The nuclear NF- κ B complexes which are formed can therefore be attributed to the effect of the Listerial PLCs on the host cell (Hauf et al., 1997).

In summary, the secretion of PLCs during listerial infection has several effects on the host cell. One of these effects has been shown to increase the permeation of phagosomal membrane. The activation of PKC β through PI-PLC facilitates the escape of the bacterium. The decreased affinity of *L. monocytogenes* for the glycan linker of the GPI-anchored protein due to the lack or absence of the Vb β -strand also increases the ability for the bacterium to escape during infection. The degradation of I κ B β by listerial phospholipases PI-PLC and PC-PLC leads to the activation of NF- κ B, which allows the bacteria to exploit the host cell machinery.

Charge! The Main Act: ActA Protein Fills the Role

Quickly after *Listeria* enters the host cell's cytosol, the protein ActA interacts with the cell's proteins to mediate actin-based motility. ActA is a 610-amino-acid protein containing a charged N-terminal end, proline rich repeats, and a C-terminal end to anchor the protein to the bacteria surface (Cossart et al., 2000). Specifically, ActA directly activates the Arp2/3 complex, followed by other proteins that exploit the host cell's machinery for actin polymerization

(Portnoy et al., 2002). The ActA protein activates the Arp2/3 complex by mimicking proteins of the WASP family. Research is underway to understand the nature of the binding between ActA and the Arp2/3 complex.

In order to determine what is most important in actin polymerization, many different proteins and their functions have been examined in past studies. The Arp2/3 complex has been found to organize signals of actin cytoskeletons and initiate actin assembly because of its specific function. The Arp2/3 complex is comprised of the actin related proteins Arp2 and Arp3, along with p41-Arc, p34-Arc, p21-Arc, and p16-Arc. Arp2 and Arp3 both have insertions in loops that are exposed to the cytosol, and Arp2 contains a profiling binding site. However, neither Arp2 nor Arp3 are capable of the polymerization of actin alone. The Arp2/3 complex nucleates actin filaments, elongating the barbed ends. Also, the complex binds to other actin filaments and produces a branching formation when present at a filament pointed end. Once the polar actin tails become long enough, LM is propelled inter/intracellularly with actin-based motility (Fig 4).

Why Only Act A Is Needed

WASP family proteins become activated by interactions with Cdc42 and PIP2 region and other proteins. This interaction opens the given protein exposing the C-terminal region. The Arp2/3 complex and the C-terminal ends of these proteins interact. Arp2/3 binds to the CA-like region which all of the WASP family proteins share. Equivalent regions of the Act protein are also capable of activating the Arp2/3 complex in this way. Because of this unique ability to activate the complex, LM can by-pass the machinery for polymerization and directly interact with the complex. The complex attracts G-actin or F-actin to further actin polymerization.

In addition to the Arp2/3 complex, other host cell proteins are involved in actin polymerization. Research has found that VASP proteins bind to the proline-rich region of ActA at the EVH1 domain. This was proven by mutating VASP proteins, which resulted in a slower rate bacterial locomotion. Many different ligands exist for the ActA protein. Therefore, ActA has more than one mechanism for polymerizing actin and eventually for locomotion.

Listeria monocytogenes Infects Neighboring Cells

After polymerizing actin, *Listeria* combines other mechanisms in order to spread to neighboring cells. In the areas of newly polymerized actin, two molecules have been found to concentrate. Phosphatidylinositol 3,4,5-bisphosphate (PtdIns(4,5)P₃) and phosphatidylinositol 3,4,5-bisphosphate (PtdIns(4,5)P₂) play essential roles in the actin-based motility of LM. Recent studies have found that reducing the amount of PtdIns(4,5)P₃ and PtdIns(4,5)P₂ with Akt-PH-GFP and PCL δ -PH-GFP, respectively, significantly slows the movement of actin within a cell. As expected, this also inhibits the filopod formation process.

When a PI 3-Kinase was used to allow the concentration of PtdIns(4,5)P₃ by degrading Akt-PH GFP, but not PCL δ -PH-GFP, actin based motility was completely inhibited. When this PI 3-kinase was removed, full recovery of actin based motility and filopod formation was observed. The specific kinase used was LY294002. These results imply that

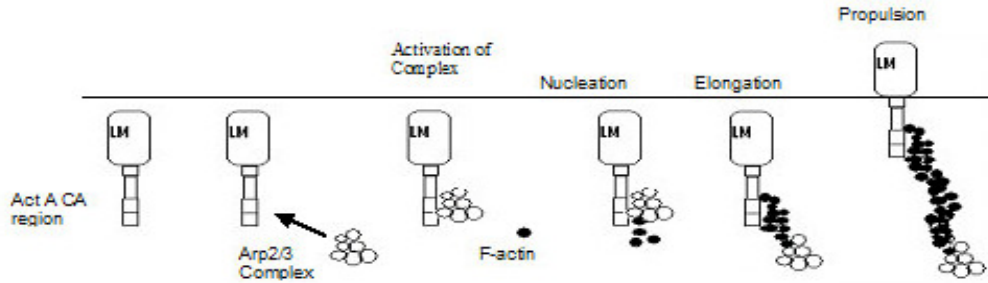


Figure 4: Activation of the Arp2/3 complex by the ActA protein

Recruitment of the Arp2/3 complex by the ActA protein followed by nucleation and elongation of the actin tail. Activation of the Arp2/3 complex results in propulsion of LM.

PtdIns(4,5)P₂ is the substrate for formation of PtdIns(4,5)P₃. Thus, the newly formed actin polymers (formed through the recruitment and activation of the Arp2/3 complex by Act A proteins) dissociate when PtdIns(4,5)P₂ and PtdIns(4,5)P₃ are not present.

This charged actin filament polymerization allows for the directional force, actin-based motility, through the cells' cytoplasm. Eventually the bacteria are propelled to the peripheral membrane of the host cell. Enough force is present to actually push the membrane outward, forming distinct filopods on the host cell using polymerized actin as support (Fig 5). The filopods are then ingested by adjacent cells, and the cell cycle continues to repeat. Filopod formation and the survival of newly polymerized actin is directly correlated with the presence of PtdIns(4,5)P₂ and PtdIns(4,5)P₃ (Vingjevic et al., 2003). Also, the PI 3-Kinase activity can play a major inhibitory role in the actin-based motility of LM and formation of filopods (Sidhu et al., 2005). The formation of filopods, as well as the specific interactions of the proteins involved, is still being researched.

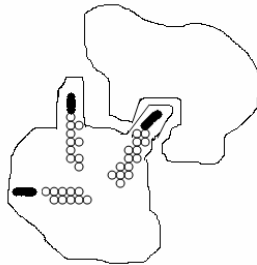


Figure 5: Formation of Filopods and cell to cell spread.

Filopod formation and entry into adjacent host cells is mediated by actin-based motility.

Attacking the Trojan horse: Treatments for Listeriosis

To diagnose Listeriosis, blood or cerebrospinal fluid cultures are used to establish bacterium growth, and characterization of the bacterium (Taege et al., 1999). The most common and current curative method of treating Listeriosis includes the use of ampicillin. Ampicillin works by targeting the cell wall of the bacterium, inhibiting the third and final stage of bacterial cell wall synthesis, which ultimately causes the cell to lyse. However, ampicillin is only

bacteriostatic, for *L. monocytogenes* (Taege et al., 1999). Often ampicillin is combined with gentamicin for synergy (Kamath et al., 2002 and Taege et al., 1999). With the interaction of these two agents, the overall effect will be greater than the sum of their individual effects. While ampicillin targets the cell wall; gentamicin hampers metabolic activities in the bacteria. Gentamicin operates by binding to a site located on the bacterial ribosome, which results in the misreading of the genetic code.

For patients who are allergic to ampicillin, an alternative treatment option including Trimethoprim and sulfamethoxazole may be used to treat Listeriosis. Trimethoprim interferes with the action of bacterial dihydrofolate reductase to prevent the synthesis of folic acid, which is an essential precursor in the new synthesis of the DNA nucleosides thymidine and uridine. When the bacterium is unable to take up folic acid from the environment, enzyme inhibition starves the bacteria of two bases necessary for DNA replication and transcription (Taege et al., 1999). These two antibiotics are used in a combination known as co-trimoxazole. Co-trimoxazole works by inhibiting the successive steps in folate synthesis (<http://www.pubmedcentral.nih.gov>).

Other treatments include chloramphenicol, which is reserved for life-threatening infections (Wei et al., 2005). This antibiotic has acute side-effects, including damage to bone marrow in humans. Chloramphenicol is used by the World Health Organization (WHO) in third world countries in the absence of cheaper alternatives. Chloramphenicol operates by stopping bacterial growth by hindering the ribosomal enzyme peptidyl transferase which assists in the formation of peptide links between amino acids during the translation process of protein biosynthesis (Wei et al., 2005).

Current Experimental Treatments via Plants

Treatment via plant extracts from *Pluchea quitoc* is in the experimental stage, studying the effects it has on Listeria infection. *Pluchea quitoc* extract is a well known remedy used in South American traditional medicine for the treatment of digestive diseases. In research, it has demonstrated strong anti-inflammatory and antioxidant activities. An experiment to study the effects of the *P. quitoc* extracts determined that it is helpful in defending the host against *L. monocytogenes* infection by increasing the number of leukocytes in the model mice (Queiroz et al., 2000).

Results indicated that the administration of *P. quitoc* increased hematopoietic recovery in the mice infected with LM. The increase in the number of

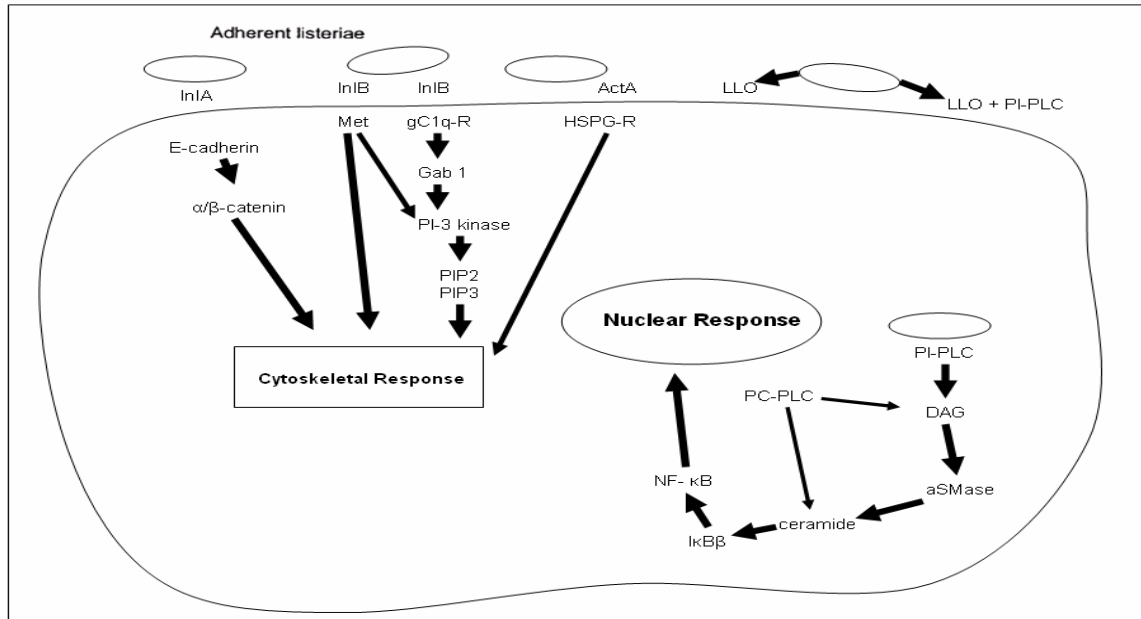


Figure 6: Model showing current understanding of *Listeria monocytogenes* infection in host cells.

Bacterial entry into the cell is facilitated via the internalin adhesion proteins. Listeriolysin O (LLO) and PI-PLC's mediate lysis of vacuoles and escape of the bacteria into the cytoplasm. Recruitment of actin filaments aids in bacterial movement and spread (Reproduced with modifications from Vaquez-Boland et al, 2001).

leukocytes enhances the host's ability to defend itself against the infection. It is still unclear what mechanism improves the survival of mice that are treated with *P. quitoc*, however, when this mechanism is discovered, further studies could make these extracts a useful remedy for humans.

Recapping the story of the Trojan Horse: Summary

Figure 6 illustrates the pathway and specific components through which LM enters, replicates and moves in mammalian cells. The main mechanisms for entry that have been described are the E-cadherin mediated Internalin pathway and the Met mediated InIB pathway. Furthermore, LLO and PC- PLC play the major role in the escape of LM from the vacuole. Once inside the cytosol, LM uses actin polymerization to gain motility and spread from cell to cell. Ampicillin, gentamicin, chloramphenicol and co-trimoxazole are currently used to treat listerial infection.

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