Trends in Microbiology

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Review

Crossing the Intestinal Barrier via *Listeria* Adhesion Protein and Internalin A

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The intestinal epithelial cell lining provides the first line of defense, yet foodborne pathogens such as *Listeria monocytogenes* can overcome this barrier; however, the underlying mechanism is not well understood. Though the host M cells in Peyer's patch and the bacterial invasion protein internalin A (InlA) are involved, *L. monocytogenes* can cross the gut barrier in their absence. The interaction of *Listeria* adhesion protein (LAP) with the host cell receptor (heat shock protein 60) disrupts the epithelial barrier, promoting bacterial translocation. InlA aids *L. monocytogenes* transcytosis via interaction with the E-cadherin receptor, which is facilitated by epithelial cell extrusion and goblet cell exocytosis; however, LAP-induced cell junction opening may be an alternative bacterial strategy for InlA access to E-cadherin and its translocation. Here, we summarize the strategies that *L. monocytogenes* employs to circumvent the intestinal epithelial barrier and compare and contrast these strategies with other enteric bacterial pathogens. Additionally, we provide implications of recent findings for food safety regulations.

Highlights

Intestinal epithelial cells are the first line of defense against enteric pathogens.

Bacterial pathogens such as *L. monocytogenes* have evolved sophisticated mechanisms to breach this barrier.

L. monocytogenes invasion protein internalin A (InlA) targets its basolateral receptor, E-cadherin, by host intrinsic mechanisms, the epithelial cell extrusion and goblet cell exocytosis allows its transcytosis across the intestinal barrier.

The *Listeria* adhesion protein (LAP) engages its surface receptor, Hsp60, and initiates a complex signaling cascade, leading to cellular redistribution of cell-to-cell junctional proteins for *L. monocytogenes* translocation.

L. monocytogenes capitalizes on two most dominant pathways, the LAP-mediated and InlA-mediated pathways, for bypassing the critical intestinal barrier and successful infection.

Foodborne *Listeria monocytogenes* Infection

L. monocytogenes is an opportunistic and highly invasive foodborne bacterial pathogen. It was first isolated from rabbits [1]. In the early part of the 20th century the pathogen was recognized as an animal pathogen and was found to infect ruminants, predominantly cows and sheep, causing circling disease and abortion. Animals suffer from ataxia, anorexia, depression, lethargy, septicemia, meningitis, head tilt, and encephalitis, which result in the loss of balance, causing the animals to walk in a circle [2]. *L. monocytogenes* was not recognized as a human foodborne pathogen until early in the 1980s, when multiple outbreaks were reported in North America [3,4]. Researchers began to link this pathogen's association with soil, manure, decaying vegetation, and the environment [5,6] as the primary mode of transmission to foods. *L. monocytogenes* is highly adaptable and uses sophisticated regulatory mechanisms to make the transition from a soil-living saprophyte to an invasive pathogen in humans and animals during foodborne infection [7,8].

The breaching of barriers – such as the host intestinal barrier [9–11], the blood–brain barrier [12,13], and the placental barrier [14,15] – is a key mechanism used by the intracellular bacterium *L. monocytogenes* [16]. Pregnant women, fetuses, newborn children, adults aged 65 and older, and people with weakened immune systems are most at risk and suffer from severe illnesses, including sepsis, meningitis, or encephalitis, often with life-long consequences. Listeriosis outbreaks are often associated with ready-to-eat (RTE) products, including deli meats, hot dogs, liver pâté, smoked fish, soft cheeses prepared from unpasteurized milk, ice cream, coleslaw, and produce such as frozen vegetables, cantaloupe, and apple [3]. In the USA, about 1600 people are infected each year, causing an estimated 260 deaths [17]. Among

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the foodborne pathogens, *L. monocytogenes* infections result in the highest hospitalization and case fatality rate (20–30%) compared to other pathogens [17,18]. In 2010, listeriosis resulted in 23 150 illnesses and 5463 deaths worldwide [19]. Recently, one of the largest outbreaks of listeriosis was reported in South Africa, with a total of 1060 cases and 216 deaths as of July 26, 2018i. The estimated infectious dose of *L. monocytogenes* is 10^6–10^7 colony-forming units (CFUs) in primates and susceptible humans [20,21], but as few as 10^4 organisms could have caused listeriosis in immunocompromised patients in a foodborne outbreak in Finland involving contaminated butter [22]. However, in the 2015 ice cream outbreak in the USA, the FDA estimated that 99.8% of ice cream samples contained <100 most probable number/g, thus implying a very low infectious dose for this pathogen [23,24]. This further indicates that *L. monocytogenes* has to be highly efficient in crossing the gut epithelial barrier and avoiding the host innate defense for its systemic spread.

Owing to the lack of knowledge of the accurate infectious dose, and high mortality among the high-risk groups, the US Food Safety Inspection Service agency established a zero-tolerance policy in RTE products in 1989, that is, 0 cells in 5 × 25 g samples. While the rationale for the zero-tolerance policy has been questioned, in the absence of solid evidence of the actual infectious dose the zero-tolerance policy continues to be enforced. Often, *Listeria*-tainted products are recalled, costing the food industry millions of dollars, and having a negative impact on brand reputation, thereby inflicting more financial damage. The annual cost of damage due to *L. monocytogenes* in the USA is about US$ 2.8 billion [25,26]. The European Food Safety Authority permits <100 CFUs/g of RTE food provided an appropriate antibacterial growth barrier is present, even though the number of confirmed human listeriosis cases is on the rise [18,27]. Thus, an understanding of how *L. monocytogenes* can breach critical host barriers to cause a systemic infection is of the foremost importance.

The Anatomy of the Intestinal Epithelial Barrier

The crossing of the gastrointestinal barrier is the first step, and a critical step, in the infectious process of a foodborne pathogen.

The mammalian intestine contains as high as 10^{14} commensal bacteria of approximately 100 different species, which form the microbiota. The microbiota not only contributes to digestive, metabolic, and immune functions of the gut, it also competes for colonization with pathogenic bacteria [28,29]. The gut microbiota can restrict the colonization of pathogenic bacteria by competing for nutrients. For example, the commensal *Escherichia coli* Nissle and HS strains outcompete and diminish colonization by the pathogenic *E. coli* O157:H7 strain by competing for carbohydrates [30]. Similarly, *E. coli* Nissle inhibits colonization by the pathogenic bacterium *Salmonella enterica* serovar Typhimurium by competing for iron [31]. The commensals in the gut microbiota can also secrete small molecules with bacteriostatic or bactericidal activity to protect against pathogens. For example, the commensal *Enterococcus faecalis* strain carrying a plasmid-encoded bacteriocin clears the vancomycin-resistant *Enterococcus* (VRE) from the intestinal tract [32]. Finally, the gut microbiota can also modulate the immune system to protect against pathogens. For example, the gut microbiota can combat systemic infections through the induction of protective immunoglobulin G (IgG) antibody response [33]. Thus, the microbiota can act as an important barrier by resisting the colonization of pathogenic bacteria.

The intestinal epithelium is a heterogeneous population of five differentiated cell types: **goblet cells** (see Glossary), **enterocytes,** enteroendocrine cells, Paneth cells, and tuft cells. These cells have important and distinct functions such as mucus production, absorption, hormone secretion, antimicrobial peptide (AMP) secretion and taste-chemosensory responses,

Glossary

Adherens junction (AJ): also known as zonula adherens. It is located below the tight junction between adjoining epithelial cells. It is an adhesive junction that maintains cell–cell adhesion and is composed of cadherin and transmembrane adhesion molecules connected to the actin cytoskeleton.
Apical junctional complex: three types of intercellular junction (TJ, AJ, and desmosomes) comprise the apical junctional complex. It consists of a network of transmembrane, scaffolding, and signaling proteins, and serves as a barrier, adhesion site, and signaling complex to control cell polarity, proliferation, and differentiation.
Caveolin: specialized invaginations of the plasma membrane that contains caveolin-1 protein and cholesterol. The caveolin aids in the uptake of some extracellular materials and is involved in cell signaling.
Clathrin: a molecular scaffold for vesicular uptake of cargo at the plasma membrane.
Desmosome: also known as macula adherens. It is located beneath the apical junctional complex. Desmosomes are composed of transmembrane cadherins of two subtypes, desmoglein and desmocollin, and adaptor proteins plakoglobin, plakophillin, and desmoplakin.
E-cadherin: belongs to the family of classical cadherins. It is a Ca^{2+}-dependent cell–cell adhesion molecule that mediates the formation of adherens junctions between polarized epithelial cells and plays an important role in cell sorting during development.
Enterocytes: columnar epithelial cells with apical microvilli. They are the most abundant cells in the small intestine. They form a major barrier to the resident intestinal microbiota and to pathogens in the gastrointestinal tract.
Goblet cell: a specialized secretory cell that synthesizes and secretes mucin glycoproteins and other components of the mucus.
Gut-associated lymphoid tissue (GALT): GALT consists of both isolated and aggregated lymphoid follicles and is part of the MALT that

respectively. The luminal surfaces of the intestinal mucosa are covered with hydrated gel composed of **mucins** secreted by the goblet cells (Figure 1A). This layer ranges from 700 μm in the stomach to 150–300 μm in the small and large intestines; it prevents large particles and intact bacteria from coming into direct contact with the underlying epithelium [34]. However, bacterial pathogens, such as *L. monocytogenes*, possess different virulence factors such as internalin (Inl) B, InlC, InlJ, InlL and the *Listeria*-mucin-binding invasin A (*lmo1413*) that binds mucins [35–37]. These virulence factors are covalently bound to the bacterial peptidoglycan via anchoring domains with an LPXTG motif [38]. The binding of *L. monocytogenes* to mucins via these virulence factors may allow the bacterium to efficiently penetrate the mucus and

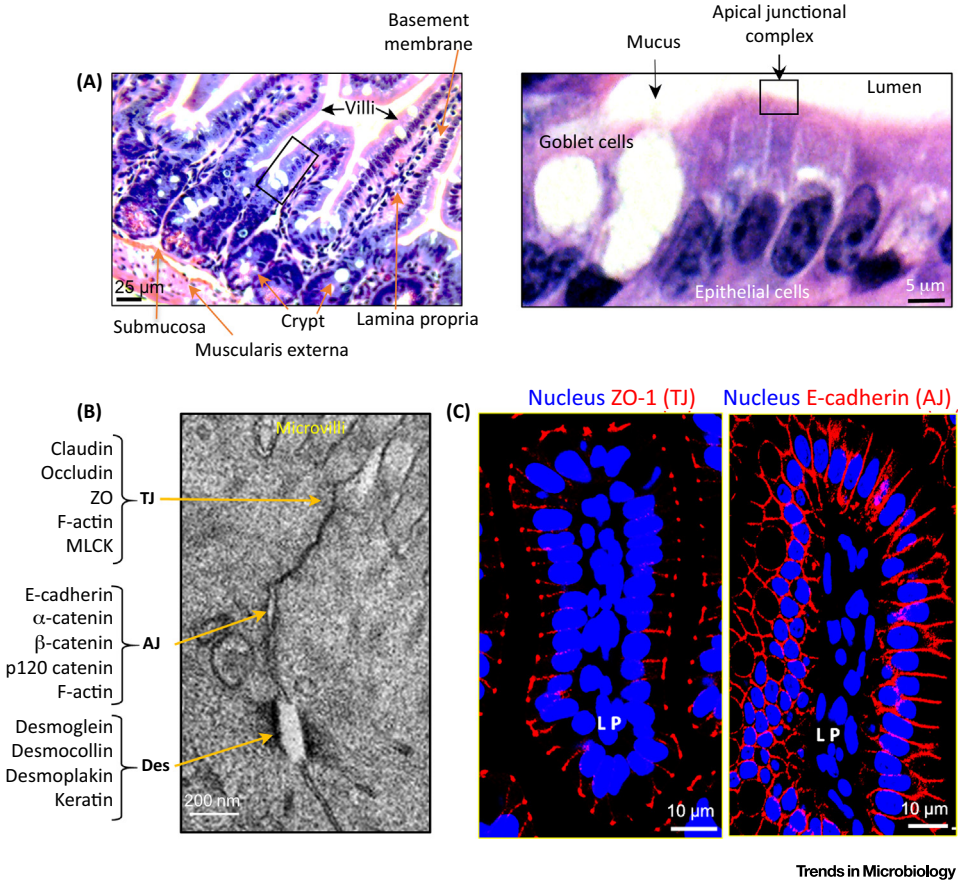

Figure 1. The Anatomy of the Mucosal Barrier. (A) Hematoxylin and eosin staining showing the morphology of the mouse intestine (left). Scale bar, 25 μm. The panels on the right are enlargements of the boxed areas. Scale bar, 5 μm. The intestine is lined with a single layer of polarized epithelial cells (right) organized into finger-like projections, called villi, and the underlying lamina propria. The lamina propria is located beneath the basement membrane and contains immune cells, including macrophages, dendritic cells, plasma cells, lamina propria lymphocytes, and neutrophils. Goblet cells (right) that release mucus, and other differentiated epithelial cell types are present. (B) Transmission electron microscopy showing the tight junction (TJ), adherens junction (AJ), and desmosome (Des) of the mouse ileum. Scale bar, 200 nm. The TJ consists of claudins, zonula occludens (ZO), and occludin that interact with F-actin. The myosin light-chain kinase (MLCK) is associated with the perijunctional actomyosin ring. The AJ consists of E-cadherin, α-catenin, β-catenin, and p120 catenin, all of which interact with F-actin. Desmosomes are located beneath the apical junctional complex and are composed of desmoglein, desmocollin, and desmoplakin, and interact with the keratin filaments. (C) Confocal microscopic images of mouse ileal villi immunostained for ZO-1(red), a TJ protein (left), and E-cadherin (red), an AJ protein (right). Nuclei (blue) are counterstained with DAPI. Scale bar, 10 μm. LP, lamina propria.

is comprised of lymphoid tissues and organs located directly beneath the mucosal epithelium.

Hsp60: a mitochondrial chaperonin that is responsible for the transportation and refolding of proteins from the cytoplasm into the mitochondrial matrix.

Lamina propria: a layer located beneath the basement membrane and contains immune cells, including macrophages, dendritic cells, plasma cells, lymphocytes, and neutrophils.

M cells: specialized epithelial cells of the MALT that transport antigens, bacteria, and viruses from the lumen to the underlying immune cells, thereby initiating a systemic immune response or tolerance.

Macropinocytosis: an invagination of the cell membrane to form a vesicle at highly ruffled regions of the plasma membrane. The internalized vesicle fuses with lysosomes or endosomes.

microRNAs (miRNAs): small noncoding, naturally occurring RNA molecules. They are complementary to one or more messenger RNA (mRNA) molecules and function to downregulate gene expression by translational repression, mRNA cleavage, and deadenylation.

Mucins: high-molecular-weight, heavily glycosylated proteins that coat the surfaces of cells that line the respiratory, digestive, and urogenital tracts. They are rich in complex O-linked oligosaccharides.

Mucosa-associated lymphoid tissue (MALT): MALT contains an array of lymphocytes such as B cells, T cells, plasma cells, and dendritic cells and macrophages. MALT is found in the mucosal linings of organs such as the gastrointestinal tract, lungs, salivary glands, and conjunctiva.

Mucus: a viscous, fluid layer that overlies the mucosal surface and contains secreted mucin glycoproteins and other molecules involved in host defense against infection.

Myosin light-chain kinase (MLCK): the Ca^{2+}-calmodulin-dependent kinase that phosphorylates myosin II regulatory light chain at serine 19 and threonine 18 to activate myosin ATPase.

Nuclear factor-κB (NF-κB): a transcription factor protein that plays

facilitate bacterial adhesion or invasion of host cells. In contrast, other bacterial pathogens, such as *Clostridium perfringens*, enteroaggregative *E. coli*, enterohemorrhagic *E. coli*, *Shigella flexneri, Vibrio cholerae*, and *Yersinia enterocolitica*, possess enzymes that degrade mucins or damage **mucus** [34].

The intestinal barrier is composed of a single layer of polarized columnar epithelial cells, called enterocytes, organized into finger-like projections called villi (Figure 1A). These cells are self-renewed every 4–5 days, making the intestinal epithelium a highly dynamic structure [39]. The intestinal epithelial cells are polarized with the apical surface facing the lumen and the basal surface facing the basement membrane. Epithelial cells are networked together through adhesive contacts called junctions, which join cells together and provide a paracellular seal. The space between two adjacent epithelial cells is called the paracellular space. From the apical to basolateral direction, **tight junctions** (**TJs**), **adherens junctions** (**AJs**), and **desmosomes** seal this space (Figure 1B,C). Together, these three types of intercellular junction comprise the **apical junctional complex** (Figure 1A) [40].

TJs are made of peripheral membrane proteins such as zonula occludens (ZO) and different classes of transmembrane protein, such as claudins, occludin, tricellulin, junctional adhesion molecules (JAMs), membrane-associated guanylate kinase, and the polarity complex (PAR) family proteins. TJs regulate the flux of ions and solutes, maintain cell polarity, and are the primary determinant of paracellular permeability. TJs are physically associated with the actin and myosin filament and are regulated by a variety of kinases and cytoskeletal proteins. One of the principal mediators of TJ regulation is long **myosin light-chain kinase** (**MLCK**) [41,42]. MLCK is the Ca^{2+} calmodulin-dependent serine-threonine kinase that phosphorylates myosin II regulatory light chain (MLC). The AJs are required for the integrity of the TJs and are located immediately subjacent to the TJs. The AJ has **E-cadherin**, α-catenin, β-catenin, and p120 catenin, and it interacts with F-actin. The desmosomes are adhesive junctions that connect adjacent epithelial cells and are located below the AJ. These junctions are composed of multiple protein subunits consisting of desmoglein, desmocollin, and desmoplakin, and are the points where keratin filaments attach to the plasma membrane.

The basolateral side of the cell–cell junction consists of a basement membrane, which is a thin network made of extracellular matrix proteins. The basement membrane initiates and maintains cell polarity and separates epithelial cells from the underlying **lamina propria**, which contains connective tissue, stromal cells, blood and lymphatic capillaries, and immune cells such as lymphocytes, dendritic cells, or resident macrophages.

The barrier function of the intestine is tightly regulated by the apical junctional complex. However, in compromised conditions, such as in response to pathological stimulus, the apical junctional complex can be endocytosed. This endocytosis of the TJ and AJ has been reported to occur via three classic pathways of endocytosis [43]: **macropinocytosis** of the transmembrane TJ proteins, **clathrin**-coated endocytosis of both TJ and AJ proteins, and **caveolin**-mediated endocytosis of the TJ protein occludin. Thus, endocytosis induces the disassembly of the epithelial apical junctional complex.

To establish defined boundaries, the mucosal epithelial cells cover the external surface and line internal compartments to form barriers and prevent unrestricted passage of bacteria. However, enteric bacterial pathogens have evolved sophisticated mechanisms to circumvent this barrier and efficiently cross the gut mucosa, allowing bacteria to spread systemically and cause infectious disease.

a pivotal role in regulating the expression of genes in many biological processes, including innate and adaptive immunity, inflammation, stress responses, B cell development, and lymphoid organogenesis. These proteins consist of five family members: NF-κB2 (p52/p100), NF-κB1 (p50/p105), c-Rel, RelA/p65, and RelB.

Nucleotide-binding oligomerization domain 2 (NOD2): an intracellular pattern-recognition receptor (PRR) that senses bacterial peptidoglycan in the cytosol and elicits a host immune response.

Peyer's patch: organized lymphoid follicles that form the interface between the **GALT** and the luminal microenvironment. Peyer's patch plays an important role in the immune surveillance of the intestinal lumen and facilitates the generation of the immune response within the mucosa.

Tight junction (TJ): also known as the zonula occludens. It is the most apical junction along the lateral surface that creates a barrier and regulates the diffusion of ions and solutes.

Toll-like receptors (TLRs): the first identified family of pattern-recognition receptors (PRRs); they are expressed either on the cell surface or associated with intracellular vesicles.

L. monocytogenes Pathogenesis and Crossing of the Gut Epithelial Barrier

L. monocytogenes is a facultative intracellular pathogen that invades and multiplies within the cellular compartment, and moves from cell to cell [44]. This provides the bacterium with a hidden lifestyle as it is protected from immune cells and antimicrobials. Elegant studies have demonstrated the role of various virulence factors during the intracellular life cycle of *L. monocytogenes*, which has been previously reviewed thoroughly [16,44,45]. However, the precise mechanism that *L. monocytogenes* employs to cross the intestinal epithelial barrier during the gastrointestinal phase of infection is not fully understood. Although **M cells** ('M' for microfold) in the **Peyer's patch** [46] and the pathways mediated by the *L. monocytogenes* invasion protein InlA [10] aid the crossing of the gut epithelial barrier, the bacterium has been shown to cross the gut barrier in animal models where these two pathways are absent, suggesting the presence of alternative routes [47,48].

The M cell pathway is a passive system which is used by many enteric pathogens, toxins, biomolecules, or even the resident microbiota. On the other hand, the InlA-mediated pathway is highly specific and it induces membrane protein reorganization to initiate bacterial uptake [49]. *L. monocytogenes* crosses the epithelial barrier by transcytosis and is released into the underlying lamina propria [50]. The bacterium reaches the mesenteric lymph nodes (MLN) through the lymphatic vessels and disseminates to the liver and spleen via the lymph and blood. It can then spread to secondary target sites of infection such as the central nervous system and the placenta. From the intestine, *L. monocytogenes* can also disseminate to the liver through the hepatic portal vein through a direct route [51].

Multiple reports indicate that *L. monocytogenes* isolates – primarily from environmental or food sources – expressing truncated InlA (due to the presence of a premature stop codon in the *inlA* ORF) are less infective in mammalian cell culture or animal models [52–54]. However, more recent studies show that such strains can indeed infect humans [55–57] and fetuses of pregnant guinea pigs and mice after oral administration [58]. These findings provide strong evidence that *L. monocytogenes* also uses alternate routes independently of InlA-mediated uptake and the M cell to translocate across the gut barrier.

LAP opens the intestinal epithelial tight junction barrier through activation of the central regulatory pathway **nuclear factor-κB** (**NF-κB)**, and MLCK, resulting in cellular redistribution of junction proteins and leading to bacterial translocation by exploiting the epithelial innate defense [9]. The dynamics of LAP-mediated NF-κB and MLCK activation is well studied and is dependent on the engagement of LAP with its surface receptor **Hsp60.** The interaction of LAP with Hsp60 leads to the internalization of surface Hsp60 to bind with the IκB kinase (IKK) complex. This activates IKK, which induces the degradation of inhibitors of kB (IkB)-α and facilitates the activation, nuclear translocation, and phosphorylation of NF-κB (p65). LAP-Hsp60-mediated NF-κB activation also results in activation of MLCK, which phosphorylates MLC for cellular redistribution of TJ proteins; claudin-1 and occludin, and the AJ protein; E-cadherin, promoting junctional opening for bacterial translocation. Thus *L. monocytogenes* executes efficient translocation across the intestinal barrier by manipulating the LAP–Hsp60–IKK–NF-κB–MLCK axis [9].

A recent study has revealed that only a minimal fraction of *L. monocytogenes* cells in the lymph nodes is intracellular, with a vast majority of cells being extracellular [59]. Additionally, *L. monocytogenes* is primarily associated with monocytes in the intestine and the MLN during the early stages (24–48 h) of infection [60]. However, these cells do not serve as an intracellular growth niche, suggesting that intracellular transport in monocytes is not a primary route of dissemination.

The host gut microbiota has an important role during orally acquired listeriosis. Treatment with *Lactobacillus* decreased the invasion capabilities of *L. monocytogenes* in the host intestine [61]. The host gut microbiota interferes with the **microRNA** (miRNA) response upon oral infection with *L. monocytogenes* [62]. In particular, five miRNA expression variations (in miRNA-143, miRNA-148a, miRNA-200b, miRNA-200c, and miRNA-378) are dependent on the presence of the microbiota [62]. Moreover, a bacteriocin produced by the *L. monocytogenes* epidemic strain modulates the host intestinal microbiota for increased host colonization [63]. A recent study has identified that intestinal commensal bacteria enhance resistance against *L. monocytogenes* [64,65].

The pattern-recognition receptors (PRRs) – such as the **Toll-like receptors** (**TLRs**) [66] and the **nucleotide-binding oligomerization domain 2** (**NOD2**) [67] in the intestine – play an important role in recognition and elimination of *L. monocytogenes*. Mice lacking MyD88, an intracellular adaptor protein involved in most TLR-mediated signaling, are more susceptible to oral challenge by *L. monocytogenes* [68]. The increased susceptibility of the MyD88 knockout mice to *L. monocytogenes* infection is attributed to the lack of production of the antibacterial peptide RegIIIγ (a bactericidal lectin) in these mice. Similarly, mice lacking the NOD2 receptors are more susceptible to oral challenge by *L. monocytogenes*, with higher dissemination rates to the liver and spleen [69]. The increased susceptibility of the NOD2- knockout mice to *L. monocytogenes* infection is attributed to decreased expression of some cryptdins (a type of AMP) in the small intestine [69]. A more recent study has also demonstrated an important role for TLR10 in intestinal epithelial cells: sensing *L. monocytogenes* and eliciting immune responses [70].

Therefore, besides the M cell-mediated pathway (Box 1), two other major pathways (Figure 2) have emerged as prevailing routes for *L. monocytogenes* to cross the intestinal epithelial barrier during the intestinal phase of infection: (i) InlA-mediated transcytosis [16], and (ii) LAP-dependent translocation [9]. Here, we provide an in-depth review of each pathway for *L. monocytogenes* translocation across the intestinal barrier.

InlA and E-cadherin: Targeting the Adherens Junction

InlA was the first protein of the internalin multigene family to be discovered, in 1991, and is involved in the invasion of epithelial cells [71]. InlA is an 80 kDa surface-invasion protein containing 15 leucine-rich repeats (LRRs) and signaling peptides, an inter-repeat (IR) region, and an LPXPTG surface-anchoring motif at the carboxy terminal that displays sortage-dependent disulfide covalent bonding with the cell wall peptidoglycan [72,73]. The host cell receptor for InlA is E-cadherin [74]. E-cadherin is a major transmembrane protein of 882 amino acids required for AJ formation in epithelial cells. E-cadherin consists of the N terminal extracellular domain of 555 amino acids, a transmembrane domain of 175 amino acids, and a short intracellular domain of 152 amino acids.

Dynamics of the InlA–E-cadherin Interaction

The dynamics of the signaling cascades activated by the InlA–E-cadherin interaction has been well studied and reviewed (Figure 3) [75]. The binding of InlA to the extracellular domain of E-cadherin results in the recruitment of the junctional proteins α- and β-catenins, p120 catenin, a Rho-GAP protein ARHGAP10, a non-conventional myosin VIIa, and a ubiquitous transmembrane protein vezatin. InlA–E-cadherin interaction induces the caveolin-dependent clustering of E-cadherin. The clustering of E-cadherin leads to the activation of the nonreceptor tyrosine kinase Src that phosphorylates E-cadherin at residues 753 and 754. The Src-dependent phosphorylation of E-cadherin initiates the release of p120 catenin to the cytoplasm, which

Box 1. M Cells: Exploiting the Natural Immune Surveillance Function

The **mucosa-associated lymphoid tissue** (**MALT**) comprises immune cells and structured lymphoid tissues. MALT is found in close contact with all mucosa throughout the body. In the intestine, it is termed **gut-associated lymphoid tissue** (**GALT**); this consists of both isolated and aggregated lymphoid follicles. These are the sites where antigen recognition and mucosal immune responses are initiated. Marco Aurelio Severino initially described aggregated lymphoid follicles in 1645 in Italy. It was later renamed Peyer's patches after the Swiss pathologist Dr Johann Conrad Peyer, who provided a detailed description in 1677.

The Peyer's patch is composed of aggregated lymphoid follicles surrounded by a follicle-associated epithelium (FAE) that forms the interface between the GALT and the luminal microenvironment. The FAE contains M cells, which are unique epithelial phagocytes that continually sample luminal contents and are located within intestinal villi above isolated or aggregated lymphoid follicles [144]. The normal function of M cells is to sample the contents of the lumen of the intestine and present antigens to the immune cells residing in the Peyer's patch. Thus, M cell transcytosis is an important first step in the initiation of a secretory immune response.

Many bacterial enteric pathogens – such as rabbit diarrheagenic *E. coli*, *S. enterica* serovar Typhimurium, *S. flexneri*, *V. cholerae*, and *Y. pseudotuberculosis* – transit through M cells into the underlying Peyer's patch by taking advantage of the natural immune surveillance function of M cells [145,146]. Like other enteric pathogens, *L. monocytogenes* also uses the M cell-mediated pathway, and the bacterium has been localized within Peyer's patches in orally infected mice [9,46,47]. This mechanism is independent of *L. monocytogenes* virulence factors, such as InlA or the pore-forming toxin listeriolysin O [147], while another invasion protein, InlB, has been implicated in accelerated listerial invasion of murine M cells on ileal Peyer's patch [47].

However, *L. monocytogenes* also translocates to deeper tissues and organs with similar efficiencies in a rat ligated ileal loop with or without Peyer's patch [48]. Moreover, in a Peyer's patch-null mouse model, *L. monocytogenes* colonized the ileum and disseminated to the MLN, liver, and spleen [47]. Additionally, systemic dissemination of *L. monocytogenes* to the peripheral tissues was also observed by an intrarectal route of infection in the absence of Peyer's patch in the large intestine [148]. These findings indicate that *L. monocytogenes* also traverses across the epithelium independently of M cells where other routes of invasion in the gastrointestinal tract are possible.

induces the activation of the actin nucleator Arp2/3 complex and actin polymerization. This is followed by recruitment of septin at the site of entry. The Src-dependent phosphorylation of E-cadherin also recruits the ubiquitin ligase Hakai, which ubiquitinates E-cadherin. E-cadherin ubiquitination induces the recruitment of clathrin to form coated pits for clathrin-mediated bacterial internalization, or caveolin for clathrin-independent bacterial endocytosis via caveosomes (Figure 3). While InlA–E-cadherin signaling is important for *L. monocytogenes* invasion of epithelial cells, *L. monocytogenes* efficiently invades cells lacking the intracellular domain of E-cadherin [76]. This suggests that the extracellular domain of E-cadherin is sufficient to mediate *L. monocytogenes* invasion [76].

Species Specificity of the InlA–E-cadherin Interaction

The InlA–E-cadherin interaction exhibits a host species specificity that is attributed to a variation at residue 16, at which proline is substituted by glutamic acid in the host species' E-cadherin [77]. Therefore, InlA does not interact with the E-cadherin of InlA-nonpermissive hosts, such as mice or rats, but it does interact with the E-cadherin of InlA-permissive hosts such as humans, guinea pigs, rabbits, and gerbils [78]. E-cadherin is located basolaterally at the AJ and is inaccessible to luminal bacteria [79]. However, two models have been proposed for InlA access to luminal E-cadherin during (i) epithelial cell extrusion, and (ii) mucus exocytosis (Figure 2). (i) During villous epithelial 'cell extrusion' [80], the apical junctional complex proteins are redistributed to the lateral membranes [81], and thus the extruding cells show a defect in epithelial polarity, which exposes E-cadherin at the cell surface. (ii) InlA access to E-cadherin occurs near the mucus-expelling goblet cells; this exposes E-cadherin luminally, where *L. monocytogenes* is internalized and rapidly transcytosed in a vacuole across the epithelial cells and exits into the underlying lamina propria and disseminates

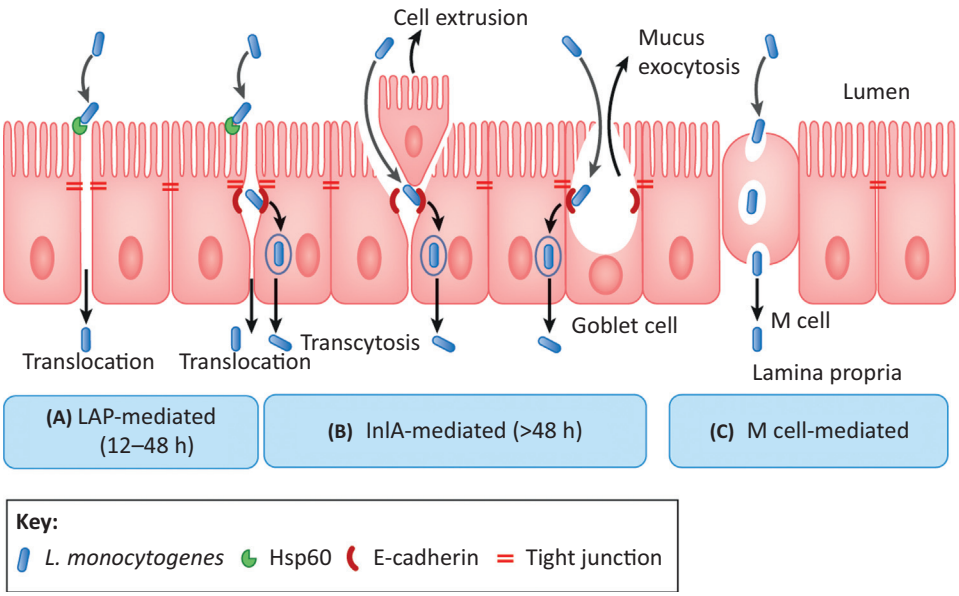

Figure 2. Schematic Representation Showing Different Routes Used by *Listeria monocytogenes* to Cross the Gut Epithelial Barrier. (A) *Listeria* adhesion protein (LAP)-mediated *L. monocytogenes* translocation involves the interaction of LAP with epithelial Hsp60 for redistribution of the tight junction proteins (claudin-1 and occludin) the adherens junction protein (E-cadherin), and subsequent epithelial barrier opening. (B) InlA/E-cadherin-mediated *L. monocytogenes* transcytosis, which occurs during epithelial cell extrusion or goblet cell mucus exocytosis, providing access of InlA to E-cadherin at the adherens junction. (C) M cell-mediated *L. monocytogenes* translocation occurs in the Peyer's patches.

systemically [50]. InlA access near mucus-expelling goblet cells is supported by observations in which treatment of humanized E-cadherin transgenic mice with IL-33 increases the number of goblet cells and mucus secretion, also resulting in increased *L. monocytogenes* invasion of the intestinal villi and systemic dissemination to the spleen [50]. Both of these mechanisms for InlA access to E-cadherin rely on the continuous self-renewal ability for eliminating damaged cells and the inherent heterogeneous nature of cells in the intestinal epithelium, respectively. Additionally, phosphoinositide 3-kinase (PI3-K) is constitutively active in the intestine [82]. Thus, another invasion protein, InlB, which is known to activate PI3-K, is not required for crossing the intestinal barrier [82,83].

The contribution of the InlA–E-cadherin Interaction: Lessons from Animal Models

Despite the key role of InlA–E-cadherin interaction in the crossing of the intestinal epithelial barrier by *L. monocytogenes*, the intragastric inoculation of an *L. monocytogenes* Δ*inlA* strain resulted in high bacterial burdens in the liver and spleen of wild-type (WT) mice [73], and in the small intestine, cecum, colon, and MLN of transgenic mice expressing humanized E-cadherin [84]. These observations suggest that the humanized E-cadherin allele is relevant only to InlA-mediated bacterial invasion, and that *L. monocytogenes* uses alternative routes to translocate across the gut mucosa. Furthermore, oral infection of mice with *L. monocytogenes* expressing murinized InlA ($InlA^m$), which binds E-cadherin with high affinity, did not show a significant difference in bacterial burdens in the liver, spleen, and MLN compared to mice that were inoculated with the WT *L. monocytogenes* for up to 48–72 h postinfection (hpi) [47,85,86]. This further suggests that the InlA–E-cadherin interaction may not be essential for *L. monocytogenes* to cross the intestinal barrier, at least during the early phase of infection. A coinfection

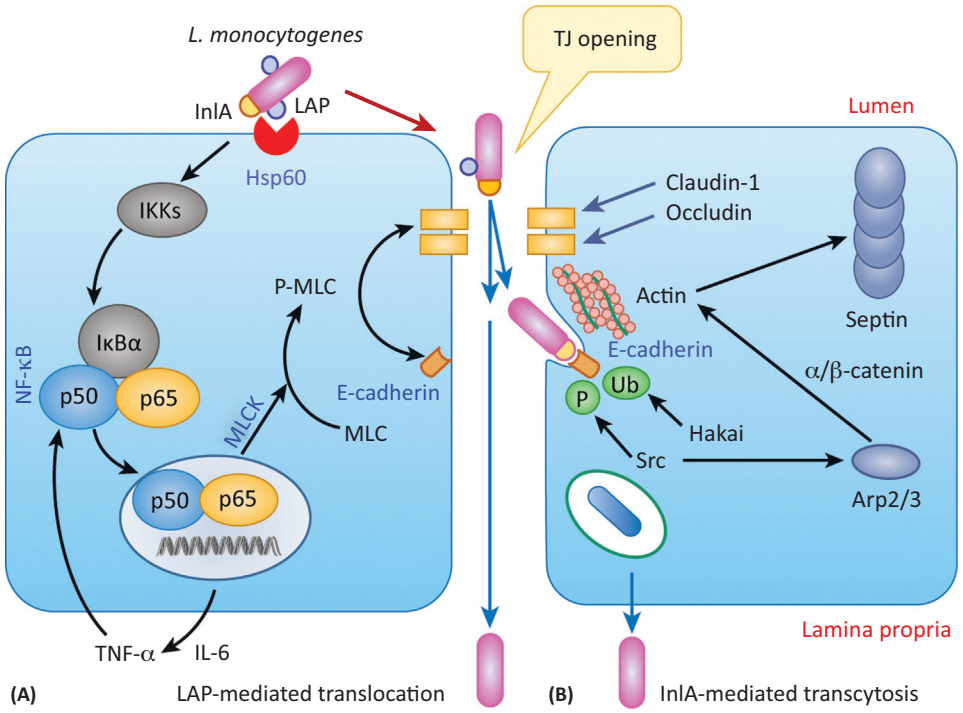

Figure 3. Detailed Schematic Showing the Dynamics of *Listeria monocytogenes* Epithelial Barrier Crossing Strategies. (A) Dynamics of the LAP/Hsp60 interaction. The *Listeria* adhesion protein (LAP) binds to its epithelial receptor Hsp60 in the plasma membrane. Upon engagement of its surface receptor, Hsp60, it is redistributed to the cytosol to directly interact with IKK, which leads to the activation of the canonical NF-κB pathway. NF-κB activation results in the secretion of proinflammatory cytokines, TNFα and IL-6, leading to increased epithelial permeability. Activated NF-κB induces activation of myosin light-chain kinase (MLCK), which phosphorylates the myosin light chain (MLC). Phosphorylated MLC triggers the redistribution of junctional proteins (claudin-1, occludin, and E-cadherin) for junctional opening, which allows for subsequent bacterial translocation. The LAP-Hsp60-mediated junctional opening may also facilitate InlA access to E-cadherin at the adherens junction (AJ) for InlA-mediated translocation. (B) Dynamics of the InlA/E–cadherin interaction. Upon interaction of InlA with E-cadherin at the AJ, the junctional machinery is activated, leading to caveolin-dependent clustering of E-cadherin. Two post-translational modifications occur at this stage: Src-dependent phosphorylation of E-cadherin followed by Hakai-dependent ubiquitination of E-cadherin. This results in a recruitment of clathrin and multiple components of the endocytic machinery, which works with the actin machinery to promote bacterial uptake. Two pathways link E-cadherin to actin polymerization: (i) direct interaction of α-catenin and β-catenin, and (ii) Src-dependent activation of the Arp2/3 complex followed by recruitment of septin at the site of entry. These events lead to the generation of caveolin and clathrin-coated pit for bacterial internalization as well as epithelial barrier crossing by transcytosis.

study using $InlA^m$, WT, or Δ*inlA* strains demonstrated that InlA is not required for the establishment of intestinal infection in mice [87].

Additionally, though the interaction between InlA and E-cadherin in mice and rats is not fully functional [10], several studies have advocated that *L. monocytogenes* can cross the intestinal barrier and disseminate to the MLN, liver, and spleen following oral infection [73,87–90]. These observations are further supported by more recent studies showing that *L. monocytogenes* strains expressing a nonfunctional InlA (encoding a premature stop codon) can indeed infect human newborns with neonatal listeriosis [55], or adults [56,57] and fetuses of pregnant guinea pigs and mice after oral administration [58]. Taken together, these observations strongly point to the existence of alternative pathway(s), independent of InlA, for *L. monocytogenes* to cross the gut epithelial barrier.

LAP and Heat Shock Protein (Hsp) 60: Targeting the Tight Junction and Adherens Junction

LAP is a 104 kDa alcohol acetaldehyde dehydrogenase, an AdhE homolog (encoded by *lmo1634*) that promotes adhesion of only pathogenic *Listeria* species to cell lines of intestinal origin [91–93]. Analysis of the *lmo1634* locus from F4244, a 4b serotype with genomic sequences from several strains, revealed 99% sequence identity with Clip80459 and F2365 (both 4b serotypes), and 97% sequence identity with EGD-e, EGD, and 10403S (all 1/2a serotypes) [94].

A Bifunctional Adhesin

LAP is a cytosolic bifunctional housekeeping enzyme containing N terminal acetaldehyde dehydrogenase (ALDH) and C terminal alcohol dehydrogenase (ADH), and does not contain a leader sequence [91]. LAP is present in both pathogenic and nonpathogenic *Listeria* species, and the sequence analysis has revealed ~98% amino acid similarity in LAP from pathogenic species (*L. monocytogenes* and *L. ivanovii*) and nonpathogenic species (*L. innocua*, *L. welshimeri*, *L. seeligeri*) of *Listeria* [91]. However, LAP contributes to bacterial adhesion to cell lines of intestinal origin only in pathogenic *Listeria* [91,92] because of inadequate secretion and surface reassociation of LAP on nonpathogenic species of *Listeria* [89,91]. LAP is prominantly reduced in intracellular and cell-surface protein fractions, and is undetectable in the extracellular milieu of nonpathogenic species. LAP production is maximal during the late logarithmic to stationary phase, and growth temperature influences its synthesis [95]. Nutrient-starving conditions and low glucose (0.4–0.8 g/l) levels increase LAP production [96]. LAP-mediated pathogenesis also depends on the effective secretion of the protein from the bacterium by SecA2, an auxiliary secretion system [89,97]. The oxygen-limiting environment increases the expression of LAP, thus augmenting the pathogenic potential of *L. monocytogenes* as observed in *in vitro* cell culture and mouse models [89].

Hsp60 is the epithelial receptor for LAP [98]. The N terminal ALDH, specifically the N2 subdomain (Gly224–Gly411), interacts and binds to Hsp60 with high affinity (K_D = 9.5 mM) [99]. Hsp60 is a mitochondrial chaperonin and plays a role in protein folding and removal of misfolded proteins from cells. Mammalian Hsp60 activates the innate immune response [100], and thus is considered a moonlighting protein [101] possessing two or more distinct biological activities. *L. monocytogenes* infection at low dosage increases plasma membrane and intracellular Hsp60 levels in epithelial Caco-2 cells independently of LAP expression [102]. Furthermore, *L. monocytogenes*-induced membrane Hsp60 expression subsequently facilitates enhanced LAP-mediated *L. monocytogenes* interaction with epithelial cells *in vitro* [102]. Interestingly, Hsp60 also acts as a cell-surface receptor for *Staphylococcus aureus* [103] and HIV [104].

LAP Promotes *L. monocytogenes* Translocation

LAP was first shown to aid *L. monocytogenes* in adhering to cells of intestinal origin as a potential colonization factor without any apparent invasion function [91,93]. Later, LAP was found to have a key role in bacterial transepithelial translocation. In a trans-well culture device, the mutant strain lacking *lap* (*lap*$^-$) showed significantly impaired translocation compared to that of the WT strain across the polarized Caco-2 cell monolayer [102]. However, a strain lacking the InlA (Δ*inlA*) exhibited translocation rates equal to the WT strain [102], suggesting that LAP-mediated paracellular translocation is independent of InlA-mediated epithelial invasion. Additionally, the translocation phenotype of the WT and Δ*inlA* strains was significantly impaired in Caco-2 cell lines with *Hsp60* knocked-down, but the translocation phenotype of the *lap*$^-$ strain had no change [102]. Conversely, overexpression of *hsp60* significantly increased

translocation of WT and Δ*inlA* but not the *lap*-deficient strain. Therefore, LAP-mediated translocation requires the engagement of its surface receptor Hsp60. Additionally, *L. monocytogenes* isolates showing high LAP secretion also showed a higher rate of epithelial adhesion and translocation across the Caco-2 epithelial cell barrier [105]. These findings suggest that interaction of LAP with the host cell receptor Hsp60 is critical for *L. monocytogenes* translocation across the epithelial barrier *in vitro*, and this event can take place independently of InlA.

In a mouse model, LAP induces intestinal barrier dysfunction for *L. monocytogenes* translocation across the epithelial barrier. In orally infected mice, the translocation of the *lap*-deficient *L. monocytogenes* strain from the lumen to the underlying lamina propria of the intestinal epithelium and to the MLN, liver, and spleen is significantly impaired [9]. In contrast, the Δ*inlA* strain translocated as efficiently as the WT strain [9]. However, in mice infected intraperitoneally, a route that bypasses the intestinal barrier, both the WT and the *lap*-deficient *L. monocytogenes* strains translocated to the liver in almost equal numbers [80], suggesting that LAP has an important role in the intestinal phase of infection but not in the late stages of infection. LAP increases intestinal epithelial permeability, which positively correlates with translocation of *Listeria* into the underlying lamina propria. In mouse ileal tissue, *L. monocytogenes* WT and Δ*inlA* cells were colocalized with ZO-1, which is a TJ protein, and these strains were observed to exit into the lamina propria while the *lap*$^{-}$ strain remained confined to the gut lumen [9] (Figure 4A,B).

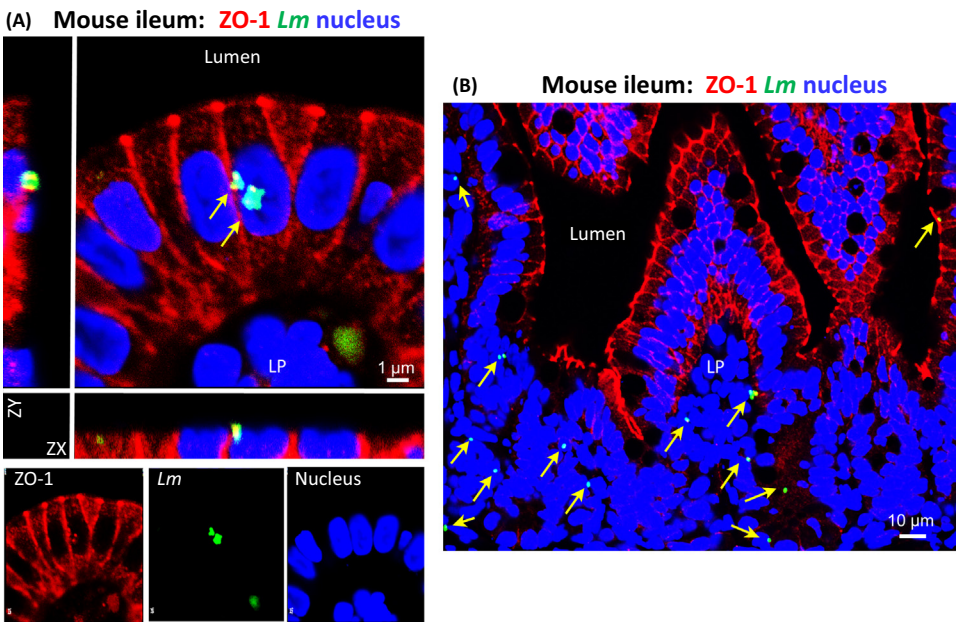

Figure 4. *Listeria monocytogenes* Translocation through the Intestinal Epithelium. (A) Confocal microscopic images showing wild-type *L. monocytogenes* (F4244) localization in the mouse ileal epithelial cell junction (48 h post-infection) immunostained for ZO-1 (red), *L. monocytogenes* (*Lm*, green, arrows), and nucleus (blue). *L. monocytogenes* (arrows) is observed exiting the epithelial cell into the underlying lamina propria (LP) and colocalized with ZO-1 (ZY axis and ZX axis images). Scale bar, 1 μm. Bottom panel displays separate channels individually. (B) Confocal microscopic images of ileal villi immunostained for ZO-1 (red), *L. monocytogenes* F4244 (*Lm*) (green, arrows), and nucleus (blue) of mice orally infected with wild-type *L. monocytogenes* for 48 h postinfection. *L. monocytogenes* (green, arrows) cells are visible in the lamina propria (LP). Scale bar, 10 μm.

Exploiting the Epithelial Innate Immunity

LAP activates canonical signaling by NF-κB, a central regulator of innate immunity [106] in Caco-2 cells and mouse ileum for increased epithelial permeability [9]. The LAP-mediated increase in epithelial permeability correlates with the increased expression of the NF-κB-regulated proinflammatory cytokines TNF-α and IL-6. LAP-mediated NF-κB activation requires the interaction of LAP to its surface receptor Hsp60. LAP induces internalization of surface-expressed Hsp60 in Caco-2 cells to facilitate interaction of Hsp60 with the IKK complex. Furthermore, NF-κB inhibitors significantly reduce LAP-mediated NF-κB activation, epithelial permeability, and *L. monocytogenes* translocation across polarized Caco-2 cells [9]. Additionally, in the mouse ileal villi, Hsp60 localizes at the plasma membrane of epithelial cells and is thus accessible to bacteria located in the intestinal lumen. Therefore, *L. monocytogenes* uses LAP to exploit epithelial innate defenses and induce intestinal barrier dysfunction.

Breaching the Cell–Cell Junction

LAP also activates MLCK [41,42], which regulates and redistributes cellular junctional proteins along the membrane architecture. LAP redistributes and endocytoses the TJ proteins claudin-1 and occludin, as well as the AJ protein E-cadherin, from the membrane, as observed in mouse ileum and Caco-2 cells [9]. This results in increased epithelial permeability and bacterial translocation across the epithelial barrier. Pharmacological inhibition of MLCK in cells, or genetic ablation of MLCK in mice, prevents phosphorylation of MLC (PMLC), epithelial cell-junction protein redistribution, and *L. monocytogenes* translocation. Thus, LAP directly binds to Hsp60 to activate canonical NF-κB (p65) signaling, thereby facilitating the MLCK-mediated opening of the intestinal cell–cell junction barrier via the cellular redistribution of the major junctional proteins claudin-1, occludin, and E-cadherin, and subsequent bacterial translocation [9] (Figure 3). Therefore, LAP-mediated MLCK activation is critical for epithelial barrier dysfunction and *L. monocytogenes* translocation.

The targeting of the cell–cell junction in not restricted to *L. monocytogenes*; many enteric bacterial pathogens have evolved different strategies for breaching the cell–cell junction in order to cause intestinal barrier dysfunction and systemic infection [107] (Table 1). Adherent-invasive *E. coli* (AIEC) infects CEABAC10 transgenic mice (expressing human CEACAMs) and increases intestinal paracellular permeability by upregulating the expression of the pore-forming TJ protein claudin-2 [108]. *Campylobacter jejuni* possesses outer-membrane vesicle-associated proteolytic activity for cleaving occludin and E-cadherin [109,110]. Enteropathogenic *E. coli* (EPEC) employs the type III secretion system (T3SS) effectors, NleA [111], EspM [112], Map [113,114], and EspF [114–117], to disrupt TJs when causing diarrheal disease. Recently, EPEC has also been shown to engage EspG1 to target tricellulin [118], and EspH to target the desmosomes [119]. *Helicobacter pylori* uses CagA to cause disorganization of gastric epithelial architecture through inhibition of PAR1 kinase activity [120–122]. More recent work suggests that other *H. pylori* virulence factors, such as HrtA [123] and urease [124], also cause disruption of cell–cell junctions. *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* increase intestinal paracellular permeability in Caco-2 and TC7 cell lines via rearrangements of F-actin microfilaments [125]. Recent studies have also demonstrated that *P. fluorescens* increases intestinal paracellular permeability by causing the release of IL-1β by immune cells and the activation of MLCK in epithelial cells in a NOD2-dependent manner *in vivo* [126]. *S. enterica* serovar Typhimurium employs SPI-1-secreted effectors (SopB, SopE, SopE2, and SipA) to stimulate Rho family GTPases for the disruption of tight junction structure and function [127]. In contrast, the *Salmonella* T3SS effector ArvA was shown to stabilize TJs [128,129]. *S. flexneri* increases paracellular permeability by regulating tight junction-associated proteins [130,131], and recently, the bacterial secreted serine protease A (SepA) has been implicated in this process [132]. *V. cholerae* uses the toxins hemagglutinin/

Table 1. Microbial Pathogens Involved in the Disruption of the Epithelial Cell–Cell Junction Barrier

Bacteria	Virulence factors	Host targets	Refs
Adherent-invasive *Escherichia coli*	Unknown	Intestinal CEACAMs and claudin-2	[108]
Campylobacter jejuni	Outer membrane vesicles	Occludin and E-cadherin	[109]
Enteropathogenic *E. coli*	NleA	COPII	[111]
	EspM	RhoA	[112]
	Map	Cdc42	[113,114]
	EspF	Crb3, occludin,	[114,116,117]
	EspG1	Tricellulin	[118]
	EspH	Rho GTPase, desmoglein-2	[119]
Helicobacter pylori	CagA	ZO-1, PAR1, β-catenin	[120–122]
	HtrA	Occludin, claudin-8, E-cadherin	[123]
	Urease	MLCK, ROCK	[124]
Listeria monocytogenes	LAP	Hsp60, NF-κB, MLCK, claudin-1, occludin and E-cadherin	[9,102]
Pseudomonas aeruginosa	Unknown	F-actin microfilaments	[125]
Pseudomonas fluorescens	Unknown	NOD2 signaling, MLCK	[67,126]
Salmonella enterica serovar Typhimurium	SipA, SopB, SopE, SopE2	Rho GTPase	[127]
Shigella flexneri	SepA	LIM kinase 1 (LIMK1), cofilin, ZO-1, ZO-2, E-cadherin and occludin	[130–132]
Vibrio cholerae	HAP and ZOT	ZO-1, occludin	[133,134]
	Cholera toxin	E-cadherin and Notch signaling	[135]
Yersinia pseudotuberculosis	YopJ	NOD2 signaling, activates caspase-1	[136]

protease (HAP) and zonula occludens toxin (ZOT) to cleave ZO-1 from the intracellular domain of occludin to disrupt barrier function [133,134]. Recent work also demonstrates that cholera toxin disrupts barrier functions by mislocalizing E-cadherin, via inhibition of exocyst-mediated trafficking of host proteins to intestinal cell junctions [135]. Finally, *Yersinia pseudotuberculosis* requires the outer membrane protein YopJ to subvert the signaling of the innate immune receptor Nod2 to induce IL-1β production, leading to the disruption of the intestinal epithelial barrier [136,137].

The E-cadherin Dilemma in Epithelial Barrier Architecture

It is now clear that *L. monocytogenes* uses the LAP-mediated pathway to establish infection by directly shifting junctional protein composition. InlA employs E-cadherin as the receptor, but *L. monocytogenes* redistributes E-cadherin to facilitate bacterial translocation during infection [9], raising an intriguing question: at what stage during the infection process does InlA interact with E-cadherin? If the initial LAP-mediated interaction causes a redistribution of membrane E-cadherin to be endocytosed for cell junction opening, there are possibly remnants of membrane E-cadherin left to interact with InlA to proceed with InlA-mediated bacterial uptake and invasion. Since earlier studies reported that InlA-mediated transcytosis takes place only after 48–72 hpi [47,85–87], it is possible that, during the early infection phase, *L. monocytogenes* capitalizes on the LAP-Hsp60-mediated translocation pathway to gain access for successful infection. This flexibility may make the bacterium far less susceptible to clearance by the innate immune defense by providing easier access to the lamina propria early in the infection process. Furthermore, LAP-Hsp60-mediated translocation may also serve as an

important precursor event for InlA-dependent epithelial translocation that provides access to E-cadherin in the adherens junction of permissive hosts, such as gerbils, guinea pigs, and humans, in addition to epithelial invasion via villous cell extrusion [80] and at the empty goblet cell junction [50] as the infection continues (Figure 2).

Concluding Remarks

The epithelial cells in the gastrointestinal tract provide the first line of defense against luminal microbes from crossing the gut wall. In healthy humans, the epithelial barrier is tightly controlled, and it allows selective passage of nutrients, drugs, and small molecules, but it prevents passage of luminal microbes, including pathogens. *L. monocytogenes*, being a foodborne pathogen, has to cross the gut barrier to enter the blood circulation and disseminate to the MLN, liver, spleen, brain, and placenta (in pregnant women) for successful infection. It has been well established that, for *L. monocytogenes* to cross the epithelial barrier, InlA is responsible and it is considered the most important protein for epithelial cell invasion [16]. However, some *L. monocytogenes* isolates from the environment or from food sources naturally carry truncated InlA with a premature stop codon, and these isolates were considered less invasive [52–54]. Conversely, in recent years, *L. monocytogenes* strains with defective InlA were responsible for neonatal listeriosis in humans [55–57], mice, and guinea pigs after oral challenge [58]. Other virulence factors, such as Vip [138] and LapB [139], have been described in the literature and help *Listeria* to invade cells, but their contribution in *L. monocytogenes* crossing of the intestinal epithelial barrier has not been well defined.

LAP aids *L. monocytogenes* in crossing the gut epithelial barrier during the early stage of infection. LAP is a housekeeping enzyme (alcohol acetaldehyde dehydrogenase) required for bacterial growth under anaerobic conditions [89,91,140], and at the same time it helps *L. monocytogenes* to attach to the intestinal cells by interacting with the cellular receptor Hsp60; thus, LAP is considered a prototypical moonlight protein [101]. LAP orchestrates a complex cell signaling event to activate regulatory molecules, NF-κB, and MLCK to regulate the opening of the epithelial cell–cell junction [9]. The intestinal cell junction opening allows *L. monocytogenes* to cross the gut barrier very early in the infection process (in a few hours) as soon as the bacterium reaches the intestine when the immune system is not very active. It is clear that *L. monocytogenes* uses two dominant pathways, LAP-mediated [9] followed by InlA-mediated [84,85,87], or their cooperative action (Figure 3), to enter the host tissues, thus making this bacterium a very successful infective foodborne pathogen, especially in high-risk immunocompromised individuals. However, the role of other *Listeria* proteins, the gut microbiota, and the immune status of the host cannot be ignored since the bacterium may employ several virulence factors [141] and multiple independent routes [142] to be highly invasive (see Outstanding Questions). This may also explain why *L. monocytogenes* has such a low infectious dose [23,24] and the highest hospitalization and mortality rate among the foodborne pathogens [17]. Thus, RTE foods containing even a few *L. monocytogenes* cells such as $<$100 CFUs/g, as defined in food regulations by the European Union [27], would be a huge risk to the growing worldwide population with underlying chronic health conditions including diabetes mellitus, cancer, HIV/AIDS, alcoholism, cirrhosis, opioid addiction, malnutrition, and diseases related to smoking [143]. Future research focused on deeper understanding of the molecular and cellular mechanisms that *L. monocytogenes* and other bacterial foodborne pathogens employ to cross the intestinal barrier will help in drug design and therapeutics for preventing fatal systemic infections.

Outstanding Questions

Does interaction of LAP with Hsp60 initiate other signaling pathway(s) besides NF-κB and MLCK?

Does the immunocompromised condition increase susceptibility to listeriosis by enhancing enterocyte Hsp60 expression?

Does *L. monocytogenes* target other tight-junction components such as tricellulin and desmosomes in order to breach the cell junction?

What other mechanism(s), besides bacteriocin, does *L. monocytogenes* employ to modulate the host gut microbiota?

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Resources

[1]www.nicd.ac.za/index.php/listeriosis-outbreak-situation-report/

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