CHAPTER ONE

NEW INSIGHTS INTO DETERMINANTS OF LISTERIA MONOCYTOGENES VIRULENCE

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Abstract

Listeria monocytogenes is the causative agent of human listeriosis, a potentially fatal foodborne infection. Clinical manifestations range from febrile gastroenteritis to more severe invasive forms including meningitis, encephalitis, abortions, and perinatal infections. This Gram-positive facultative intracellular pathogen has evolved multiple strategies to face extracellular innate defense mechanisms of the host and to invade and multiply intracellularly within macrophages and nonphagocytic cells. This chapter provides an updated panorama of recent advances in the characterization of L. monocytogenes virulence determinants in the postgenomic era.

Key Words: Listeriosis, Listeria monocytogenes, Virulence, Genome, Cell invasion, Immunity, Pathophysiology.

1. Introduction

The Listeria genus is composed of six species: L. monocytogenes, L. ivanovii, L. innocua, L. seeligeri, L. welshimeri, and L. grayi (Vazquez-Boland et al., 2001b). The pathogenic species L. monocytogenes causes disease in humans and animals. The second pathogenic species, L. ivanovii, causes disease in animals. L. innocua, L. seeligeri, L. welshimeri, and L. grayi are four nonpathogenic species. Listeria spp. are flagellated and motile Gram-positive, nonspore-forming, facultative anaerobic bacilli of low GC content (Seeliger and Jones, 1986). These ubiquitous bacteria are commonly isolated from multiple sources such as plants, soil, and water. L. monocytogenes can contaminate the agricultural environment, animal feed, and food at various stages of the production process leading to recalls (Orndorff et al., 2006; Roberts and Wiedmann, 2003). It is thus a major problem in the food industry. Ingestion of food contaminated with L. monocytogenes is the primary route of transmission to humans. L. monocytogenes is the causative agent of listeriosis. Although the incidence of the disease is low (0.1 to 11.3/1,000,000), it is a public health concern because of a high mortality rate (20–30%) and high occurrence of Listeria in food (Swaminathan and Gerner-Smidt, 2007).

L. monocytogenes causes two forms of listeriosis depending on the immunological status of the host, the pathogenic potential of the bacterial strain, and the infectious dose: noninvasive gastrointestinal listeriosis and invasive listeriosis (Vazquez-Boland et al., 2001b). In immunocompetent individuals, noninvasive listeriosis develops as a typical febrile gastroenteritis. In immunocompromised adults such as the elderly, patients with genetic or acquired defects in immunity and patients receiving immunosuppressive agents, listeriosis can manifest as septicemia and/or meningoencephalitis.
Invasive listeriosis can also be acquired by the fetus from the infected mother by transplacental transmission. Perinatal listeriosis can lead to abortion, birth of a stillborn fetus or a baby with generalized infection (granulomatosis infanseeptica), and meningitis in neonates. Clinical features of invasive listeriosis derive from the unique capacity of *L. monocytogenes* to cross three barriers: the intestinal, blood–brain, and placental barriers (Lecuit, 2005). The clinical outcome of listeriosis is influenced by the pathogenic potential of the infecting strain. Among *L. monocytogenes* strains, those of the serovars 1/2a, 1/2b, and 4b are responsible for 95% of human infections and most outbreaks are caused by strains of serovar 4b (Swaminathan and Gerner-Smidt, 2007). The remarkable capacity of *L. monocytogenes* to invade and multiply in epithelial cells and professional phagocytic cells is central to listeriosis pathophysiology (Fig. 1.1). *L. monocytogenes* uses various receptors to enter these cells. After internalization, the bacterium lyzes the vacuole, escapes in the cytosol, and replicates. *L. monocytogenes* then exploits the actin machinery to move within the cell and to neighboring cells where it is internalized in a double-membrane vacuole that is lyzed, allowing the bacterium to access the cytosol and start a new intracellular infection cycle (Tilney and Portnoy, 1989).

For more than 40 years, *L. monocytogenes* and experimental listeriosis have been used to study the immune response and the biology of the cell leading to major discoveries (Cossart, 2007; Garifulin and Boyartchuk, 2005; Hamon *et al.*, 2006; Mackaness, 1962; Pamer, 2004). More recently, the extensive characterization of the mechanisms used by *L. monocytogenes* to

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**Figure 1.1** Schematic representation of the infectious cycle of *Listeria monocytogenes*. Bacteria first adhere to cells, induce entry, and are internalized in a vacuole (1). The vacuole is lyzed by *L. monocytogenes* virulence factors (2). Once free in the cell cytoplasm, bacteria start to replicate (3). *L. monocytogenes* then exploits the actin polymerization machinery of the cell to propel itself (4). When bacteria reach a neighboring cell, they induce the formation of a protrusion characterized by a double membrane (5). This secondary vacuole is finally lyzed (6), allowing a new infection cycle.
manipulate the host cell contributed to the creation of the field of cellular microbiology (Cossart et al., 1996). The advanced knowledge of the specificity of *L. monocytogenes* interactions with the host culminated in 2001, with the creation of the first transgenic mouse to model human listeriosis in animals (Lecuit, 2007; Lecuit and Cossart, 2002; Lecuit et al., 2001). The same year, the first comparison of the genome sequences of a pathogenic bacterium and a related nonpathogenic species, *L. monocytogenes* and *L. innocua*, respectively, allowed to envision the identification of the complete arsenal used by *Listeria* to cause disease (Dussurget et al., 2004; Glaser et al., 2001). Here, we review *L. monocytogenes* major virulence determinants that have been currently characterized.

## 2. Acquisition of Virulence Genes and Their Expression

### 2.1. Acquisition of virulence genes

Acquisition and loss of genetic elements lead to bacterial speciation and provide the properties necessary for a particular lifestyle. Cumulative acquisition of virulence genes provides pathogenic bacteria the functions required for survival, growth and damage in the infected host.

The genome organization is remarkably conserved between different *Listeria* species (Hain et al., 2007; Schmid et al., 2005). However, comparative genomic analysis of pathogenic and nonpathogenic *Listeria* species reveals scattered genes specific to virulent strains that are isolated or form pathogenicity islands (Glaser et al., 2001; Vazquez-Boland et al., 2001a). The unusual base composition of some of these sequences could be the consequence of horizontal transfer (Begley et al., 2005; Dussurget et al., 2002). Interestingly, *Listeria* genomes contain open reading frames homologous to *Bacillus subtilis* competence genes (Buchrieser, 2007; Glaser et al., 2001). Although the function of this putative DNA uptake system has not been demonstrated yet, it could be hypothesized that competence may play a role in acquisition of virulence genes by *L. monocytogenes*. Lysogenic bacteriophages, plasmids, and transposons, which could play critical roles in the evolution of pathogenicity, have been characterized in *Listeria* species but they have not been associated to virulence (Hain et al., 2007). Nonpathogenic species, including *L. innocua* appear to have evolved from a *L. monocytogenes* ancestor after multiple deletions of virulence genes (Buchrieser, 2007; Hain et al., 2006). Recently, analysis of the complete genome sequence of the nonpathogenic *L. welshimeri* revealed deletions of all the genes required for virulence and of some genes encoding
transcription factors, surface proteins, and proteins involved in carbohydrate transport and metabolism (Hain et al., 2006). Comparison of L. welshimeri and L. innocua suggests similar evolutionary paths from an ancestor.

### 2.2. Regulation of virulence gene expression

Adaptability of L. monocytogenes that is central to the infectious process is determined by the genetic elements allowing bacteria to survive and multiply within multiple tissues and by the mechanisms required for the tight and coordinate regulation of their expression.

#### 2.2.1. PrfA

PrfA is the master regulator of virulence gene expression in L. monocytogenes. PrfA is a protein of 233 amino acids that binds to a 14-bp palindromic sequence in the −41 region of the genes from the PrfA regulon and activates their transcription. The activity of PrfA itself is tightly controlled by multiple mechanisms (Vazquez-Boland et al., 2001b). Translation of PrfA is regulated by temperature (Johansson and Cossart, 2003; Johansson et al., 2002). At a temperature lower than 30 °C, the untranslated region of prfA mRNA adopts a stable secondary structure that prevents binding of the ribosome and blocks translation. In the host, the temperature of 37 °C induces melting of the secondary structure. Consequently, PrfA is translated and activates virulence gene expression. Determination of L. monocytogenes genome sequence allowed analysis of the transcriptome and identification of the PrfA regulon (Milohanic et al., 2003). The transcriptomic analysis identified a total of 73 genes regulated directly or indirectly by PrfA. This study confirmed that the expression of important virulence genes such as hly, actA, plcA, plcB, mpl, inlA, inlB, inlC, hpt, and prfA itself is activated by PrfA. Interestingly, the expression of all these genes is increased intracellularly after infection of macrophages and epithelial cells (Chatterjee et al., 2006; Joseph et al., 2006).

#### 2.2.2. Sigma B

Other regulatory elements have been demonstrated to be necessary for full virulence of L. monocytogenes. The stress-responsive alternative sigma factor encoded by sigB contributes to invasion (Kim et al., 2004) and virulence (Garner et al., 2006; Nadon et al., 2002). The sigma B regulon contains stress response and virulence genes such as gadB, opuCA, bsh, inlA, and inlB (Kazmierczak et al., 2003; McGann et al., 2007; Sue et al., 2003) and stress and virulence gene regulators Hfq (Christiansen et al., 2004; Nadon et al., 2002) and PrfA (Nadon et al., 2002).
2.2.3. MogR
Temperature-dependent expression of the flagellin gene flaA is controlled by the transcriptional regulator DegU and by the antagonist activity of the repressor MogR (Grundling et al., 2004). At 37 °C, flagellin synthesis is repressed by the regulator MogR. At 30 °C and below, DegU activates expression of GmaR that forms a complex with MogR and prevents binding of the repressor to its target DNA sequences (Shen et al., 2006). GmaR is a bifunctional protein that functions as an antirepressor and an O-linked N-acetylglucosamine transferase that glycosylates flagellin (Schirm et al., 2005; Shen et al., 2006). The role of flagellin glycosylation remains to be determined. MogR contributes to L. monocytogenes virulence (Grundling et al., 2004; Shen and Higgins, 2006) and its expression is induced in macrophages (Chatterjee et al., 2006). Overproduction of FlaA in mogR mutants leads to defects in bacterial division, intracellular spread, and virulence in mice.

2.2.4. CtsR
The class III stress gene repressor CtsR regulates the expression of class III heat-shock genes encoding the Clp ATPases ClpB, ClpC, ClpE, and ClpP, which are required for virulence (Chastanet et al., 2004; Gaillot et al., 2000; Nair et al., 1999, 2000; Rouquette et al., 1998). Interestingly, the expression of CtsR and the four ATPases is induced in infected macrophages (Chatterjee et al., 2006).

2.2.5. PerR and Fur
The Fur family of regulators includes sensors of iron (Fur), zinc (Zur), manganese (Mur), nickel (Nur), as well as metal-dependent reactive oxygen species sensors such as the peroxide sensor PerR (Lee and Helmann, 2007). The iron-responsive transcriptional regulator Fur is responsible for coordinating the expression of genes involved in iron uptake and storage (Lee and Helmann, 2007). The regulator PerR senses peroxides by metal-catalyzed oxidation and regulates the expression of inducible genes involved in defense against reactive oxygen species (Lee and Helmann, 2006). L. monocytogenes perR and fur mutants are more sensitive to hydrogen peroxide and have a significantly reduced virulence of in mice (Rea et al., 2004, 2005). Interestingly, the PerR regulon includes the ferritin gene fri that contributes to survival of L. monocytogenes in vivo (Dussurget et al., 2005; Mohamed et al., 2006; Olsen et al., 2005). Thus, regulation of iron uptake and oxidative stress response is an important determinant for the infectious process.

2.2.6. LisRK, AgrA, VirR, and DegU
Several two-component regulatory systems contribute to L. monocytogenes survival in the infected host. LisRK is important for bacterial response to acid and hydrogen peroxide stresses and for osmotolerance mediated by the
HtrA-like serine protease (Cotter et al., 1999; Stack et al., 2005). The response regulators AgrA (Autret et al., 2003) and VirR (Mandin et al., 2005) play a role in virulence, which was identified by signature-tagged mutagenesis. A transcriptomic approach led to the identification of 12 genes regulated by VirR, including the dlt operon, which is required for L. monocytogenes full virulence. However, a dltA mutant is not as impaired in virulence as a virR mutant, suggesting that the response regulator may control the expression of other virulence determinants (Mandin et al., 2005). Indeed, another member of the VirR regulon, the mprF gene, has recently been shown to contribute to L. monocytogenes virulence (Thedieck et al., 2006). The response regulator DegU is a transcriptional activator of the expression of the flagellin gene flaA at low temperature and regulates virulence-associated genes (Knudsen et al., 2004; Williams et al., 2005).

2.2.7. Stp
Analysis of L. monocytogenes genome sequence revealed 9 signal transduction systems based on reversible phosphorylation in addition to the 16 two-component systems: 4 putative tyrosine phosphatases, 3 putative serine-threonine kinases, and 2 putative serine-threonine phosphatases (Archambaud et al., 2005; Glaser et al., 2001). One of the latter enzyme is an Mn$^{2+}$-dependent serine-threonine phosphatase that has an important role in regulating the elongation factor EF-Tu and controlling bacterial survival in the infected host (Archambaud et al., 2005). Stp was recently shown to control L. monocytogenes manganese dependent-superoxide dismutase (MnSOD) an enzyme that is required for full virulence (Archambaud et al., 2006).

2.2.8. Hfq
The RNA-binding protein Hfq regulates multiple important processes such as stress tolerance and virulence. Hfq contributes to virulence in mice possibly by interacting with mRNA and/or small regulatory RNA, playing a role in the survival and multiplication of L. monocytogenes in vivo (Christiansen et al., 2004; Mandin et al., 2007).

3. Adaptation to Host Extracellular Compartments

Following ingestion, the capacity of L. monocytogenes to survive and multiply successfully under the multiple and dynamic environments found in the host is an essential factor in the infectious process.
3.1. GAD

The glutamate decarboxylase system GAD is essential for survival in the stomach after ingestion (Cotter et al., 2001). Depending on the strain, it is composed of two or three glutamate decarboxylases and one or two glutamate/γ-aminobutyrate antiporters (Cotter et al., 2005). The GAD system transports and converts glutamate to γ-aminobutyrate consuming a proton, allowing L. monocytogenes to survive in acidic environments.

3.2. BSH

Bile is essential to emulsify lipids and has important antimicrobial properties. L. monocytogenes is well equipped to tolerate high concentration of bile (Begley et al., 2002, 2003, 2005; Dussurget et al., 2002; Sleator et al., 2005). Analysis of L. monocytogenes genome sequence revealed the presence of a gene encoding a bile salt hydrolase (BSH) that was absent from the genome of the nonpathogenic species L. innocua (Dussurget et al., 2002). BSH is produced by commensal enteric bacteria and lactic bacteria. Deconjugation of conjugated bile salts by BSH could be a protective mechanism against bile toxicity. L. monocytogenes BSH is controlled by sigma B (Kazmierczak et al., 2003; Sue et al., 2003) and activated by PrfA (Dussurget et al., 2002). Its activity is induced at low oxygen tension that could be a signal sensed by bacteria after ingestion to express the bsh as well as other virulence genes (Dussurget et al., 2002). L. monocytogenes BSH confers resistance to bile (Begley et al., 2005; Dussurget et al., 2002). Deletion of the bsh gene results in dramatically reduced fecal carriage in guinea pigs after intragastric inoculation and decreased survival in the liver of mice after intravenous injection (Dussurget et al., 2002). BSH is therefore a new type of virulence determinant that is important for both intestinal persistence and hepatic colonization.

3.3. BilE

Analysis of L. monocytogenes genome revealed a two-gene operon, bilEA–bilEB, which is critical for bile tolerance (Sleator et al., 2005). The expression of the operon is controlled by sigma B and PrfA. The operon encodes a bile exclusion system providing a protection against bile and contributing to L. monocytogenes virulence in mice infected orally.

3.4. BtlB

A third locus, btlB, has been shown to contribute to bile tolerance and L. monocytogenes virulence in mice (Begley et al., 2005). BtlA and Pva that encode a putative transporter and a penicillin V amidase, respectively, are other important determinants conferring tolerance to bile but do not contribute significantly to virulence in mice (Begley et al., 2003, 2005).
3.5. OpuC

Once in the intestinal lumen, *L. monocytogenes* has to cope not only with the presence of bile salts but also with an increased osmolarity. *L. monocytogenes* produces several osmolyte uptake systems increasing osmotolerance, such as the glycine betaine transporters BetL and Gbu and the carnitine transporter OpuC (Ko and Smith, 1999; Sleator *et al.*, 1999, 2001; Wemekamp-Kamphuis *et al.*, 2002). While deletion of betL and gbu does not affect virulence, OpuC is required for full virulence in mice infected orally (Sleator *et al.*, 2001; Wemekamp-Kamphuis *et al.*, 2002).

3.6. OppA

Uptake of oligopeptides by the OppA transporter could also contribute to osmotolerance and is required for intracellular survival in macrophages and bacterial growth in mice (Borezee *et al.*, 2000).

4. **Adhesion, Cell Invasion, and Intracellular Multiplication**

Following gastrointestinal passage of *L. monocytogenes*, some of the bacteria that survived nonspecific defense mechanisms of the host in the stomach and intestinal lumen invade the intestinal tissue. Crossing of the intestinal barrier prevents their mechanical elimination by peristalsis and competition with the commensal flora. *L. monocytogenes* has the capacity to invade both intestinal epithelial cells and M cells of Peyer’s patches. After intestinal translocation, bacteria reach the liver, spleen, and mesenteric lymph nodes by the blood and lymph. In the liver, the major site of *L. monocytogenes* multiplication is the hepatocyte. If the multiplication is not controlled by the host immune response, bacteria access the bloodstream and infect secondary target organs. Although *L. monocytogenes* has a strong neurotropism, it can infect a wide range of tissues (Vazquez-Boland *et al.*, 2001b). *L. monocytogenes* has an exceptional repertoire of virulence determinants involved in cellular adhesion, entry, and survival (Bierne and Cossart, 2007; Hamon *et al.*, 2006; Seveau *et al.*, 2007).

4.1. Adhesion

4.1.1. Ami

Ami is a 102-kDa autolytic amidase of 917 amino acids that is involved in adhesion to cells and virulence (Milohanic *et al.*, 2000, 2001). It was identified by transposon mutagenesis in an *inlAB* deletion mutant.
(Milohanic et al., 2000, 2001). One of the mutants severely defective in adhesion to eukaryotic cells had five insertions, one of which was upstream from the ami gene. Construction of an ami null mutant demonstrated that Ami significantly contributed to L. monocytogenes adhesion capacity (Milohanic et al., 2000, 2001). Ami has an N-terminal region containing the amidase domain and C-terminal cell wall-anchoring domain composed of eight modules containing the dipeptide GW (Milohanic et al., 2004). Adhesion to cells is promoted by the cell wall-anchoring domain (Milohanic et al., 2000, 2001). L. monocytogenes attachment mediated by Ami may contribute to colonization of host tissues.

4.1.2. DltA
Lipoteichoic acids are highly anionic cell wall-associated polymers. The dltABCD operon is responsible for d-alanine esterification of lipoteichoic acids. Inactivation of the d-alanine-polyphosphoribitol ligase gene dltA, leading to synthesis of d-alanine-deficient lipoteichoic acids, attenuates L. monocytogenes virulence in mice (Abachin et al., 2002; Mandin et al., 2005). DltA deficiency decreases adherence of bacteria to macrophages, hepatocytes, and epithelial cells, possibly by modulation of the charge of the bacterial surface and/or by alteration of adhesin-binding activity (Abachin et al., 2002).

4.1.3. FbpA
FbpA is an adhesin that is important for L. monocytogenes pathogenesis. FbpA has been identified using signature-tagged mutagenesis (Dramsi et al., 2004). It was shown to be required for liver colonization of mice inoculated intravenously as well as intestinal and liver colonization of mice expressing human E-cadherin after intragastric inoculation. FbpA is a protein of 570 amino acids homologous to atypical fibronectin-binding proteins. It binds to human fibronectin and increases L. monocytogenes adhesion to eukaryotic cells in the presence of exogenous fibronectin. FbpA is secreted by the SecA2 pathway and exposed on the bacterial surface. In addition to its fibronectin-binding capacity, FbpA coprecipitates with the virulence factors listeriolysin O (LLO) and InlB. Expression of FbpA modulates the protein levels of LLO and InlB, suggesting that it could function as a chaperone to prevent the degradation of virulence factors (Dramsi et al., 2004).

4.1.4. Flagella
L. monocytogenes produces up to six peritrichous flagella (Leifson and Palen, 1955). Flagella are composed of a basal body, hook/junction proteins, a flagellar motor/switch, a flagella export apparatus, and a flagellar filament containing mostly the flagellin protein FlaA. Flagellin is a potent proinflammatory protein that activates Toll-like receptor (TLR) 5 (Hayashi et al., 2001). Moreover, flagellin has been reported to have peptidoglycan
hydrolyzing activity (Popowska and Markiewicz, 2004). While many flagella are produced at 20 °C, the expression of flagellar motility genes is repressed at 37 °C (Griffin and Robbins, 1944; Grundling et al., 2004; Peel et al., 1988; Way et al., 2004). However, the temperature control of flagellar motility is less stringent in some \textit{L. monocytogenes} strains (Grundling et al., 2004; Way et al., 2004). Flagellin expression at 37 °C is maintained in 20% of clinical isolates (Bigot et al., 2005; Way et al., 2004). Flagella contribute to \textit{L. monocytogenes} adhesion and invasion of epithelial cells. Indeed, the nonmotile \textit{flaA} mutant, \textit{fliF} and \textit{fliI} mutants, lacking the basal body and the ATPase of the flagellar export apparatus, and the \textit{cheYA} chemotaxis mutant are strongly impaired in adhesion and invasion (Bigot et al., 2005; Dons et al., 2004). It has recently been demonstrated that flagella do not function as adhesins but that flagella–dependent motility promotes \textit{L. monocytogenes} invasion of epithelial cells (O’Neil and Marquis, 2006). The specific role of flagellar motility and flagellin in the infectious process is not completely understood. Liver and spleen colonization of a \textit{flaA} deletion mutant has been shown to be similar to that of a parental strain expressing flagellin constitutively, after intravenous infection of mice (Way et al., 2004). However, survival of the parental strain producing flagellin seemed to be decreased compared with that of the \textit{flaA} mutant, 7 days after intragastric inoculation of mice. The LD50 of \textit{fliF} and \textit{fliI} mutants was very modestly affected compared with that of the EGDe wild-type strain after intravenous infection of Swiss mice (Bigot et al., 2005). Interestingly, the survival of the wild-type strain was lower than that of the \textit{fliI} mutant in the spleen of BALB/c mice, 3 days after intragastric infection. A similar observation was reported with a \textit{flaA} mutant that was recovered in higher numbers than the wild-type strain from the spleen of BALB/c mice, 3 days after intragastric inoculation (Dons et al., 2004). However, the fact that this difference was not detected at 1 or 7 days postinfection is puzzling. Recently, flagellin was shown to be required for intestinal and liver colonization in the early phase of murine listeriosis, between 4 and 18 h after intragastric inoculation (O’Neil and Marquis, 2006). It could be hypothesized that \textit{L. monocytogenes} regulates flagella synthesis in time and space, producing flagella to colonize the gastrointestinal tract after ingestion and repressing their synthesis as a means of innate immune evasion at later stages of the infectious process.

4.2. Internalization

4.2.1. Internalin

The internalin is a protein of 800 amino acids encoded by the \textit{inlA} gene. It is composed of a typical N-terminal signal sequence followed by 15 leucine-rich repeats (LRRs) of 22 amino acids, a conserved interrepeat region and a second repeat region, the B repeat region. The C-terminus displays the
sequence LPTTG, which is recognized by the sortase A, a transamidase that covalently links LPXTG-containing proteins to the peptidoglycan. This surface protein is an invasin that mediates internalization of *L. monocytogenes* in epithelial cells. It was identified by screening a bank of transposon mutants for reduced entry in Caco-2 cells (Gaillard *et al.*, 1991). The intercellular adhesion glycoprotein E-cadherin was subsequently identified as the internalin ligand using affinity chromatography (Mengaud *et al.*, 1996). If E-cadherin ectodomain is sufficient for adherence of *L. monocytogenes* to cells, the intracytoplasmic β-catenin-binding domain is required for entry (Lecuit *et al.*, 2000). Bacterial interaction with E-cadherin triggers actin polymerization mediated by β-catenin and α-catenin interaction, leading to membrane extension and internalization. Recently, ARHGAP10, a Rho-GAP domain protein that interacts with the small GTP-binding protein Arf6 and is a new ligand of α-catenin identified by a two-hybrid screen, has been shown to be critical for recruitment of α-catenin and bacterial entry (Sousa *et al.*, 2005). The internalin-dependent entry pathway requires several other proteins including myosin VIIA, Src, cortactin, and Arp2/3. The myosin VIIA, a molecular motor recruited at adherens junctions by the transmembrane protein vezatin, could contribute to the contractile force necessary for internalization of *L. monocytogenes* (Sousa *et al.*, 2004). The tyrosine kinase Src and the small GTPase Rac1 promote the recruitment of cortactin leading to activation of the actin nucleator Arp2/3 necessary for E-cadherin-mediated bacterial entry (Sousa *et al.*, 2007).

Although internalin plays a major role in bacterial internalization into specific cell lines, the protein had a minor contribution to virulence in the murine models that were first used, irrespective of the route of infection, that is intravenous or intragastric inoculations (Gaillard *et al.*, 1996). It was later shown that the mouse E-cadherin does not interact efficiently with InlA (Lecuit *et al.*, 1999). Indeed, the interaction requires recognition of the proline 16 of the first extracellular domain of E-cadherin as found in human or guinea pig E-cadherins. However, the murine E-cadherin has a glutamic acid at position 16. A transgenic mouse expressing the human E-cadherin in the intestine was created and used to demonstrate the major role of internalin in the specific crossing of the intestinal barrier by *L. monocytogenes* (Lecuit *et al.*, 2001). Recently, a strain of *L. monocytogenes* expressing an internalin with two amino acid substitutions allowing efficient binding to murine E-cadherin was created (Wollert *et al.*, 2007). This new strain could be a powerful tool to study listeriosis in nontransgenic mice, circumventing limitations, and problems inherent to humanized mice.

In addition to its established role in crossing of the intestinal barrier, InlA is involved in the crossing of the maternofetal barrier (Lecuit *et al.*, 2004). Internalin is required for *L. monocytogenes* entry into E-cadherin-expressing syncytiotrophoblasts and crossing of the trophoblastic barrier in human placental explants (Lecuit *et al.*, 2004). Interestingly, the InlA protein is
truncated in some *L. monocytogenes* isolates. Truncation of InlA has been involved in defective invasion capacity of *L. monocytogenes* isolates from healthy carriers (Olier et al., 2003). An epidemiological survey demonstrated that a full-length InlA was produced by 96% of *L. monocytogenes* clinical isolates and only 65% of the strains isolated from food products (Jacquet et al., 2004). Another study confirmed that *inlA* mutations leading to premature stop codons were common in food isolates but rare in clinical isolates (Nightingale et al., 2005). These results strongly suggest that a functional internalin is a key determinant in the pathogenesis of human listeriosis.

4.2.2. InlB

InlB is a 630-amino acid protein encoded by the gene *inlB*, which is located directly downstream of *inlA* in a two-gene operon (Gaillard et al., 1991). The operon is regulated by PrfA and absent from *L. innocua* (Dramsi et al., 1993; Glaser et al., 2001; Lingnau et al., 1995; Milohanic et al., 2003). In contrast to internalin, InlB is required for *L. monocytogenes* internalization into a wide range of cells including epithelial cells, endothelial cells, hepatocytes, and fibroblasts (Braun et al., 1998; Dramsi et al., 1995; Greiffenberg et al., 1998; Parida et al., 1998). The InlB protein displays a signal sequence followed by seven LRRs, a B repeat, and three C-terminal GW modules. The GW modules interact noncovalently with lipoteichoic acids mediating loose attachment of InlB to the bacterial cell wall (Jonquieres et al., 1999). The LRR region of the protein is sufficient to allow entry of noninvasive *L. innocua* or latex beads into cells (Braun et al., 1999). However, the GW modules enhance internalization triggered by the LRR region. Binding of InlB to cellular glycosaminoglycans by its GW modules is required for efficient invasion (Banerjee et al., 2004; Jonquieres et al., 2001; Marino et al., 2000, 2002, 2004). The GW modules of InlB also interact with the receptor for the globular head domain of the complement component C1q, gC1qR (Braun et al., 2000). This interaction is not sufficient to allow entry but cooperates with the hepatocyte growth factor, also known as the tyrosine kinase receptor Met, for invasion (Khelef et al., 2006). Met has been identified as the main receptor of InlB (Shen et al., 2000). Interaction of InlB and Met results in transient phosphorylation of Met (Shen et al., 2000), and recruitment and phosphorylation of the adaptor proteins Cbl, Gab1, and Shc leading to activation of the PI3-kinase (Ireton et al., 1996, 1999). The PI3-kinase converts PI(4,5)P2 into PI(3,4,5)P3, which results in successive activation of Rac and LIM kinase. The LIM kinase regulates the actin depolymerizing factor cofilin and thus internalization of *L. monocytogenes* (Bierne et al., 2001). The WAVE complex, N-WASP, Ena/VASP, and the Arp2/3 complex are other key effectors of the Met signaling pathway that are important for cytoskeletal rearrangements necessary for InlB-mediated entry (Bierne et al., 2005). It has been demonstrated that
InlB induces monoubiquitination of Met by the ubiquitin ligase Cbl resulting in endocytosis of Met (Veiga and Cossart, 2005). \textit{L. monocytogenes} exploits the endocytic machinery to invade the cell (Bonazzi and Cossart, 2006; Veiga and Cossart, 2006). Indeed, bacterial internalization was shown to be dependent on major components of the endocytic machinery such as clathrin, dynamin, eps15, Grb2, CIN85, cortactin, and Hrs (Veiga and Cossart, 2005, 2006; Veiga \textit{et al.}, 2007).

Activation of Met by InlB is species-specific (Khelef \textit{et al.}, 2006). InlB activates human and murine Met but not guinea pig and rabbit Met. In mice, InlB contributes slightly to colonization of the liver and spleen. In contrast, a role for InlB in \textit{L. monocytogenes} virulence could not be detected in guinea pigs and rabbits (Khelef \textit{et al.}, 2006).

### 4.2.3. SrtA and SrtB

Surface proteins displaying a C-terminal LPXTG motif are covalently linked to the bacterial cell wall peptidoglycan by sortases. Analysis of \textit{L. monocytogenes} genome sequence revealed the presence of two genes encoding sortases, \textit{srtA} and \textit{srtB} (Bierne \textit{et al.}, 2002). SrtA anchors InlA and several other LPXTG proteins to the peptidoglycane (Bierne \textit{et al.}, 2002; Garandeau \textit{et al.}, 2002; Pucciarelli \textit{et al.}, 2005). Consequently, the sortase A is necessary for efficient entry into epithelial cells (Bierne \textit{et al.}, 2002; Garandeau \textit{et al.}, 2002). Interestingly, it has been shown that in contrast to deletion of \textit{inlA}, inactivation of \textit{srtA} leads to impaired colonization of the liver and spleen of mice after intragastric inoculation (Bierne \textit{et al.}, 2002). Thus, the sortase A could be required for the anchoring of additional LPXTG proteins involved in virulence.

In \textit{L. monocytogenes}, SrtB anchors a small group of proteins and may recognize two different sorting motifs, NXZTN and NPKXZ (Pucciarelli \textit{et al.}, 2005). Inactivation of \textit{L. monocytogenes} SrtB does not affect virulence in mice after intravenous inoculation (Bierne \textit{et al.}, 2004). One of SrtB substrate is SvpA (Bierne \textit{et al.}, 2004), a surface protein first reported to be involved in bacterial escape from the phagosome of macrophages and in virulence (Borezee \textit{et al.}, 2001). It was later shown that the \textit{svpA-srtB} locus does not contribute to virulence in mice after intravenous inoculation, but is required for efficient colonization of the liver, spleen, and intestine of mice infected by the oral route (Newton \textit{et al.}, 2005).

### 4.2.4. Auto

The gene \textit{aut} was identified by a comparative genomic approach (Cabanes \textit{et al.}, 2002, 2004; Glaser \textit{et al.}, 2001). It is absent from the genome of the nonpathogenic species \textit{L. innocua}. It encodes Auto, a surface protein of 572 amino acids. The N-terminus of the protein contains a signal sequence and
an autolysin domain. The C-terminus displays a cell wall attachment domain composed of four GW modules. Inactivation of Auto decreases bacterial entry into cells. However, expression of the autolysin in *L. innocua* does not confer invasivity. Thus, Auto is necessary but not sufficient for entry. The decreased invasive potential of the *aut* deletion mutant correlates with its attenuation *in vivo*. Indeed, Auto is required for *L. monocytogenes* virulence in mice infected intravenously and in guinea pigs after intragastric inoculation (Cabanes *et al.*, 2004). The precise function of Auto remains to be determined. The autolytic activity of the protein could possibly play a role in pathogenicity, for example, by controlling the composition and structure of the bacterial surface during the infectious process.

### 4.2.5. Vip

The gene encoding the surface protein Vip was also identified by comparative genomics of *Listeria* species (Cabanes *et al.*, 2002, 2005; Glaser *et al.*, 2001). PrfA regulates the expression of the gene *vip*, which is absent from the genome of *L. innocua* (Cabanes *et al.*, 2005). The Vip protein contains a C-terminal LPXTG motif and is anchored to the peptidoglycane by the sortase A (Cabanes *et al.*, 2005). Vip is required for invasion of several cell lines and contributes to virulence in mice infected intravenously. In contrast to InlA, it is required for virulence in mice after intragastric inoculation independently of the expression of human E-cadherin at the intestinal level. It is also an important determinant of virulence in the guinea pig. The endoplasmic reticulum resident chaperone Gp96 has been identified as a ligand of Vip (Cabanes *et al.*, 2005). Recently, the creation of a macrophage-specific gp96-deficient mouse allowed to establish that Gp96 is an important chaperone for all TLRs that have been tested (Yang *et al.*, 2007). Interestingly, these gp96-deficient mice were highly susceptible to listeriosis. In wild-type mice, interaction of Vip with Gp96 could possibly interfere with TLRs trafficking resulting in the control of the innate immune response by *L. monocytogenes*.

### 4.2.6. LpeA

The *lpeA* gene encoding a 35-kDa lipoprotein was identified by analysis of *L. monocytogenes* genome sequence (Glaser *et al.*, 2001; Reglier-Poupet *et al.*, 2003b). The LpeA (for lipoprotein promoting entry) protein is homologous to PsaA, a lipoprotein involved in *Streptococcus pneumoniae* adherence to cells. LpeA is not involved in adherence but is required for entry of *L. monocytogenes* into nonprofessional phagocytic cells. However, the impaired invasion of an *lpeA* mutant is not correlated with a decrease in virulence in mice (Reglier-Poupet *et al.*, 2003b).
4.3. Vacuolar escape, intracellular survival and multiplication

4.3.1. Listeriolysin O

Listeriolysin O (LLO) is one of the major virulence determinants of *L. monocytogenes* (Kayal and Charbit, 2006; Schnupf and Portnoy, 2007; Vazquez-Boland et al., 2001b). The *hly* gene encoding LLO was the first virulence gene identified in *Listeria*. Identification was based on transposon mutagenesis. Characterization of the *hly* genomic locus led to identification of the *L. monocytogenes* main virulence gene cluster composed of *prfA*, *plcA*, *hly*, *mpl*, *actA*, *plcB*, and *orfX*. LLO is a secreted protein that belongs to the cholesterol-dependent cytolysin (CDC) toxin family. It is responsible for bacterial escape from primary and secondary vacuoles (Gedde et al., 2000; Portnoy et al., 1988). *L. monocytogenes* mutants lacking LLO fail to reach the cytoplasm and are nonvirulent (Cossart et al., 1989; Gaillard et al., 1986, 1987; Kathariou et al., 1987; Portnoy et al., 1988). The activity of LLO is optimal at the acidic pH of the phagosome. It is less active at the neutral pH of the cytoplasm, preventing excessive cell damage. LLO binds to the cell plasma membrane as monomers that oligomerize into large complexes that penetrate the membrane and contribute to pore formation. As other CDCs, LLO is a potent signaling protein that can activate important signaling pathways such as NF-κB (Kayal et al., 1999), MAP kinase (Tang et al., 1996), and protein kinase C (Wadsworth and Goldfine, 2002) and induce proinflammatory cytokine secretion (Kayal et al., 1999). Interestingly, LLO is also required for *L. monocytogenes* entry into cells (Dramsi and Cossart, 2003). The specific functions of LLO in the signaling and entry processes remain to be elucidated.

4.3.2. Phospholipases

*L. monocytogenes* secretes two phospholipases C (PLC), PlcA and PlcB, involved in the bacterial escape from the vacuoles (Goldfine et al., 1998). PlcA is a secreted phosphatidylinositol-specific PLC (PI-PLC) encoded by the *plcA* gene (Leimeister-Wachter et al., 1991; Mengaud et al., 1991). PlcB is a secreted phosphatidylcholine PLC (PC-PLC) of broad substrate range encoded by the *plcB* gene (Geoffroy et al., 1991; Vazquez-Boland et al., 1992). PlcB is expressed as a proenzyme. The zinc metalloprotease encoded by the gene *mpl* is required for maturation of PlcB (Domann et al., 1991; Ravenneau et al., 1992). The two phospholipases act in synergy with LLO to lyse primary and secondary vacuoles allowing *L. monocytogenes* to escape into the cytoplasm (Camilli et al., 1993; Grundling et al., 2003; Smith et al., 1995). PlcB can also promote lysis of the primary vacuole in the absence of LLO (Grundling et al., 2003; Marquis et al., 1995). Both phospholipases are required for virulence in mice (Camilli et al., 1991, 1993; Ravenneau et al., 1992; Schluter et al., 1998; Smith et al., 1995).
Recently, it has been demonstrated that *L. monocytogenes* phospholipases are necessary for evasion of autophagy (Birmingham et al., 2007; Py et al., 2007). Cellular invasion by *L. monocytogenes* first induces autophagy, a host degradative pathway important for both cell physiology and innate immunity. Expression of LLO is necessary for the induction of the autophagic response at the early time points after infection, suggesting a role for permeabilization of the vacuole in the induction of the degradative pathway. The expression PlcA and PlcB is then required for *L. monocytogenes* escape from autophagic degradation in nonprofessional phagocytic cells and macrophages (Birmingham et al., 2007; Py et al., 2007). The phospholipases may prevent autophagic killing by mediating escape from the double-membrane autophagosome or by inhibiting recognition of the target of the degradative pathway.

4.3.3. Lsp

The signal peptidase II Lsp is responsible for the maturation of lipoproteins in *L. monocytogenes* (Desvaux and Hebraud, 2006; Reglier-Poupet et al., 2003a). A deletion mutant of the *lsp* gene fails to process lipoproteins and has a reduced virulence. Interestingly, the expression of *lsp* is strongly induced in the phagosome of infected macrophages. This induction correlates with the important role of Lsp, and thus lipoprotein maturation, in *L. monocytogenes* escape from the phagosome (Reglier-Poupet et al., 2003a).

4.3.4. SipX and SipZ

*L. monocytogenes* genome contains three contiguous type I signal peptidase genes, *sipX*, *sipY*, and *sipZ*, for cleavage of signal peptides proteins exported and secreted by the general secretory pathway (Bonnemain et al., 2004; Desvaux and Hebraud, 2006). The expression of the three genes is induced in the phagosome of infected cells (Raynaud and Charbit, 2005). The signal peptidases SipX and SipZ are required for full virulence (Bonnemain et al., 2004). In contrast, inactivation of SipY did not impaired *L. monocytogenes* virulence. In addition, SipZ is required for efficient secretion of LLO and PC-PLC. Consequently, inactivation of SipZ restricts bacterial intracellular multiplication (Bonnemain et al., 2004).

4.3.5. Hpt

Once free in the cytoplasm, *L. monocytogenes* expresses specific determinants to acquire nutrients necessary for intracellular multiplication. Uptake of glucose-1-phosphate, a source of carbon and energy available in the cytosol, depends on the PrfA-regulated hexose phosphate transporter Hpt (Chico-Calero et al., 2002). Interestingly, Hpt is a structural and functional homologue of the eukaryotic glucose-6-phosphate translocase required for transport of glucose-6-phosphate from the cytosol into the endoplasmic
reticulum. Hpt has been shown to be required for intracellular replication of *L. monocytogenes* and for virulence in mice (Chico-Calero *et al.*, 2002).

### 4.3.6. LplA1

*L. monocytogenes* is a lipoate auxotroph. In order to scavenge this important cofactor, bacteria produce lipoate ligases to lipoylate specific metabolic enzymes. Analysis of *L. monocytogenes* genome sequence reveals two genes encoding putative lipoate ligases, *lplA1* and *lplA2* (Keeney *et al.*, 2007). However, only *lplA1* is required for intracellular replication and virulence (Keeney *et al.*, 2007; O’Riordan *et al.*, 2003). LplA1 is critical for utilization of host lipoyl peptides as a source of lipoate by *L. monocytogenes*.

### 4.3.7. Fri

*L. monocytogenes* genome encodes a single ferritin, Fri, which is involved in iron storage. Expression of the *fri* gene is controlled by the hydrogen peroxide regulator PerR and sigma B (Olsen *et al.*, 2005). The ferritin is required for protection against reactive oxygen species and contributes to *L. monocytogenes* survival and replication in macrophages and nonprofessional phagocytic cells (Dussurget *et al.*, 2005; Mohamed *et al.*, 2006; Olsen *et al.*, 2005). The impaired survival of a *fri* deletion mutant in macrophages correlates with decreased virulence of the mutant in mice (Dussurget *et al.*, 2005; Mohamed *et al.*, 2006; Olsen *et al.*, 2005). The capacity to prevent excessive production of reactive oxygen species and control the level of iron is an important component of *L. monocytogenes* intracellular survival strategy.

### 4.3.8. HupC

*L. monocytogenes* does not secrete siderophores but can use siderophores from other microorganisms or transferrin, hemin, and hemoglobin to obtain iron (Jin *et al.*, 2006; Newton *et al.*, 2005; Simon *et al.*, 1995). The permease HupC is an ABC transporter required for hemin and hemoglobin uptake (Jin *et al.*, 2006; Newton *et al.*, 2005). The LD50 of a mutant *L. monocytogenes* lacking *hupC* was strongly increased in Swiss mice infected intravenously, suggesting that acquisition of iron from blood or other infected sites facilitates *L. monocytogenes* host colonization.

### 4.3.9. MnSOD

SOD plays an important role in protection against oxidative stress and has been shown to contribute to the pathogenic potential of many bacterial species. *L. monocytogenes* produces a single MnSOD encoded by the gene *sod* (Archambaud *et al.*, 2006; Brehm *et al.*, 1992; Glaser *et al.*, 2001). A *sod* deletion mutant is impaired in survival within macrophages and in virulence in mice (Archambaud *et al.*, 2006). Cytoplasmic MnSOD is phosphorylated on serine and threonine residues and can be dephosphorylated by the serine/threonine phosphatase Stp resulting in an increased SOD activity.
4.3.10. RelA
The relA gene encodes a (p)ppGpp synthetase. An L. monocytogenes relA transposon insertion mutant was unable to accumulate (p)ppGpp in response to amino acid starvation (Taylor et al., 2002). The virulence of the mutant was strongly attenuated in mice, indicating an essential role of the stringent response in the survival and multiplication of L. monocytogenes in the host. Recently, RelA has been shown to be important for bacterial growth in macrophages and nonprofessional phagocytic cells, suggesting that the ability of L. monocytogenes to mount a stringent response is required for efficient intracellular multiplication (Bennett et al., 2007).

4.3.11. Lgt
The lipoprotein diacylglycerol transferase Lgt catalyzes transfer of an N-acyl diglyceride group from a glycerophospholipid to the sulfhydryl moiety of a cysteine residue conserved in the signal peptides of lipoprotein precursors. The product of the reaction is then cleaved by the signal peptidase Lsp. Deletion of lgt impairs intracellular growth of L. monocytogenes (Baumgartner et al., 2007), confirming the importance of lipoprotein processing for pathogenicity (Reglier-Poupet et al., 2003a).

4.4. Cell–cell spread
4.4.1. ActA
After synthesis of the determinants responsible for entry, intracellular survival, lysis of the vacuole, and cytotoxic replication, L. monocytogenes induces polymerization of actin filaments to move in the cytoplasm and to spread from cell to cell (Mounier et al., 1990; Theriot et al., 1992; Tilney and Portnoy, 1989; Tilney et al., 1990). The surface protein ActA is the only bacterial determinant necessary for actin-based motility of L. monocytogenes (Fig. 1.2) (Domann et al., 1992; Kocks et al., 1992). Indeed, L. innocua expressing ActA and latex beads coated with ActA acquire the capacity to polymerize actin and move (Cameron et al., 1999; Kocks et al., 1995). ActA is one of the major virulence determinants of L. monocytogenes (Domann et al., 1992). ActA is a protein of 639 amino acids containing an N-terminal signal sequence and a C-terminal transmembrane domain (Domann et al.,
The central part of the protein presents a domain composed of four proline-rich repeats that binds proteins of the Ena/VASP family, which modulate speed and directionality of bacterial movement (Auerbuch et al., 2003; Chakraborty et al., 1995; Geese et al., 2002; Lasa et al., 1995; Laurent et al., 1999; Niebuhr et al., 1997). The N-terminal region of ActA is sufficient to induce motility (Lasa et al., 1997). It binds and activates the Arp2/3 complex inducing actin polymerization, mimicking proteins of the WASP family (Boujemaa-Paterski et al., 2001; Skoble et al., 2000, 2001). Actin tails induced by L. monocytogenes are composed of branched filaments similar to those of Shigella flexneri, in contrast to Rickettsia conorii actin tails which contain long and unbranched filaments (Gouin et al., 1999, 2004, 2005).

ActA is also involved in cell attachment and entry by recognition of heparan sulfate (Alvarez-Dominguez et al., 1997). Inactivation of ActA impairs L. monocytogenes invasion in macrophages and epithelial cells (Alvarez-Dominguez et al., 1997; Suarez et al., 2001). In addition, expression of ActA in L. innocua is sufficient to confer the capacity to enter epithelial cells (Suarez et al., 2001).

A third role has been assigned to ActA in preventing bacterial autophagy in the cytosol of macrophages (Birmingham et al., 2007; Rich et al., 2003). Some L. monocytogenes are targeted by autophagy during early stages of infection by an LLO-dependent process. ActA expression is sufficient to promote autophagy evasion in the cytosol at later stages of infection (Birmingham et al., 2007). ActA could possibly lead to escape from autophagy by actin-based movement or by actin masking of the bacteria, inhibiting recognition of autophagy targets.
4.4.2. **SecA2**
The auxiliary SecA paralogue protein SecA2 was identified by analysis of spontaneous rough variants of *L. monocytogenes*, which grew in chains (Lenz and Portnoy, 2002). In contrast to SecA, SecA2 is not essential for cell viability. SecA2 is required for virulence in mice and cell–cell spread in cultured cells (Lenz and Portnoy, 2002; Lenz et al., 2003). Using a proteomic approach, 17 SecA2-dependent secreted and surface proteins were identified including the autolysin p60 and the N-acetylmuramidase NamA (Lenz et al., 2003). These two peptidoglycane hydrolases and other SecA2 targets, such FbpA (Dramsi et al., 2004) and MnSOD (Archambaud et al., 2006), are important determinants of the infectious process. Thus, SecA2 could have evolved in part to mediate secretion of a subset of proteins contributing to virulence.

## 5. IMMUNOMODULATION AND PERSISTENCE

### 5.1. Evasion and manipulation of host immune response

#### 5.1.1. PgdA
Bacterial cell wall peptidoglycan is the pathogen-associated molecular pattern detected by the nucleotide-binding oligomerization domain (NOD) protein family of pattern-recognition receptors, resulting in activation of the NF-κB pathway (Chamaillard et al., 2003; Girardin et al., 2003a,b, Inohara et al., 2003). Analysis of *L. monocytogenes* peptidoglycan revealed deacetylation of N-acetylglucosamine residues (Boneca et al., 2007; Kamisango et al., 1982). *L. monocytogenes* genome contains a single peptidoglycane N-deacetylase gene, *pgdA* (Boneca et al., 2007; Glaser et al., 2001). Inactivation of *pgdA* dramatically increases *L. monocytogenes* sensitivity to lysozyme *in vitro* and strongly attenuates virulence in mice infected intravenously and in transgenic mice expressing human E-cadherin after intragastric inoculation (Boneca et al., 2007). PgdA is required for survival within macrophage vacuoles (Fig. 1.3) and prevents proinflammatory cytokine and interferon-β secretion (Boneca et al., 2007). Thus, peptidoglycan N-deacetylation is critical for evasion of host innate defenses.

#### 5.1.2. p60
The autolysin p60, also known as the invasion–associated protein Iap or the cell wall hydrolase A CwhA, is a 60-kDa protein secreted by the SecA2 pathway. This peptidoglycan hydrolase promotes *L. monocytogenes* infection *in vivo* (Faith et al., 2007; Lenz et al., 2003). The mechanism of virulence attenuation of p60-deficient mutants is not completely understood. Recently, the reduced capacity of a p60 mutant to cause systemic infection...
of mice after intragastric inoculation was correlated to a diminished ability to enter and multiply within epithelial cells (Faith et al., 2007). Interestingly, p60 has also been shown to indirectly increase NK cell activation and interferon-γ production (Humann et al., 2007). It was suggested that p60 could promote early bacterial multiplication by subversion of interferon-γ-mediated immune responses and manipulation of deleterious and protective effects of interferon-γ production. The bacterial components that are released by the catalytic activity of p60 and directly modulate host innate response remain to be identified.

5.1.3. LLO

*L. monocytogenes* infection leads to modulation of expression of host genes. Posttranslational modifications of histones play an essential role in chromatin remodeling and gene expression regulation. It has been shown that infection of human endothelial cells by *L. monocytogenes* induces a p38 MAPK and MEK1-dependent acetylation of histone H4 and phosphorylation and acetylation of histone H3 globally as well as specifically at the promoter of IL8 (Schmeck et al., 2005). LLO is required for upregulation of adhesion molecules and chemokines in endothelial cells infected by *L. monocytogenes* (Kayal et al., 1999). Recently, LLO was shown to be critical for dephosphorylation of histone H3 and deacetylation of histone H4 during early phase of infection (Hamon et al., 2007). Indeed, decreased LLO-mediated histone modifications were associated to modulation of host cell gene expression (Hamon et al., 2007). Interestingly, transcription of the chemokine gene *cxcl2* and of other specific immunity genes was decreased, suggesting that LLO genetic reprogramming of the host cell could be an additional mechanism by which *L. monocytogenes* manipulate the host immune response.
5.1.4. MprF

*L. monocytogenes* multiple peptide resistance factor MprF is a membrane protein of 98 kDa regulated by the response regulator VirR (Mandin et al., 2005; Thedieck et al., 2006). MprF is required for synthesis of lysylphosphatidylglycerol and for lysinylation of diphosphatidylglycerol, two-membrane phospholipids (Thedieck et al., 2006). Inactivation of MprF results in a decreased invasivity in both epithelial cells and macrophages and in attenuation of the virulence in mice. MprF is critical for resistance to cationic antimicrobial peptides and could be another mechanism of *L. monocytogenes* to escape host innate immune response.

5.2. Persistence

*L. monocytogenes* is a common transient colonizer of the human gastrointestinal tract that does not cause invasive disease unless a combination of host susceptibility factors, bacterial virulence determinants, and a high infective dose is met. Asymptomatic fecal carriage in healthy individuals has a prevalence of 2–10% (Schlech, 2000). The mechanisms used by *L. monocytogenes* to persist in the host are not fully understood.

*L. monocytogenes* infection of the gallbladder has been documented in humans (Allerberger et al., 1989; Gluck et al., 2002; Gordon and Singer, 1986; Loupa et al., 2007). In addition, *L. monocytogenes* was isolated from liver, bile, and feces of mice inoculated subcutaneously, suggesting that bacteria reached the intestine by biliary excretion (Briones et al., 1992). *L. monocytogenes* can replicate extracellularly in the gallbladder of mice after oral or intravenous inoculation (Hardy et al., 2004). Bacteria growing in the lumen of the gallbladder can transit through the bile duct into the intestine as soon as 5 min after induction of gallbladder contraction by food or cholecystokinin (Hardy et al., 2006). Bacteria then move through the intestinal lumen, are excreted in the environment, and possibly reinfect mice. *L. monocytogenes* strains causing human disease express a BSH conferring resistance to bile antimicrobial activity and the capacity to colonize the gastrointestinal tract (Dussurget et al., 2002). *L. monocytogenes* is particularly well equipped to survive in presence of bile as several other important genetic loci involved in bile resistance have been identified (Begley et al., 2002, 2003, 2005; Sleator et al., 2005). Thus, gallbladder could represent a niche where *L. monocytogenes* grows in the absence of commensal competitors and specific immune response. Dissemination of *L. monocytogenes* from the gallbladder to the intestine and the environment could play an important role in transient or chronic shedding and in transmission.
6. Virulence Determinants of Unknown Function

6.1. InlC

InlC (also designated internalin-related protein A, IrpA) is a secreted protein of 297 amino acids containing a central region composed of 6 LRRs followed by a C-terminal Ig-like domain (Domann et al., 1997; Engelbrecht et al., 1996; Ooi et al., 2006). The *inlC* gene, which is absent from the genome of *L. innocua*, is transcribed by PrfA-dependent and -independent mechanisms (Domann et al., 1997; Luo et al., 2004). InlC contributes to *L. monocytogenes* virulence in mice (Domann et al., 1997; Engelbrecht et al., 1996). The expression of *inlC* is strongly induced in the cytoplasm of infected macrophages (Engelbrecht et al., 1996). However, deletion of *inlC* does not affect invasion, intracellular survival, or cell spread (Domann et al., 1997; Engelbrecht et al., 1996; Greiffenberg et al., 1998). The function and binding partners of InlC have yet to be discovered.

6.2. InlGHE

A gene cluster encoding the three internalins InlG, InlH, and InlE has been identified in some *L. monocytogenes* strains (Raffelsbauer et al., 1998). An in-frame deletion of the *inlGHE* operon had no effect on cellular invasion and its function remains unknown. However, the mutant showed reduced colonization of the spleen and liver after infection of mice by the oral route (Raffelsbauer et al., 1998). A specific role for InlH in virulence was later demonstrated in mice infected intravenously (Schubert et al., 2001).

6.3. InlJ

Another internalin encoding gene, *inlj*, was identified by analyzing *L. monocytogenes* genome sequence (Cabanes et al., 2002; Glaser et al., 2001; Sabet et al., 2005). InlJ is required for full virulence of *L. monocytogenes* in mice infected intravenously and after intragastric inoculation in transgenic mice expressing the human E-cadherin at the level of the intestine (Sabet et al., 2005). However, inactivation of *inlj* does not affect *L. monocytogenes* capacity to infect cells. The function of this internalin remains to be determined.

7. Conclusion

The advent of comparative genomics and transcriptomic technologies allowing analysis of host cell and bacterial gene expression during the infectious cycle coupled to the development of new animal models of
infection have greatly improved our knowledge of \textit{L. monocytogenes} pathogenesis. Here, we have highlighted some of the important bacterial determinants that have been involved in the infectious process. However, our understanding of listeriosis is still far from complete. As more virulence determinants are identified, determination of their specific function, their host partners, and where and when they are expressed during the infectious process will become the next challenge. Identification of the key components of host immune response involved in listeriosis and how they can be manipulated by \textit{L. monocytogenes} should benefit from the recent advances in the field of innate immunity. Dynamic gene profiling \textit{in vivo}, noninvasive imaging in relevant animal models (Fig. 1.4), and real-time imaging in living cells will surely help to address the complexity of \textit{L. monocytogenes} interactions with the host and bring us a step closer to a comprehensive understanding of the disease.

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