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# 1        **Listeriosis, a model infection to study host-pathogen interactions *in vivo***

2  
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## 16 17       **Highlights**

- 18       • Humanized animal models allow deciphering *L. monocytogenes* interactions with host  
19       tissues.
- 20       • *L. monocytogenes* adapts to challenging environments inside and outside the host.
- 21       • Sequence-tagged *L. monocytogenes* are instrumental to study infection within-host  
22       dynamics.
- 23       • *L. monocytogenes* evades immune responses, favoring its systemic dissemination.

## 24 25       **Key future questions**

- 26       - What are the mechanism(s) of *L. monocytogenes* immune escape at the mucosal  
27       level?
- 28       - What are the respective roles of microbiota, host and *Listeria* factors involved in *L.*  
29       *monocytogenes* fecal carriage and shedding in the environment?
- 30       - What are the mechanism(s) underlying *L. monocytogenes* hypervirulence?

31  
32

33 **Abstract**

34 *Listeria monocytogenes* (*Lm*) is a foodborne pathogen and the etiological agent of listeriosis.  
35 This facultative intracellular Gram-positive bacterium has the ability to colonize the intestinal  
36 lumen, cross the intestinal, blood-brain and placental barriers, leading to bacteremia,  
37 neurolisteriosis and maternal-fetal listeriosis. *Lm* is a model microorganism for the study of the  
38 interplay between a pathogenic microbe, host tissues and microbiota *in vivo*. Here we review  
39 how animal models permissive to *Lm*-host interactions allow deciphering some of the key  
40 steps of the infectious process, from the intestinal lumen to the crossing of host barriers and  
41 dissemination within the host. We also highlight recent investigations using tagged *Lm* and  
42 clinically relevant strains that have shed light on within-host dynamics and the purifying  
43 selection of *Lm* virulence factors. Studying *Lm* infection *in vivo* is a way forward to explore  
44 host biology and unveil the mechanisms that have selected its capacity to closely associate  
45 with its vertebrate hosts.

## 46 **Introduction**

47 *Listeria monocytogenes* (*Lm*) is a Gram-positive bacterium and the etiological agent of  
48 listeriosis, one of the deadliest foodborne infections. Listeriosis manifests as bacteremia and  
49 neurolisteriosis in elderly and immunocompromised patients, with a high fatality rate of 20 to  
50 30% even with appropriate antibiotic treatment, and maternal-fetal infection in pregnant  
51 women [1]. *Lm* is a highly diverse species that can be classified into clonal complexes (CCs)  
52 of uneven virulence [2,3] (reviewed in [4]). The CCs most prevalent in food samples and under-  
53 represented in clinical cases tend to be hypovirulent compared to reference strains, invading  
54 less efficiently host tissues in a mouse model of infection, while they form more biofilm on  
55 food-processing environments [2,5]. In contrast, CCs over-represented in clinical cases tend  
56 to be hypervirulent compared to reference strains, colonize better the gut lumen, invade more  
57 efficiently host tissues and lead to higher fecal shedding [2,5,6].

58

59 *Lm* is widespread in the environment where it can thrive as a saprophyte, and is able to infect  
60 a wide range of hosts including vertebrates, from birds to mammals [7]. Importantly, *Lm* is  
61 frequently isolated from domestic ruminants, in which it can lead to rhombencephalitis and  
62 abortion, and be a source of contamination of dairy products [5,8-10]. Even though *Lm* host  
63 range is wide, murine laboratory animals are not permissive to orally-acquired listeriosis, due  
64 to the species specificities of *Lm* interactions with mammalian cells. Indeed, the *Lm* surface  
65 protein internalin (InIA), which mediates *Lm* internalization in epithelial cells [11], interacts with  
66 its receptor E-cadherin (Ecad) [12] in a species-specific manner that relies on the nature of its  
67 16<sup>th</sup> amino-acid [13]. It is a proline in permissive species and a glutamic acid in non-permissive  
68 species [13]. Mouse and rat Ecad exhibit a glutamic in position 16 and are therefore not able  
69 to serve as a receptor for InIA, in contrast to human, guinea pig, rabbit and gerbil [13,14]. InIB,  
70 another virulence factor of *Lm*, interacts with c-Met, the hepatocyte growth factor receptor [15],  
71 in human, gerbils and mice but not guinea pigs and rabbits [16]. Ideally, human listeriosis  
72 should therefore be studied in animals that are naturally permissive to both InIA and InIB, such  
73 as ruminants, gerbils, or non-human primates [14,17,18]. However, ethical considerations and  
74 the relative unavailability of molecular tools limits the use of these models. To circumvent  
75 these limitations, two genetically modified mouse models have been generated: iFABP-hEcad  
76 transgenic mice, which express human Ecad under the control of the enterocyte specific  
77 promoter iFABP [19], and the KIE16P knock-in mouse line in which mouse Ecad is specifically  
78 modified and harbors a proline at position 16 in place of a glutamic acid [14]. A "murinized"  
79 *Lm* expressing a modified version of InIA (InIA<sup>m</sup>) so that it interacts with mouse Ecad has also  
80 been generated [20] to overcome InIA-Ecad species specificity. However, InIA<sup>m</sup> happens to  
81 interact with both mouse E-cad and N-cadherin, which modifies *Lm* cell tropism at the intestinal  
82 level, leading to gut inflammation that delays and decreases *Lm* systemic dissemination [21].

83 Upon ingestion of contaminated food, *Lm* colonizes the gut lumen and can cross the intestinal  
84 barrier. *Lm* has the ability to spread systemically to the spleen and liver, and breach the blood-  
85 brain and placental barriers, leading to neurolisteriosis and maternal-fetal listeriosis,  
86 respectively [22]. Recent studies have shown that *Lm* is shed back from infected tissues to  
87 the gut lumen, in particular via the bile duct, favoring its release in the feces and the  
88 environment [23,24]. Investigating how *Lm* crosses host barriers, amplifies in host tissues and  
89 is shed back to the environment is critical to understand the pathophysiology of listeriosis and  
90 the forces that have shaped its genome and selected its virulence factors.

91

## 92 ***Lm* adaptation to the host environment**

93 ***Sensing and adapting to the host*** — Once in the intestinal lumen, *Lm* has to adapt to the  
94 physical and chemical conditions of the gut. Sensing its environment is key for *Lm* to switch  
95 from saprophytism to a host-adapted phenotype [25]. The alternative sigma factor B (SigB) is  
96 the major *Lm* transcriptional factor of the general stress response and is activated through the  
97 stressosome, a multi-protein complex linked to the membrane by Prli42, a small protein  
98 essential for stress sensing by the stressosome [26] (reviewed in [27]). SigB has been shown  
99 to be required for *Lm* adaptation to the gut lumen [25]. It activates *bsh* transcription, which  
100 gene product allows bacteria to hydrolyze bile salts [28], and *inlA* and *inlB* transcription [29].  
101 These genes, as well as the major virulence genes *hly* (encoding Listeriolysin O, LLO)  
102 and *actA* from *Listeria* pathogenicity island 1 (LIPI-1), are also under the control of the  
103 transcriptional factor PrfA, which is itself regulated by SigB, and is key to allow *Lm* transition  
104 from an extracellular to an intracellular environment (reviewed in [30]). *prfA* translation is  
105 activated at 37°C via an RNA thermosensor in its 5'UTR [31]. Activation of PrfA also requires  
106 the presence of its co-factor glutathione (GSH) synthesized by the host or the *Lm* glutathione  
107 synthase GshF [32,33]. GSH synthesis by GshF requires sensing the intracellular  
108 environment and host metabolites, as *Lm* is auxotrophic for cysteine, a precursor of GSH. It  
109 has been recently shown that methylglyoxal, a toxic byproduct of metabolism produced by the  
110 host and *Lm*, induces the expression of *gshF* [34]. The Opp *Lm* transporter imports peptides  
111 from the host cell cytosol that provides cysteine for GSH synthesis [35]. Another ABC  
112 transporter, TcyKLMN, is a cystine/cysteine importer also involved in GSH synthesis and  
113 activation of PrfA in infected cells [36]. *Lm* can also adapt to the redox state of its  
114 environment [37]. The transcriptional regulator Rex senses the ratio of reduced and oxidized  
115 nicotinamide adenine dinucleotides (NADH/and NAD<sup>+</sup>). It represses the fermentative  
116 metabolism and allows *Lm* to adapt to an intracellular oxygen-rich environment. Rex is  
117 dispensable for gut lumen colonization but is involved in spleen, liver and gallbladder  
118 infection [38].

119

120 **Interaction with the host microbiota** — The gut microbiota, which is constituted of >100  
121 bacterial species and >10<sup>13</sup> bacterial cells per individual [39], exerts a protective role against  
122 orally acquired pathogens, a process called colonization resistance (reviewed in [40]).  
123 Conditions associated with an immature or disbalanced microbiota (e.g. young age or after  
124 antibiotic treatment) can increase host susceptibility to incoming pathogens such as group B  
125 streptococcus [41], *Enterococcus faecium* [42], *Salmonella* [43] or *Clostridium difficile* [44,45].  
126 The microbiota can act directly through competition for nutrients, the production of  
127 bacteriocins, contact-dependent killing [46-48], or indirectly through stimulation of the host  
128 immune system [49,50].

129 The gut microbiota also plays a protective role against *Lm* infection in mice [23,51]: microbiota  
130 depletion by antibiotics increases susceptibility to *Lm*, but this effect can be rescued by a group  
131 of four *Clostridia* species, potentially by directly inhibiting or killing *Lm* [51]. Depleting the  
132 microbiota with antimicrobials can however also increase host protection against a second  
133 infectious challenge. Indeed, it leads to an increase in tissue resident memory CD8+ T cells,  
134 which protects the host against a second encounter with *Lm* [52]. It has also been shown that  
135 antibiotic-resistant commensal *E. coli* can indirectly protect sensitive *Lm* from anti-bacterial  
136 treatment, by favoring antibiotic degradation in the intestine [53]. The impact of microbiota  
137 composition on listeriosis has also been observed in aged mice, in which age-related dysbiosis  
138 favors *Lm* inflammation and infection [54].

139 Some *Lm* isolates, mostly belonging to Lineage I, also express bacteriocins which are active  
140 on the host microbiota and potentially decrease colonization resistance. Two *Lm* bacteriocins  
141 have been described so far: the bacteriocin LLS (encoded by LIPI-3), synthesized by strains  
142 from hypervirulent CCs [55,56], which induces membrane permeabilization of Gram-positive  
143 bacteria by direct contact [57] and Lmo2776, which targets *Prevotella* species, and is, for  
144 reasons which are not fully understood, associated to a decrease in *Lm* intestinal colonization  
145 [58] (Figure1).

146 *Lm* might also compete with the microbiota when it is shed back into the intestinal lumen from  
147 host tissues after infection. Indeed, recent results show that *Lm*, but not its non-pathogenic  
148 related species can establish asymptomatic carriage in humans and other animals, being  
149 present in up to 10% of asymptomatic human stool samples. This process depends on the  
150 composition of the microbiota: *Lm* abundance in feces positively correlates with the ratio of  
151 abundance of (i) Firmicutes and (ii) Actinobacteria to Bacteroidetes phyla [59].

152 Considering the wide range of interactions between this model microorganism and the host  
153 microbiota, *Lm* stands as a powerful model to further investigate the tripartite interplay  
154 between foodborne pathogens, their host and microbiota.

155

156 **Intestinal barrier crossing**

157 **Transcytosis across goblet cells at the intestinal villus level** — *In vitro* studies have  
158 demonstrated that *Lm* enters epithelial cells upon the interaction of InIA with its receptor E-  
159 cadherin (Ecad) [11]. The critical role of InIA in crossing the intestinal barrier was shown *in*  
160 *vivo* in animal models naturally permissive to InIA-Ecad interaction (guinea pig, gerbils) and in  
161 genetically modified iFabp-hEcad and KIE16P mice [14,19]. However, Ecad forms *adherens*  
162 junctions, which are located below tight junctions on polarized epithelia, and is therefore  
163 inaccessible from the intestinal lumen. This apparent paradox was addressed by whole mount  
164 tissue imaging, which showed that Ecad can be lumenally accessible around mucus-expelling  
165 goblet cells [60]. Upon inoculation of *Lm* into the intestinal lumen, *Lm* specifically associates  
166 with goblet cells and is transferred across these cells within vacuoles and is released in the  
167 *lamina propria*. This translocation is solely dependent on InIA, and is in particular independent  
168 of LLO and ActA, involved respectively in escape from the vacuole and intracytosolic  
169 movement (Figure1). Moreover, the translocation of *Lm*-containing vacuole also requires host  
170 microtubule dynamics and the exocytic machinery [60]. These results collectively indicate that  
171 *Lm* breaches the intestinal epithelium by transcytosis through goblet cells [60]. The molecular  
172 mechanism of *Lm* transcytosis has been studied in intestinal organoids in which *Lm* can be  
173 microinjected. The advantage of intestinal organoids also referred to as “minigut” is that it  
174 allows the differentiation in an *in vitro* context of goblet cells surrounded by classical  
175 enterocytes, and that they are easily genetically amenable [61]. In this experimental setting,  
176 *Lm* translocation across the intestinal epithelium can be imaged in real-time, and has been  
177 shown to last less than 12 minutes [62]. *Lm*-containing vesicle remains associated with the  
178 cell apex when endocytosis is inhibited, while transcytosis towards the cell basal pole requires  
179 functional host microtubules, in line with *in vivo* studies [60,62]. Finally, in organoids  
180 expressing a dominant-negative version of Rab11 (Rab11DN), *Lm* is stuck at the basal pole  
181 of goblet cells, enwrapped with mucus material, and fails to finalize its translocation [62].  
182 These results collectively indicate that *Lm* hijacks the recycling of its receptor Ecad to cross  
183 the intestinal barrier through goblet cells [62] (Figure 1).

184

185 **Crossing of the intestinal barrier via M cells** — The gut-associated lymphoid tissue (GALT)  
186 is distributed along the gastrointestinal tract. It consists of Peyer’s patches (PP) in the small  
187 intestine, cecal and colonic patches, and isolated lymphoid follicles. These immune structures  
188 sample luminal antigens and orchestrate the induction of mucosal immune responses.  
189 Microfold cells (M cells) are specialized epithelial cells that overlay the GALT, which  
190 phagocytic activity allows sampling luminal antigens.

191 *Lm* can cross PP through M cells in InIA-independent manner, and this has been shown to be  
192 sufficient to trigger *Lm* intestinal tissue response to infection [63-65] (Figure 1). An *in vitro*  
193 study has reported that M cell infection by *Lm* may also propagate to neighboring enterocytes

194 [66]. Once translocated *via* M-cells, *Lm* infects CX3CR1<sup>+</sup> myeloid cells in PP, leading to the  
195 expression of IL-12 and IL-23 and subsequent decrease in the number of mature goblet cells.  
196 As a consequence, it locks InIA-dependent entry, while sensitizing the host to colitis [67].  
197 Adaptive immune response to *Lm* at the PP level has been recently shown to be complement-  
198 dependent. Indeed, lysozyme-expressing dendritic cells close to M cells in PPs express the  
199 complement C5a receptor (C5aR), and induce *Lm*-specific CD8<sup>+</sup> T cells response in a C5a-  
200 dependent manner [68].

201 As indicated above, *Lm* expressing a murinized version of InIA (InIA<sup>m</sup>) interacts not only with  
202 mouse Ecad [20], but also with lumenally accessible N-cadherin expressed by villus M cells  
203 [21,64]. This artefactual entry elicits host immune responses at the villus level and epithelial  
204 damage, which are not observed with wild-type *Lm* infection [21]. This indicates a role for villus  
205 M cells in the induction of immune responses, in contrast to goblet cells [21,65]. Comparison  
206 of the mechanisms by which *Lm*-InIA<sup>m</sup>- and wt-*Lm*-dependent host responses are either  
207 triggered or prevented is therefore a way forward to investigate how host responses are  
208 differentially induced by M cells and goblet cells.

209

## 210 **Systemic dissemination**

211 ***Within-host dynamics of Lm infection*** — Once *Lm* has crossed the intestinal barrier, it  
212 spreads to inner organs (Figure 2). The standard method to follow a bacterial infection is to  
213 dissect animals at sequential time points post inoculation and count CFUs, and also to image  
214 bacteria in host tissues [69]. Use of bioluminescent bacteria allows following qualitatively and  
215 over time *Lm* infection within individual animals [14,70,71]. In order to follow and model the  
216 spatiotemporal dynamics of infection within the host, several methods based on tagged clones  
217 have been developed (reviewed in [72]).

218 *Lm* within-host dynamics has been studied based on sequence tag-based analysis of  
219 microbial populations (STAMP), a methodology initially set up to study *Vibrio cholerae*  
220 intestinal phase of infection [73]. This technique makes use of hundreds of individually tagged  
221 bacterial clones, whose numbers and relative proportions are measured over time in various  
222 anatomical locations by high throughput DNA sequencing. This, combined to mathematical  
223 modelling allows evaluating the barrier exerted by each organ (bottleneck effect), by analyzing  
224 the clonality and the number of bacteria from the inoculum that reach the organs (founding  
225 population). It also allows comparing clones retrieved from various host organs and retrace  
226 their dissemination routes along the infectious process.

227 The modelling of *Lm* infection was performed using STAMP with the 10403S reference strain  
228 modified so that it expressed InIA<sup>m</sup>, upon oral and intravenous (iv) inoculation. The founding  
229 populations in liver and spleen were relatively high, but decreased drastically in the  
230 gallbladder, which collects the bile produced by the liver to release it in the duodenum, the



231 proximal part of the intestine. Comparison of the tags between the gallbladder and the gut  
232 upon iv inoculation indicates that the gallbladder is a reservoir fueling *Lm* fecal shedding in  
233 this model [24], a phenomenon which is dependent on both LLO and ActA, two core virulence  
234 genes of *Lm* [3,23]. The observation of the low number of tags and the implication of LLO and  
235 ActA in gallbladder-mediated shedding of *Lm* in the gut suggests that *Lm* release by the biliary  
236 tract is involved in the purifying selection of virulence genes [3,23].

237

238 ***Infection of mesenteric lymph nodes and spleen*** — The mesenteric lymph nodes (MLN)  
239 are lymphoid organs that drain lymph irrigating the intestinal mucosa. As such, they become  
240 rapidly infected upon oral inoculation of *Lm* [19] (Figure 2). A recent study using *Lm* InIA<sup>m</sup>  
241 suggests that migration to and bacterial accumulation into MLN occurs in a dendritic cell- (DC)  
242 dependent manner and triggers a robust CD8<sup>+</sup> T cell response [74]. However, it remains to be  
243 determined where *Lm* mostly disseminates from (intestinal villi vs. PPs vs. cecum vs. colon),  
244 and if the infection route plays a role in host responses.

245 The spleen is the major lymphoid organ filtering the systemic circulation and plays a critical  
246 role in controlling bloodborne infections (reviewed in [75]). The histological structure of the  
247 spleen includes the red and white pulps and marginal zone (MZ), which contain different  
248 populations of immune cells. Upon crossing the intestinal barrier, *Lm* disseminates rapidly  
249 from the bloodstream to the spleen (Figure 2). It is taken up mainly by MZ macrophages.  
250 Depletion of MZ macrophages leads to the spread of *Lm* to the red pulp and exacerbation of  
251 the infection [76]. These cells are therefore crucial for early bacterial control and represent a  
252 critical line of defense against *Lm*, in line with their strategic location in the spleen [77]. MZ  
253 macrophages closely interact with MZ CD8 $\alpha^+$  DCs to initiate *Lm* transfer to T cell zones and  
254 induce effective adaptive immune responses, while the mechanism of *Lm* transmission from  
255 MZ macrophages to DCs remains to be elucidated [77]. In infected phagocytes, *Lm* escape  
256 from the vacuole induces the production of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), which is necessary for  
257 an effective T-cell priming [78]. Splenic DCs can also initiate innate immune responses by  
258 inducing TNF and iNOS to clear bacteria through CCR2-dependent signaling [79].

259 While the spleen plays a major role in inducing protective immunity to *Lm*, splenectomized  
260 mice are actually more resistant to infection and have less bacteria in the liver compared to  
261 non-splenectomized controls [80]. This paradoxical phenotype reflects the fact that *Lm* takes  
262 advantage of the splenic environment to increase its survival and replication and promote  
263 infection. Indeed, *Lm* can grow exponentially in MZ CD8 $\alpha^+$  DCs during the first 18 hours of  
264 infection, and the lack of these cells leads to a rapid bacterial clearance in MZ [81]. This growth  
265 is at least partly due to the release of the regulatory cytokine IL-10 upon *Lm* infection by MZ  
266 B cells in the spleen. IL-10 exerts multiple suppressive effects on myeloid and T cells [82]. In

267 *Lm* infected spleen, IL-10 inhibits iNOS production and favors the bacterial burden in MZ  
268 macrophages, which in turn transfer *Lm* to CD8 $\alpha^+$  DCs, as described above. This leads to two  
269 opposite effects: a high bacterial load in the spleen, but also a strong CD8 T<sup>+</sup> cell response  
270 induced by infected CD8 $\alpha^+$  DCs [83]. In addition, *Lm* can also take advantage of IL-10  
271 produced by regulatory NK cells to promote host susceptibility, by blocking the accumulation  
272 and activation of inflammatory myeloid cells [84] [85].

273

274 **Liver invasion** — In the liver, *Lm* is rapidly taken up by tissue-resident macrophages called  
275 Kupffer cells, leading to their rapid death [86]. This triggers monocyte recruitment and induces  
276 a type-1 antimicrobial inflammatory response that controls the infection. In addition, Kupffer  
277 cell death also induces secretion of IL-33 by hepatocytes, which induce IL-4 expression in  
278 basophils that orchestrates the switch from an inflammatory type-1 to a type-2 response that  
279 dampens inflammation, allowing the replacement of embryo derived Kupffer cells by myeloid-  
280 derived macrophages and return to homeostasis [86].

281 As for the spleen, *Lm* may benefit from the immune response elicited in the liver to promote  
282 infection. Indeed, it has been observed that mice lacking type I interferon receptor are  
283 paradoxically more resistant to *Lm* infection, highlighting its role in promoting *Lm* pathogenesis  
284 [87]. Further studies reviewed in [88] have investigated the mechanisms involved in this  
285 atypical facilitating effect of type I IFN on infection. Recently, it has been shown that type-I IFN  
286 expressed by myeloid cells impairs the degradation of *Lm* virulence factors (ActA and LLO) in  
287 phagosomes, which promotes intracellular bacterial growth and cell-to-cell spread in the liver  
288 [89,90]. It also inhibits proinflammatory cytokine signaling and immune cell recruitment *via* the  
289 IFN-induced transmembrane protein 3 (IFITM3) [90].

290 Liver, as the producer of bile, also fuels the bile duct with *Lm* [23,24,70], even though *Lm*  
291 clinically patent bile infection in human is extremely rare [91].

292

### 293 **Brain and placenta infection**

294 Neurolisteriosis and maternal-fetal listeriosis are the two most severe presentations of human  
295 listeriosis [1,92,93].

296 **Brain infection** — Crossing of the blood-brain barrier is considered as the way by which *Lm*  
297 enters the CNS in human, while retrograde nervous infection is likely to be at play in ruminants  
298 [9]. The blood-brain barrier is actually highly efficient to prevent brain infection [24] and several  
299 mechanisms of neuroinvasion have been proposed, using rodents as animal models. Infection  
300 of monocytes by *Lm* is involved in brain infection with reference strains in mice non-permissive  
301 to InlA-Ecad interaction [94,95]. Extracellular bacteria have also been proposed to cross the  
302 blood-brain barrier, and InlF, expressed by some but not all *Lm* strains, has been reported to

303 be involved in brain infection in mice, by interacting with Vimentin present on the surface of  
304 endothelial cells [96,97] (Figure 2).

305 Seminal studies on *Lm* pathogenesis have been performed with commonly used laboratory  
306 reference strains EGD, 10403S, EGDe or L028. However, strains from the CCs most  
307 associated to neurolisteriosis and maternal-fetal listeriosis (CC1, 4 and 6) are hypervirulent  
308 compared to these reference *Lm*, which are actually very poorly neuroinvasive [2]. In contrast,  
309 hypervirulent strains infect the brain and fetal-placental unit to a high level in humanized mice,  
310 suggesting that factors specific to these strains are involved in neuro- and placental invasion  
311 [2]. A comparative genomic analysis has shown that the LIPI-4 locus coding for a putative  
312 cellobiose-family phosphotransferase system (PTS) is involved in hypervirulence and  
313 neuroinvasiveness of CC4 [2] (Figure2). However, the mechanism by which LIPI-4 mediates  
314 hypervirulence remains unknown and ongoing studies are under way to decipher the  
315 neuroinvasion of *Lm*.

316

317 **Placenta infection** — The maternal-fetal interface is constituted by the syncytiotrophoblast,  
318 a tight syncytial epithelial barrier of fetal origin in direct contact with the maternal blood  
319 (reviewed in [98]). As for the intestine, InIA is involved in the crossing of this barrier by  
320 interacting with Ecad apically accessible at the surface of the syncytiotrophoblast [99].  
321 However, in contrast to the intestine, InIA acts in a conjugated manner with InIB for placental  
322 invasion [14]. Indeed, InIA-dependent entry requires phosphoinositide 3-kinase (PI3-K)  
323 activity, which is induced by InIB-c-Met interaction [64]. Goblet cells targeted by *Lm* exhibit a  
324 detectable basal level of PI3-K activity (PI3K is involved in mucus production), which makes  
325 InIB-c-Met interaction dispensable for intestinal barrier crossing [64]. In contrast, PI3-K activity  
326 is low at the syncytiotrophoblast level, and InIB stimulates, *via* c-Met, PI3-K activation and  
327 therefore allows InIA-dependent entry, leading to placental and fetus invasion [64]. InIP is  
328 another *Lm* factor reported as involved in *Lm* crossing of the placental barrier, through its  
329 interaction with Afadin and is also involved in other steps of listeriosis, including spleen and  
330 liver infection [100,101]. As for the CNS infection, the LIPI-4 locus, which is associated with  
331 CC4 hypervirulence, is also involved in placental invasion [2] (Figure 2).

332

### 333 **Concluding remarks**

334 As illustrated in this review, many steps of listeriosis, from ingestion of contaminated food,  
335 intestinal barrier crossing to brain and placenta infection, have been thoroughly investigated  
336 and deciphered *in vivo*. This allowed to deepen knowledge in infection biology, and also  
337 revealed unexpected properties of host tissues (as previously reviewed in [4,22,102,103]).  
338 However, many questions remain unanswered, for which animal models of listeriosis will be  
339 instrumental, especially regarding the natural history of *Lm* association with its hosts and the

340 forces that have shaped and select its core and accessory genes. *Ex vivo* models such as  
341 organoids will also help to further study the cell biology of the successive steps of *Lm* infection  
342 of mammalian cells.

343 **Figure legends:**

344

345 **Figure 1: Interaction of *Lm* with the intestine**

346 *Lm* competes with the microbiota for survival by producing bacteriocins.

347 *Lm* crosses the intestinal barrier via mucus-producing goblet cells in villi and M cells in Peyer's  
348 patches.

349

350 **Figure 2: *Lm* dissemination within the host**

351 *Lm* crosses the intestinal barrier, spreads systemically and crosses the placental and blood-  
352 brain barriers to induce maternal-fetal listeriosis and neurolisteriosis, respectively. It is  
353 released via the biliary tract back into the intestinal lumen.

354 *Lm* virulence factors are indicated in red, followed by their host receptor in black.

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359

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