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Understanding how *Listeria monocytogenes* targets and crosses host barriers

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ABSTRACT

Human listeriosis is caused by the Gram-positive bacterium *Listeria monocytogenes*. In humans, this pathogen has the ability to cross the intestinal, placental and blood–brain barriers, leading to gastroenteritis, maternofetal infections and meningoencephalitis, respectively. The entry of *L. monocytogenes* into cultured human epithelial cells is mediated by the interaction of an *L. monocytogenes* surface protein, internalin, with its human receptor, E-cadherin. The internalin–E-cadherin interaction is species-specific, and relies on the nature of a single amino-acid in the E-cadherin molecule, which is proline in permissive species such as humans, and glutamic acid in non-permissive species such as the mouse. In a transgenic mouse model that expresses human E-cadherin in enterocytes, internalin allows *L. monocytogenes* to cross the intestinal barrier. Epidemiological evidence also supports a role for internalin in human listeriosis, not only for crossing the intestinal barrier, but also for targeting and crossing the placental and blood–brain barriers. Consistent with these epidemiological data, infection with *L. monocytogenes* of trophoblastic cell lines, primary trophoblast cultures and human placental villous explants demonstrates that bacterial invasion of the syncytiotrophoblast barrier is mediated by the internalin–E-cadherin interaction, leading to histopathological lesions that mimic those seen in the placentas of women with listeriosis. Thus, the internalin–E-cadherin interaction that plays a key role in the crossing of the intestinal barrier in humans is also exploited by *L. monocytogenes* to target and cross the placental barrier. Further investigations are currently focusing on the molecular mechanisms by which *L. monocytogenes* targets and crosses the blood–brain barrier.

Keywords Animal model, cadherin, host barriers, *Listeria monocytogenes*, pathogenicity, review

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INTRODUCTION

Listeria monocytogenes is a Gram-positive food-borne pathogen that is responsible for listeriosis, a human infection with an overall mortality rate of 30%, which ranges from clinically asymptomatic faecal carriage to febrile gastroenteritis, severe mother-to-child infections, and central nervous system infections [1]. The organism has the amazing ability to cross three significant barriers in humans, namely the intestinal barrier, the blood–brain barrier and the fetoplacental barrier. *L. monocytogenes* is a facultative intracellular bacterium that has the unusual capacity to enter, survive and multiply in both phagocytic and non-phagocytic cells [2]. This property, which has been studied in detail in cultured cells, is considered to be central for the pathophysiology of human listeriosis.

Several *L. monocytogenes* factors mediating the key steps in cell infection have been identified. These include: (1) internalin (also called InlA) and InlB (another member of the internalin multigene family, characterised by the presence of leucine-rich repeats), which are responsible for internalisation of *L. monocytogenes* in cul-

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tured non-phagocytic cells; (2) listeriolysin, which acts in concert with two phospholipases (PlcA and PlcB) to allow escape from the phagocytic vacuole; and (3) ActA, which mediates actin-based intracytoplasmic movement and cell–cell spreading (Fig. 1) [2].

In mice, intravenous inoculation of *L. monocytogenes* induces dose-dependent lethality. This infection model has been used successfully for decades as a model for intracellular bacterial infections, and played a key role in the discovery of cell-mediated immunity [3]. In contrast, oral inoculation is a very inefficient way to trigger systemic listeriosis, because *L. monocytogenes* translocation across the intestinal barrier is inefficient and is similar to that of the closely related non-pathogenic species *Listeria innocua*. The few detectable foci of bacterial multiplication are restricted to Peyer's patches, areas that contain M-cells (i.e., cells possessing phagocytic activity). Thus, *L. monocytogenes* is not an enteropathogen for mice. Moreover, in mice, the brainstem and the fetoplacental unit do not appear to be elective targets, as is the case in humans. Following intravenous inoculation of mice, listeriolysin, ActA, PlcA and PlcB act as virulence factors. This is not the case for internalin [2]; indeed, despite the well-established prominent role of internalin in the internalisation process *in vitro*, its role *in vivo* has long remained elusive, with an internalin mutant behaving in the same way as its wild-type parent after intravenous or oral inoculation of mice [4].

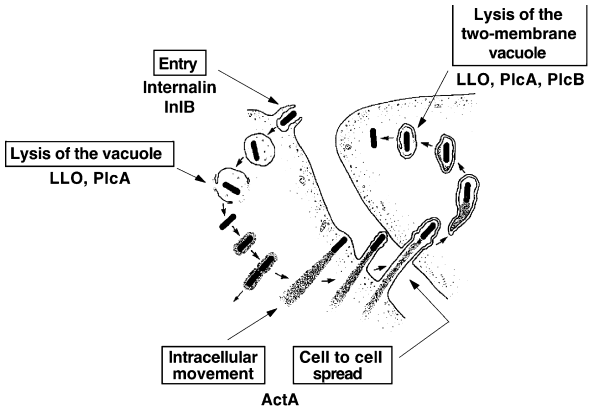

Fig. 1. Schematic representation of the process of cellular infection by *Listeria monocytogenes*. LLO, listeriolysin; PlcA, phospholipase A; PlcB, phospholipase B. Adapted with permission from [2].

SPECIES-SPECIFICITY OF THE INTERNALIN–E-CADHERIN INTERACTION

Shortly after the discovery, via an affinity chromatography approach, that human E-cadherin was the internalin receptor in a human epithelial cell line (Caco-2 cells) [5], it was established that, in contrast to human E-cadherin, mouse E-cadherin was unable to promote entry of *L. monocytogenes* into cells [4]. This specificity was shown to depend on the nature of a single amino-acid, the sixteenth, in the mature E-cadherin peptide chain, which is proline in permissive species (humans, guinea-pigs, ovines, bovines) and glutamic acid in non-permissive species (mice, rats) (Fig. 2a) [4]. Replacement of this glutamic acid with proline leads to a gain in function, thereby establishing the critical role of this residue in the internalin–E-cadherin interaction [4]. Determination of the crystal structures of the functional domain of internalin alone, and in a complex with the extracellular N-terminal domain of human E-cadherin, demonstrated that proline-16 is involved directly in the internalin–E-cadherin interaction and is essential for inter-molecular recognition [6]. This result led to the conclusion that *L. monocytogenes* exhibits a stringent species-specificity towards its host, and that the mouse model was inappropriate for the study of internalin function [4].

ROLE OF THE INTERNALIN–E-CADHERIN INTERACTION IN CROSSING OF THE INTESTINAL BARRIER

In a search for a more appropriate animal species in which to study the putative role of the internalin interaction with E-cadherin *in vivo*, cultured guinea-pig epithelial cells were shown to allow internalin-dependent entry of *L. monocytogenes* and to express E-cadherin with a proline at position 16 (Fig. 2a) [4]. In this small animal model, contrary to observations in mice and rats (whose E-cadherin also has a glutamic acid at position 16, and which are also resistant to oral infection with *Listeria*), *L. monocytogenes* is able to induce a gastroenteritis resembling that observed in humans [7,8]. Moreover, it is able to cross the intestinal barrier and induce a dose- and internalin-dependent lethality following dissemination to the systemic circulation [9].

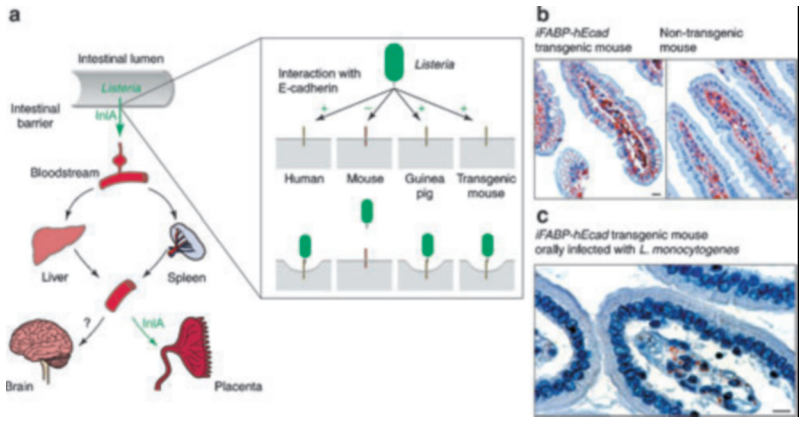

Fig. 2. (a) In-vivo consequences of the species-specific interaction of internalin with E-cadherin. The successive steps of human listeriosis are shown on the left. The internalin–E-cadherin interaction is critical for *L. monocytogenes* entry into enterocytes and syncytiotrophoblasts, and thus for the crossing of the intestinal and placental barriers *in vivo*. The putative role of the internalin–E-cadherin interaction in the crossing of the blood–brain barrier has not yet been tested. (b) Small-intestine sections of an iFABP–hEcad transgenic mouse (left) and a non-transgenic littermate (right), immunolabelled with anti-hEcad HECD1 mouse monoclonal antibody (red). HECD1 recognises human, but not mouse E-cadherin. The background signal in the lamina propria is caused by the reactivity of the secondary goat anti-mouse IgG antibody towards endogenous mouse IgG. A cross-section of a transgenic intestinal epithelial sheet allows detection of the typical honeycomb-like pattern of E-cadherin labelling. Scale bar = 10 μm. (c) *Listeria monocytogenes* multiplication within the lamina propria of a small-intestine villus of an iFABP–hEcad transgenic mouse 48 h after intragastric inoculation. A small-intestine section of an iFABP–hEcad transgenic mouse infected orally with *L. monocytogenes* and immunolabelled with rabbit anti-*L. monocytogenes* R11 polyclonal antibody (red). *L. monocytogenes* cells have crossed the intestinal barrier and replicate in the lamina propria, a phenomenon that is never observed in non-transgenic mice. Scale bar = 10 μm. Adapted with permission from [3].

In order to investigate the role of the internalin–E-cadherin interaction in the ability of *L. monocytogenes* to cross the intestinal barrier, a transgenic mouse model was designed [9]. The human E-cadherin cDNA was placed under the control of the promoter of the intestinal fatty-acid-binding protein (iFABP) gene, which is active exclusively in post-mitotic non-proliferative small intestinal enterocytes (Fig. 2b). In this transgenic model, *L. monocytogenes* targets enterocytes directly by interacting with enterocyte E-cadherin [9]. This interaction leads to *Listeria* internalisation into these cells, and allows subsequent crossing of the intestinal barrier, followed by bacterial multiplication in the small intestine lamina propria, and dissemination to mesenteric lymph nodes, liver and spleen [9]. This is the first transgenic model to reveal the role of a bacterial virulence factor and to demonstrate its critical involvement in a key step of an infection process [3]. The fact that expression of human E-cadherin is restricted to enterocytes has been critical in demonstrating the direct in-vivo targeting of enterocytes by *L. monocytogenes* at the molecular level, and its genuine enteropathogenicity. Furthermore, this transgenic model provides a molecular explanation for the lack of pathogenicity of *L. monocytogenes* in mice following oral infection, and also explains the enteropathogenicity of *L. monocytogenes* in guinea-pigs and, most probably, in humans.

In contrast to non-transgenic mice, transgenic mice expressing human E-cadherin are highly permissive to listeriosis acquired orally, thereby demonstrating an essential role for internalin in the ability of *L. monocytogenes* to cross the intestinal barrier. Thus, a mouse model for listeriosis acquired orally is now available, in which the host response to listeriosis can be studied in depth from its starting point (i.e., the intestinal lumen), using the combined approaches of microbial genetics, transgenesis, gnotobiology and functional genomics coupled to laser capture microdissection, as described previously for studies of the host response to commensal bacteria [10,11]. The availability of the *L. monocytogenes* genome sequence, together with that of its non-pathogenic counterpart *L. innocua*, will probably be very helpful in identifying additional virulence factors in conjunction with this new model [12,13].

However, this system also has its limitations, which were anticipated when the transgenesis strategy was designed [3]. Indeed, because human E-cadherin expression is restricted to enterocytes in the iFABP–hEcad transgenic mice, the role of E-cadherin expressed on cell types other than enterocytes-such as dendritic cells, hepatocytes, microvascular endothelial cells, epithelial cells of the choroid plexus, and cyto trophoblastic cells, which are all putative *L. monocytogenes* targets during human listeriosis-cannot be studied. Transgenic mice that overcome this limitation are now being generated by modifying, at the endogenous mouse E-cadherin locus Cdh1 on mouse chromosome 8, the codon for mouse E-cadherin glutamic acid 16 into a codon for proline. This unique change in murine E-cadherin has been shown to be sufficient to convert mouse E-cadherin into an internalin receptor in transfected cultured cells expressing this modified Glu16Pro mouse E-cadherin [4]. The new transgenic mouse line should permit the study of the tropism of *L. monocytogenes* for the central nervous system and the fetoplacental unit, which is responsible for the lethality of human listeriosis, and further investigations of the role of the internalin–E-cadherin interaction in extra-intestinal tissues.

EPIDEMIOLOGICAL DEMONSTRATION OF THE ROLE OF INTERNALIN IN HUMANS

In order to validate the contribution of internalin in humans, an epidemiological approach has been adopted [14]. Isolates of *L. monocytogenes* exist that express a truncated form of internalin, which is no longer anchored to the surface of the bacteria and is thus non-functional. In-vitro, such isolates enter cultured human intestinal cells very inefficiently and behave as an internalin gene deletion mutant. An epidemiological study determined the respective frequencies of isolates expressing a full-length or a truncated internalin within two sets of isolates, of food and clinical origin, respectively, that were collected prospectively over the course of 1 year by the French National Reference Centre for Listeriosis (Institut Pasteur, Paris) [14]. The working hypothesis was that there would be a higher frequency of isolates expressing a functional internalin among the isolates of clinical origin than among the isolates of food origin, and that this would constitute an epidemiological demonstration of the role of internalin in human listeriosis. This assumption was confirmed without ambiguity. Indeed, of 300 clinical isolates studied, 96% expressed a wild-type full-length internalin, compared with only 65% of the 150 isolates of food origin ($p < 10^{-7}$; OR 12.73; 95% CI 6.27–26.34) [14]. Moreover, all the isolates responsible for fetoplacental infections (61/61), and all but one of those responsible for infections of the central nervous system (55/56), expressed a wild-type full-length internalin. These results not only demonstrated the crucial role of internalin in human listeriosis, but also suggested that it is implicated in crossing of the fetoplacental barrier and the blood–brain barrier. Moreover, all 110 isolates belonging to serovar 4b, the serovar implicated most frequently in human listeriosis, expressed full-length internalin, providing a molecular explanation for the predominance of serovar 4b among clinical isolates. Overall, these epidemiological results supported the usefulness of studies on the expression of internalin as an indication for *L. monocytogenes* virulence in humans [14].

ROLE OF THE INTERNALIN–E-CADHERIN INTERACTION IN CROSSING OF THE PLACENTAL BARRIER

The above epidemiological findings, the species-specificity of the internalin–E-cadherin interaction, and the absence of a transgenic mouse model in which human E-cadherin is expressed in all epithelial lineages, prompted a direct examination of the role of the internalin–E-cadherin interaction in crossing of the maternofetal barrier in humans. This was done by means of immunohistopathological studies of placentas from women with listeriosis, in conjunction with the use of human primary trophoblasts and placental explants [15].

The human maternofetal barrier contains two anatomically distinct components, namely the chorioallantoic placenta and the chorioamnion. The barrier is formed at the placental level by the villous syncytiotrophoblast (Fig. 3). This specialised epithelial lineage is in direct contact with maternal blood circulating through the intervillous space. In a subjacent layer,

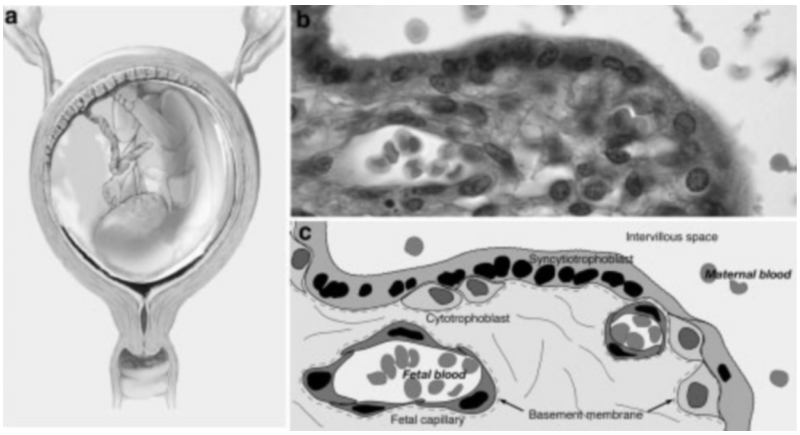

Fig. 3. Anatomy of the maternofetal interface in humans. (a) View of a pregnant human uterus. (b) Expanded view of a small segment of the placental villous tree. A cross-section of a villus with an associated diagrammatic outline of the histology (c) reveals a relatively empty intervillous space surrounding a villus in cross-section. This space is normally filled with maternal blood. The multinucleated syncytiotrophoblast, a true syncytium without lateral cell membranes, covers the villi and an underlying discontinuous layer of cytotrophoblasts in the second half of pregnancy. The subjacent cytotrophoblasts are mitotically active and, through a process of differentiation and fusion, give rise to more syncytium. The two villous trophoblast cell types share a basement membrane that delimits the villous core connective tissue where fetal vessels pass. The placental 'barrier' in the villous tree thus includes the apical microvillous surface membrane of the syncytiotrophoblast, the basal surface membrane of the syncytiotrophoblast, the trophoblastic basement membrane, and the villous core connective tissues.

mononuclear cytotrophoblasts divide, differentiate and fuse to renew overlying multinucleated syncytiotrophoblasts. A basement membrane separates these trophoblastic cells from a connective tissue core that contains fetal capillaries. The amniotic epithelium forms the maternofetal interface in the chorioamnion. The apical surface of this epithelium is exposed to amniotic fluid, whereas its basal surface sits on a basement membrane that overlies the amniotic mesoderm.

Immunohistochemical studies of multiple sections prepared from each placenta revealed blood-borne bacteria in the intervillous spaces. Bacteria were also detected on the surfaces of syncytiotrophoblasts and cytotrophoblasts, in the cytoplasm of syncytiotrophoblasts, and in the villous core adjacent to fetal capillaries. Isolated villi and villous clusters contained foci of bacteria forming abscesses. Bacteria were also detected on and in amniotic epithelial cells, but were not apparent in the connective tissue of the subjacent chorion that overlies the maternal decidua. These findings suggested that *L. monocytogenes* infection of the human fetoplacental unit follows a transplacental route. They also suggested a testable hypothesis, namely that extracellular bacteria present in the maternal blood that bathes placental villi can recognise and bind to a specific surface receptor, leading to penetration of the syncytiotrophoblast layer and subsequent invasion of the fetal vascular compartment in the villous core.

Investigation of the cellular patterns of expression of internalin receptor E-cadherin at the maternofetal interface demonstrated that it is expressed on the basal and apical plasma membranes of syncytiotrophoblasts (Fig. 4a). Quantitative assays of cellular invasion in trophoblastic cell lines (Fig. 4b), primary trophoblast cultures and placental villous explants (Fig. 4c) demonstrated that bacterial entry into syncytiotrophoblasts occurs via the apical membrane in an internalin–E-cadherin-dependent manner. In human placental villous explants, bacterial invasion of the syncytiotrophoblast barrier and underlying villous tissue, and subsequent replication, produces histopathological lesions that mimic those seen in placentas from women with listeriosis (Fig. 4d). Thus, the internalin–E-cadherin interaction, which plays a key role in the crossing of the intestinal barrier, is also exploited by *L. monocytogenes* to target and cross the human placental barrier [15].

CONCLUSIONS

The studies described above show that *L. monocytogenes* uses a common strategy to recognise

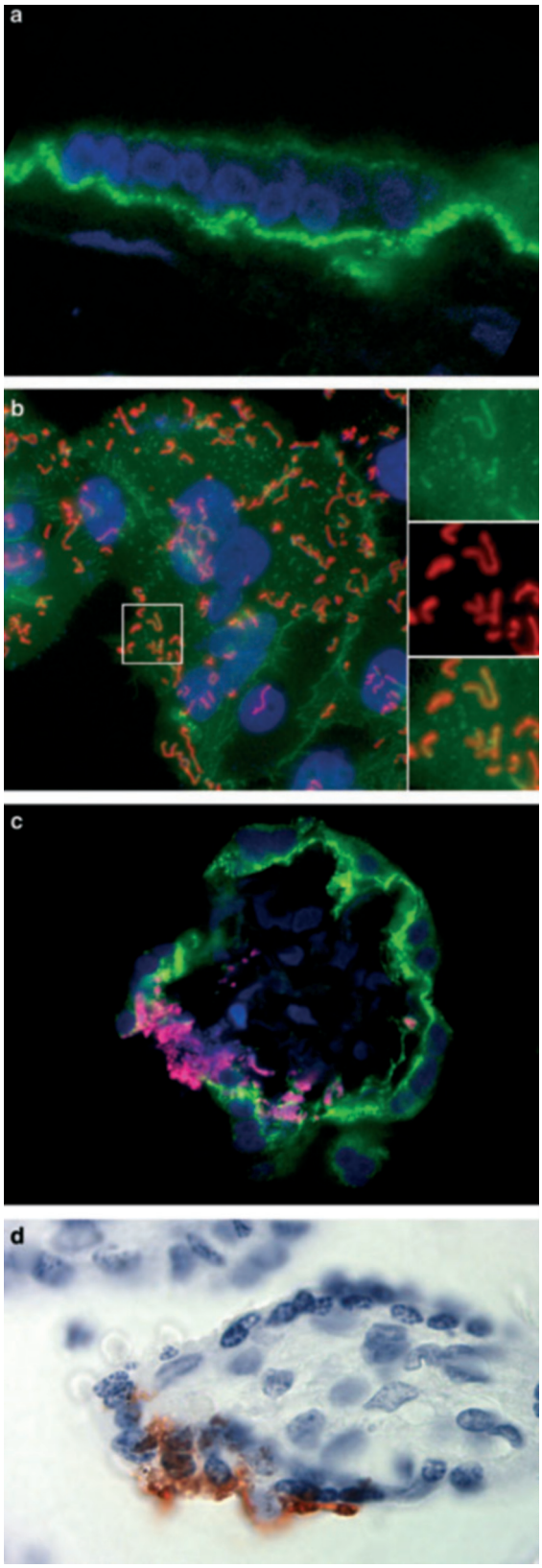

Fig. 4. (a) Section of a human placental villus centred on the syncytiotrophoblast and immunolabelled with an antibody directed against human E-cadherin. E-cadherin appears in green and nuclei in blue. (b) Adhesion and invasion of bacteria expressing internalin in cultured human syncytiotrophoblast. Bacteria are shown in red, E-cadherin in green, and nuclei in blue. There is a recruitment of E-cadherin to the site of entry of the bacteria in the syncytiotrophoblast. (c) Section of a placental villus infected *ex vivo*. *L. monocytogenes* has invaded the syncytiotrophoblast and crossed the placental barrier. This has led to the constitution of villous microabscesses similar to those observed within placentas obtained from pregnant women with listeriosis (d). Bacteria are shown in purple in (c) and in red in (d).

and cross the intestinal and placental barriers. This raises the possibility that *L. monocytogenes* placental tropism may be a consequence of its evolved mechanism for targeting the intestinal epithelium. The results also show that, in addition to pregnancy-associated immunosuppression, a specific mechanism allows *L. monocytogenes* to directly target and invade the human placental barrier. Interestingly, the blood–brain barrier is also composed of E-cadherin-expressing cells, the microvascular endothelium and choroid plexus epithelium. Thus, it is tempting to speculate that targeting and invasion of the central nervous system by *L. monocytogenes* may also be mediated by the interaction between internalin and E-cadherin. Other human pathogens, such as *Toxoplasma gondii* or human cytomegalovirus, exhibit a similar tropism for these three barriers. Future studies focused on deciphering the similarities and specificities of these barriers may extend our understanding of the strategies that microbes use to breach them.

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