Murinization of Internalin Extends Its Receptor Repertoire, Altering Listeria monocytogenes Cell Tropism and Host Responses

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Abstract

Listeria monocytogenes (Lm) is an invasive foodborne pathogen that leads to severe central nervous system and maternal-fetal infections. Lm ability to actively cross the intestinal barrier is one of its key pathogenic properties. Lm crosses the intestinal epithelium upon the interaction of its surface protein internalin (InlA) with its host receptor E-cadherin (Ecad). InlA-Ecad interaction is species-specific, does not occur in wild-type mice, but does in transgenic mice expressing human Ecad and knock-in mice expressing humanized mouse Ecad. To study listeriosis in wild-type mice, InlA has been “murinized” to interact with mouse Ecad. Here, we demonstrate that, unexpectedly, murinized InlA (InlA™) mediates not only Ecad-dependent internalization, but also N-cadherin-dependent internalization. Consequently, InlA™-expressing Lm targets not only goblet cells expressing luminaly-accessible Ecad, as does Lm in humanized mice, but also targets villous M cells, which express luminaly-accessible N-cadherin. This aberrant Lm portal of entry results in enhanced innate immune responses and intestinal barrier damage, both of which are not observed in wild-type Lm-infected humanized mice. Murinization of InlA therefore not only extends the host range of Lm, but also broadens its receptor repertoire, providing Lm with artifactual pathogenic properties. These results challenge the relevance of using InlA™-expressing Lm to study human listeriosis and in vivo host responses to this human pathogen.

Introduction

Co-evolution of microbes with their hosts can select stringently specific host-microbe interactions at the cell, tissue and species levels [1]. Species-specific host-microbe interactions, which are the rule rather than the exception, pose a challenge for the use of laboratory animal models to study human pathogens, including Listeria monocytogenes (Lm), the etiological agent of listeriosis, a deadly foodborne infection. Lm is able to actively cross the intestinal barrier, reach the systemic circulation and cross the blood-brain and placental barriers, leading to its dissemination to the central nervous system and the fetus [2].

The mouse is a genetically amenable model that is widely used to investigate human diseases [3,4]. To obtain a mouse model in which the pathogenic properties of a given pathogen are similar to what is observed in human, species specificity can be circumvented by humanizing the mouse by transgenesis [5,6,7,8], knock-out [9], knock-in [10] or xenograft techniques [11]. One can also adapt the pathogen to the mouse by multiple passages on cell lines [12,13] or in vivo [14], or specifically “murinize” a pathogen ligand so that it interacts with the mouse ortholog of a species-specific human receptor [15,16].

The Lm surface protein InlA interacts with E-cadherin (Ecad) and mediates Lm entry into epithelial cells, which express this adherens junction protein [17,18]. Cadherins constitute a family of calcium-dependent cell adhesion receptors. Ecad is expressed mainly in epithelia, whereas N-cadherin (Ncad) is found primarily in neuronal cells and endothelial cells together with VE-cadherin [19,20]. Ncad can also be coexpressed with Ecad in epithelial cells [21]. Importantly, Ncad has been reported to not act as a receptor for InlA, and so far Ecad is the only known classical cadherin acting as a receptor for InlA [18]. In contrast to Ecad from human, guinea pig, rabbit and gerbil, mouse Ecad (mEcad) and rat Ecad are not recognized by InlA and do not promote bacterial entry [9,22]. The interaction of InlB, another Lm invasion protein, with its host receptor is also species-specific [23]. InlB recognizes the hepatocyte growth factor receptor Met of human, mouse, rat and gerbil but not that of guinea pig and rabbit [9,23,24].

Two mouse lines have been established to study InlA-Ecad interaction in vivo: a transgenic mouse line expressing human...
InlA-Ecad interaction [6,9,16]. Moreover, the ability of InlAm or orally inoculated with increased intestinal, spleen and liver bacterial loads of wt mice barrier and would render wt mice orally permissive to entering human Ecad and knock-in mice expressing human Ecad. To study listeriosis in wild-type mice, InlA has been “murinized” to interact with mouse Ecad. Here, we demonstrate that in addition to interacting with mouse Ecad, InlAm also uses N-cadherin as a receptor, whereas InlA does not. This artificial InlA-N-cadherin interaction promotes bacterial translocation across villous M cells, a cell type which is not targeted by InlA-expressing bacteria. This leads to intestinal inflammation and intestinal barrier damage, both of which are not seen in humans and humanized mouse models permissive to InlA-Ecad interaction. These results challenge the relevance of using InlAm-expressing Lm as a model to study human listeriosis and host responses to this pathogen. They also illustrate that caution must be exercised before using “murinized” pathogens to study human infectious diseases.

Ecad (hEcad) in enterocytes (hEcad Tg) [6], and a humanized mEcad knock-in mouse line (E16P KI) with an E16P amino acid substitution which enables mEcad to interact with InlA without affecting Ecad homophilic interactions and allows Lm internalization [9,22]. Using these two humanized mouse models, we have demonstrated that InlA mediates Lm crossing of the intestinal epithelium upon targeting of luminal-accessible Ecad around goblet cells [6,9,25], and that InlA and InlB act interdependently to mediate the crossing of the placental barrier [9]. Epidemiological investigations have confirmed the relevance of these experimental findings, and shown that InlA is implicated in Lm crossing of human intestinal and placental barriers [9,26].

In 2007, Wollert et al. engineered a genetically modified InlA with the purpose of increasing its binding affinity to hEcad [16]. Two amino acid substitutions in InlA, S192N and Y369S, were shown to enhance InlA binding affinity to hEcad [16]. Neither S192N nor Y369S substitution has been observed in the more than 500 Lm isolates InlA sequences we have checked (our unpublished results). Wollert et al. published that this increased affinity for hEcad translates into an increased bacterial entry into human epithelial cells (Caco-2) [16]. Importantly, Wollert et al. also showed that this modified InlA binds the extracellular cadherin domain 1 (EC1) of mEcad in solution with a comparable affinity to that of the wild-type (wt) InlA for hEcad EC1 [16]. They hypothesized that this interaction would allow Lm expressing this “murinized” InlA (InlAm) to cross intestinal barrier and would render wt mice orally permissive to Lm infection, a phenotype which is mediated by InlA in permissive models [6]. In support of this hypothesis, Wollert et al. found an increased intestinal, spleen and liver bacterial loads of wt mice orally inoculated with Lm expressing InlAm, yet only after 3 to 4 days post infection, which is later than in models permissive to InlA-Ecad interaction [6,9,16]. Moreover, the ability of InlAm to mediate mEcad-dependent Lm internalization into host cells has never been tested. In addition, InlAm unexpectedly promoted pronounced inflammation and intestinal epithelial cell damages in wt mice [16], whereas wt InlA mediates the crossing of the intestinal barrier without inducing significant intestinal response and tissue damage in hEcad transgenic mice [6,27].

This prompted us to investigate the detailed properties of InlAm in cultured cells, as well as the in vivo cell and tissue tropisms of bacteria expressing InlAm, as compared to that of its isogenic parental Lm strain that expresses wt InlA. Here, we demonstrate that InlAm promotes bacterial entry not only into mEcad-positive but also into mEcad-negative mouse cells. We show that InlAm-mediated entry into mEcad-negative cells is mouse Ncad (mNcad)-dependent. Importantly, InlAm-mNcad interaction allows bacteria to specifically target Ncad-positive villous M cells in vivo, a cell type which is not targeted by Lm in humanized mouse models permissive to InlA-Ecad interaction. This leads to enhanced intestinal inflammatory responses and disruption of the intestinal barrier integrity, both of which are not observed in Lm-infected humanized mice and human listeriosis. Together, these results demonstrate that the murinization of InlA not only extends Lm host range, but also broadens its receptor repertoire, consequently changing Lm cell tropism and enhancing host immune responses to Lm. These results challenge the relevance of using InlAm-expressing Lm to study human listeriosis and in vivo host responses to this human pathogen.

**Results**

**Murinization of InlA promotes bacterial entry into mEcad-expressing cells but has no impact on bacterial entry into hEcad-expressing cells**

We first investigated whether the increased affinity of InlAm to hEcad translates into an enhanced invasion of hEcad-expressing cells, as proposed by Wollert et al. [16]. To this end, we assessed InlAm-dependent entry into LoVo cell, a human epithelial cell line expressing hEcad [22]. Lm wt strain and Lm expressing InlAm (Lm-inlAm) invaded LoVo cells at similar levels (Figure 1A). Because Lm can be internalized by InlA-independent pathways such as InlB-Met, we transferred either inlA or inlAm onto the chromosome of Listeria innocua (Li), a naturally non-invasive and non-pathogenic Listeria species, in which heterologous expression of inlA has been shown to confer invasiveness [17,18,28]. Li expressing either InlA (Li-inlA) or InlAm (Li-inlAm) were equally invasive in LoVo cells (Figure 1B). These results indicate that contrary to what is reported by Wollert et al. [16], the increased affinity of InlAm to hEcad does not translate into an increased level of bacterial entry. Both Li-inlA and Li-inlAm recruited hEcad when incubated with LoVo cells, suggesting that hEcad is involved in both InlA- and InlAm-mediated entries (Figure 1E, upper panel). Because purified InlAm interacts with the purified EC1 domain of mEcad, Wollert et al. have proposed, although not tested, that InlAm would mediate bacterial entry into mEcad-expressing cells [16]. We therefore tested the ability of InlAm to promote bacterial entry into the mouse epithelial cell line Nme, which expresses mEcad [29]. InlAm promoted bacterial entry into mEcad-expressing Nme cells, although to a lower level than InlA in hEcad-expressing LoVo cells (Figure 1C and D), Li-inlAm also recruited mEcad during cell invasion, whereas as expected, Li-inlA does not (Figure 1E, lower panel). Together, these results show that (i) the increased affinity of InlAm to hEcad does not enhance bacterial entry into hEcad-expressing cells, and (ii) the murinization of InlA confers to Lm an enhanced ability to be internalized into mEcad-expressing cells [16].
Figure 1. Respective ability of InlA and InlAm to promote bacterial entry into hEcad- and mEcad-expressing cells. Bacterial entry into hEcad-expressing human epithelial cells (LoVo) (A for Lm and B for Li) and mEcad-expressing mouse epithelial cells (Nme) (C for Lm and D for Li) was performed by counting intracellular gentamicin resistant bacteria following 1 hr of infection and 1 hr of gentamicin incubation. No significant difference was observed between InlA- and InlAm-expressing bacteria when invading LoVo cells, whereas InlAm expression promoted bacterial entry into Nme cells. Values are expressed as a mean ± SD (n = 3). Statistical analysis was performed with the unpaired Student's t test. (E) Recruitment of Ecad was performed by incubating Li-inlA or Li-inlAm with the cells cultured on coverslips for 1 hr, followed by PBS wash and fixation before staining. The cells were stained with the anti-Li antibody and anti-hEcad or anti-mEcad antibody. Right panels show separated channels and merge of boxed regions, showing the recruitment of cadherin by bacteria. Both Li-inlA and Li-inlAm recruit hEcad when incubated with LoVo cells. Li-inlAm but not Li-inlA is able to recruit endogenous mEcad in Nme cells. Scale bar, 20 μm. doi:10.1371/journal.ppat.1003381.g001
mNcad is a receptor for InlA<sup>m</sup> but not InlA<sup>m</sup>

To investigate if mNcad serves as a receptor for InlA<sup>m</sup>-mediated entry into CT26 cells, CT26 cells were treated with mNcad-specific siRNAs or scrambled control siRNAs. Treatment of CT26 cells with mNcad siRNAs led to a reduced expression of mNcad which correlated with a significantly decreased InlA<sup>m</sup>-dependent entry (Figures 3A and B). To directly assess the ability of mEcad and mNcad to act as receptors for InlA<sup>m</sup>, we used the BHK21 cell line, which is of hamster origin and does not express any known classical cadherin [32], and transfected this cell line with plasmids encoding either hEcad, mEcad or mNcad. As in CT26 cells, InlA<sup>m</sup> promoted bacterial entry into HeLa and 104C1 cells, although these two cell lines do not express Ecad and are therefore not permissive to InlA-dependent entry (Figure S2) [23]. These results suggest that the murinization of InlA confers to this protein the ability to interact with Ncad from different species, and to enter into host cells expressing Ncad.

InlA<sup>m</sup>-mNcad interaction has an impact on Lm systemic dissemination in orally inoculated mice

To investigate the impact of InlA<sup>m</sup>-mNcad interaction on the infection process, we inoculated orally wt and E16P KI mice with Lm-inlA<sup>m</sup> or Lm. In Lm-infected E16P KI mice in which InlA-Ecad interaction is functional, InlA promoted Lm invasion of the small intestinal tissue and bacterial dissemination to spleen and liver as early as 2 dpi (Figure 6). In contrast, in Lm-inlA<sup>m</sup> infected wt mice, in which both InlA<sup>m</sup>-Ecad and InlA<sup>m</sup>-Ncad interactions are functional, Lm bacterial loads in the small intestinal tissue, spleen and liver were significantly increased at 2 dpi compared to Lm-infected wt mice, but were at 4 dpi (Figure 6). This delayed systemic dissemination was also observed when comparing Lm-inlA<sup>m</sup> to Lm inlA<sup>m</sup> in E16P KI mice (Figure S7). These results demonstrate that while InlA<sup>m</sup>- and InlA<sup>m</sup>-Ecad interactions both contribute to the targeting of goblet cells, InlA<sup>m</sup>-mNcad interaction allows bacteria to target villous M cells, a cell type which is not targeted when InlA interacts only with its native receptor Ecad.

InlA<sup>m</sup>-mNcad interaction leads to enhanced intestinal response and compromised intestinal barrier function

Given the changes in infection kinetics induced by InlA<sup>m</sup>, and the artifactual route of translocation taken by InlA<sup>m</sup>-expressing bacteria at the intestinal epithelium level, we investigated whether InlA<sup>m</sup>-Ncad-mediated targeting of villous M cells would have an impact on host responses. Strikingly, oral inoculation of Lm-inlA<sup>m</sup> led to a significant neutrophil recruitment in wt (Figures 7A and B), E16P KI (Figures S8A and B) and hEcad Tg mice (Figures S8A and B), which was not observed with Lm in E16P KI (Figures 7A and B). These results suggest that InlA<sup>m</sup> may allow bacteria to target villous M cells upon mouse oral inoculation.

Munirization of InlA receptors and cell tropism

InlA<sup>m</sup> promotes mEcad-independent entry into mouse cells

Monk et al. have reported that Lm-inlA<sup>m</sup> invades mouse CT26 cells more efficiently than Lm [13]. Strikingly, CT26 cells do not express mEcad (Figure 2A) [30], yet we confirmed that InlA<sup>m</sup> mediates bacterial entry into these cells (Figure 2B). Because classical cadherins exhibit a high level of conservation in their EC1 domains (Figure S1A), we tested whether Li-inlA<sup>m</sup> would recruit another classical cadherin than mEcad in CT26 cells. We labeled CT26 cells with a pan-cadherin antibody, which recognizes the cytoplasmic domain of classical cadherins [31]. CT26 cells were strongly stained with the pan-cadherin antibody (Figure S1B), indicating that they likely express classical cadherin proteins. Furthermore, this pan-cadherin-immunoreactive protein was recruited in CT26 cells by Li-inlA<sup>m</sup> but not Li-inlA (Figure S1B). Immunoblotting and immunostaining revealed that CT26 cells express Ncad (Figures 2C and D), a classical cadherin known to be expressed in endothelial cells, neurons and some transformed epithelial cells [20]. Importantly, Li-inlA<sup>m</sup>, but not Li-inlA, recruited Ncad in CT26 cells (Figure 2D). We next tested other cell lines for Ncad expression. We found that Nine cells (which also express mEcad and are permissive to InlA<sup>m</sup>-mediated entry), human HeLa cells, and guinea pig 104C1 cells all express Ncad (Figure 2C). As in CT26 cells, InlA<sup>m</sup> promoted bacterial entry into HeLa and 104C1 cells, although these two cell lines do not express Ecad and are therefore not permissive to InlA-dependent entry (Figure S2) [23]. These results suggest that the murinization of InlA confers to this protein the ability to interact with Ncad from different species, and to enter into host cells expressing Ncad.

Murinization of InlA extends the cell tropism of Lm at the intestinal level

In order to investigate if these in vitro results translate into an in vivo phenotype, and study in particular the cell tropism of InlA<sup>m</sup>-expressing bacteria, we investigated Ncad luminal accessibility at the intestinal epithelium level, which is the portal of InlA-mediated entry of Lm. In contrast to luminally-accessible Ecad which is mostly observed as rings surrounding goblet cells [25], mNcad was accessible on the apical pole of villous M cells (Figure 4, Movie S1), but not M cells of Peyer’s patches (Movie S2) in wt mice. The expression of luminally-accessible Ncad was also detected on the apical pole of villous M cells in E16P KI mice (Figure S3, Movie S3). These results suggest that InlA<sup>m</sup> may allow bacteria to target villous M cells upon mouse oral inoculation.

To specifically investigate whether InlA<sup>m</sup>-expressing bacteria target cells that express luminally-accessible Ncad, we inoculated orally wt mice with Li-inlA or Li-inlA<sup>m</sup>, and for comparison we inoculated humanized E16P KI mice orally with Li-inlA<sup>m</sup>. As expected from our recent results [25], Li-inlA were found in goblet cells 5 hr post oral inoculation of E16P KI mice (Figures 5C and D). In contrast, Li-inlA<sup>m</sup> targeted both goblet cells (Figures 5A and D) and villous M cells (Figures 5B and D, Movie S4) in wt mice. We next performed a detailed quantification of the location of bacteria in the intestinal epithelium (i.e. goblet cells, villous M cells, other epithelial cells). This demonstrated that, contrary to InlA, which targets almost exclusively goblet cells in E16P KI mice (82%), InlA<sup>m</sup> preferentially targets villous M cells (56%) in wt mice, and to a lower degree goblet cells (34%) (p<0.001, χ<sup>2</sup> test analysis) (Figure 5D). In agreement with these results obtained with Li-inlA<sup>m</sup>, Lm-inlA<sup>m</sup> also targeted both goblet cells (Figures S4A and D, S5A, Movie S5) and villous M cells (Figures S4B and D, S5B, Movie S6) in both wt and E16P KI mice, in contrast to Lm which exclusively targeted goblet cells, only in E16P KI mice (Figures S4C and D, S5C, Movie S7). Together, these results demonstrate that while InlA- and InlA<sup>m</sup>-Ecad interactions both contribute to the targeting of goblet cells, InlA<sup>m</sup>-mNcad interaction allows bacteria to target villous M cells, a cell type which is not targeted when InlA interacts only with its native receptor Ecad.
Figure 2. InLA^{m} promotes bacterial entry into mEcad-negative mouse cells and recruits endogenous Ncad. (A) Nme cells express mEcad, whereas no mEcad expression can be detected in CT26 cells. Actin expression was used as a loading control. (B) Bacterial entry was performed as described in Figure 1. InLA^{m} but not InLA-expression promotes bacterial entry into mouse CT26 cells. Values are expressed as a mean ± SD (n = 3). Statistical analysis was performed with the unpaired Student's t test. (C) Nme cells, CT26 cells, HeLa cells and 104C1 cells express Ncad. The lysate of BHK21 cells which do not express any detectable classical cadherins was used as a negative control. (D) Recruitment of Ncad was performed as described in Figure 1. The coverslips were stained with the anti-Li antibody and anti-Ncad antibody. Right panels show separated channels and merge of boxed regions, showing the recruitment of Ncad by Li-inLA^{m}. Scale bar, 20 μm. See also Figures S1 and S2. doi:10.1371/journal.ppat.1003381.g002
Importantly, neutrophil infiltration correlated only with InlA m-mediated invasion, and did not reflect bacterial load in the villi, which was actually the highest in Lm-infected humanized mice, in which no neutrophil infiltration was observed (Figures 7A–C, S8A–C).

Moreover, a significant increase in IFN-γ and IL-1β expression was observed in the intestinal tissue of wt mice infected with Lm-inlAm, whereas no significant increase was observed in Lm-infected wt and humanized mice (Figures 7 D and E). Together, these results indicate that InlA m-Ncad-mediated intestinal invasion per se leads to exacerbated host responses compared to InlA-Ecad-mediated intestinal invasion, and are not a reflect of enhanced bacterial tissue invasion.

We next assessed intestinal barrier integrity upon infection by testing the intratissular diffusion of biotin administered intraluminally (see Material and Methods) [33]. In wt and humanized mice infected with Lm for two days, biotin localized exclusively to the luminal side of the small intestine (Figures 7F and S8D). In contrast, although the intestinal villi of Lm-inlAm infected wt and humanized mice were not heavily infected, biotin accessed the lamina propria (Figures 7F and S8D). These findings indicate that InlA m-Ncad-mediated intestinal invasion leads to a disruption of intestinal barrier integrity. Together, these results demonstrate that the murinization of InlA profoundly modifies the pathogenic properties of Lm by altering its intestinal portal of entry, host intestinal responses and intestinal barrier integrity.
Figure 4. Ncad expression is detected on the apical pole of villous M cells. Immunolabeling of the small intestine tissue section (A) and whole mount tissue of a part of ileum (B and C) of wt mice was performed. (A) The small intestine tissue section was stained by anti-mouse IgG followed by anti-Ncad mouse IgG and anti-mouse IgG conjugated with another fluorophore to distinguish mouse IgG-positive cells and Ncad-positive cells. Scale bar, 50 μm. (B and C) Intestinal tissue of wt mice was fixed and stained for luminally accessible (acc) Ncad with antibody against extracellular domain of Ncad (clone GC4) before tissue permeabilization. M cells with NKM 16-2-4 antibody, wheat germ agglutinin (WGA) and nuclei followed by anti-Ncad mouse IgG and anti-mouse IgG conjugated with another fluorophore to distinguish mouse IgG-positive cells and Ncad-positive cells. Scale bar, 20 μm. See also Figure S3, Movies S1, S2 and S3. doi:10.1371/journal.ppat.1003381.g004

Discussion

InLA interaction with Ecad allows Lm translocation across the intestinal epithelium and is therefore a critical event in the development of systemic listeriosis, one of the deadliest foodborne infections in human. Because InLA does not interact with mEcad, the discovery and characterization of this key step were made in species permissive to InLA-Ecad interaction (guinea pig, gerbil) and humanized mouse models (hEcad Tg and E16P KI mouse lines) [6,9]. A genetically engineered Lm strain expressing a murinized InLA (InLA') enabling interaction with mEcad in vitro has been proposed to constitute an alternative model to study human listeriosis in wt mice [16]. A practical advantage of this latter system is that it can be readily used to infect several different mouse lines. However, a systematic study comparing the properties of Lm expressing InLA' to that of its isogenic parental strain has not been performed, neither in vitro nor in vivo.

Here we show that InLA' is able to recruit mEcad and mediate mEcad-dependent entry into cultured cells. We also show that InLA' mediates entry into goblet cells of wt mice, which express luminally-accessible mEcad. These results confirm that the S192N and Y369S substitutions confer to InLA a phenotype in wt mice which is observed in humanized mice permissive to InLA-Ecad interaction [25].

Importantly, we also uncover that InLA' is able to recruit Ncad and mediate Ncad-dependent internalization. This artificial interaction translates in vivo into InLA'-dependent targeting of villous M cells, intestinal inflammatory responses, disruption of intestinal barrier integrity and delayed bacterial systemic dissemination in wt mice, as well as in humanized mice. Such stricking phenotypes are not observed in humanized mice orally-inoculated with wt Lm, suggesting that they depend on InLA'-Ncad interaction and invasion of villous M cells, but not on InLA'-Ecad interaction and invasion of goblet cells (Figure 8). It is important to note that these phenotypes are also present in E16P KI and hEcad Tg mice infected with Lm-inlAm', indicating that intestinal inflammation is a direct consequence of InLA'-mediated intestinal invasion, and proving that the absence of inflammation in Lm-infected humanized mice is not a side effect of mouse humanization, but is a genuine property of InLA-dependent intestinal invasion. These results are in agreement with the observation by Wollert et al. that infection with Lm-inlAm' leads to severe intestinal inflammation and tissue damage in wt mice [16], and with our earlier observation that InLA has little impact on Lm intestinal responses in mice permissive to InLA-Ecad interaction [6,27]. This indicates that the murinization of InLA, in addition to broadening the host range of Lm, also extends its receptor repertoire to another member of the classical cadherin family, Ncad, therefore modifying its cell tropism, host responses and the dynamics of infection.

The engineering of InLA' was based on the rational protein design of a modified InLA that would increase InLA-hEcad binding affinity [16]. Indeed, S192N and Y369S substitutions in InLA lead to a 6,700-fold increase in the binding affinity of InLA to hEcad [16]. Here we have shown that this does not translate into increased invasion of hEcad-expressing cells. Before drawing this conclusion, we ensured that the BHK21 cell line we used does not express other cadherins than the one we intended to study. A possible reason for the observed increased level of invasion of Lm-inlAm' in Caco-2 cells observed by Wollert et al. is the coexpression of Ecad and Ncad in these cells [21]. These results suggest that InLA-hEcad interaction, although it is of relatively low affinity (Kd = 8.4 μM) [16], has been naturally selected to mediate an optimal level of infection.

We have shown that InLB, another major invasion protein of Lm, does not play a significant role for the crossing of the intestinal barrier [23]. In contrast, InLB has been reported to promote Lm expressing InLA' to invade intestinal villi [34]. Our results shed light onto these apparent contradictory results and raise the possibility that InLA'-Ncad mediated invasion of villous M cells may involve the InLB pathway.

Shigella flexneri, the etiological agent of bacillary dysentery is associated with strong polymorphonuclear infiltration, severe local inflammation, disruption of intestinal barrier integrity, yet no systemic dissemination [35,36]. In contrast, listeriosis in human and humanized mice is characterized by the paucity of intestinal symptoms, the absence of polymorphonuclear intestinal infiltration, little local inflammation, the absence of intestinal barrier disruption, but systemic dissemination [6,27,36,37]. We have demonstrated that Lm-inlAm' triggers pro-inflammatory response and disrupts epithelial integrity in intestinal tissue of wt and humanized mice, and exhibits a delayed systemic dissemination, compared to Lm-infected humanized mice. These observations strongly suggest that the targeting of villous M cells by InLA', expressing bacteria triggers pro-inflammatory host responses which contain bacterial invasion but lead to intestinal epithelium damages. This fits with the observation that antigen delivery via villous M cells stimulates immune responses [38]. Like InLA', Als3 is a Candida albicans invasin that binds both Ecad and Ncad to invade host cells [39]. Candida albicans has been shown to favor gut inflammation and promotes food allergy accompanied by gut epithelial barrier hyperpermeability, the underlying mechanisms of which are so far unclear [40,41]. Our study indicates that Candida albicans may use Als3 to target Ncad-positive villous M cells, and thereby trigger intestinal inflammation. The specific functions of villous M cells remain poorly understood, yet villous M cells are a particularly abundant constituent of the intestinal epithelium. Our results show that InLA' and Als3-expressing microorganisms would be particularly instrumental to study villous M cell functions.

Repeated infection of mice in vivo or mouse cells in vitro allows the observation of “murinized” pathogens adapted to the mouse. Despite the great adaptability of microbes, evolutionary constraints limit pathogen variability [42]. A mutation beneficial under certain environmental conditions may end up as disadvantageous in another, highlighting the fine-tuning of host-microbe
interactions. The structure-based rational design of InlAm was proposed as a subtle and elegant way to electively “murinize” a microbial ligand with least impact on the pathogen. However, we provide here evidence that the rationally designed InlAm has gained the unfortunate ability to interact with another surface protein than its cognate receptor Ecad. Even though InlAm mediates Lm crossing of the intestinal barrier, a phenotype which is strictly dependent on InlA-Ecad interaction, the way by which Lm crosses the intestinal barrier in an InlA-dependent manner differs from what observed with wt Lm in humanized mice and humans, as does the resulting infection process. This illustrates that murinization of human-specific pathogens, although an elegant and rational approach, may unfortunately mislead rather than ease the understanding of human infectious diseases’ pathophysiology. Caution must therefore be exercised before engineering and using “murinized” pathogens to study human infectious diseases.

Materials and Methods

Bacterial and cell culture

Bacterial strains, plasmids and primers are listed in Table S1. Note that the sequences of inlA, inlAm in Lm and in Li were confirmed by sequencing, as well as the integration sites of inlA and

2 dpi

Small intestine

|     | CFUs |     |
|-----|------|-----|
| Lm  | 10^9 |     |
| Lm inlAm | 10^9 |     |

Spleen

|     | CFUs |     |
|-----|------|-----|
| Lm  | 10^7 |     |
| Lm inlAm | 10^7 |     |

Liver

|     | CFUs |     |
|-----|------|-----|
| Lm  | 10^6 |     |
| Lm inlAm | 10^6 |     |

4 dpi

Small intestine

|     | CFUs |     |
|-----|------|-----|
| Lm  | 10^8 |     |
| Lm inlAm | 10^8 |     |

Spleen

|     | CFUs |     |
|-----|------|-----|
| Lm  | 10^5 |     |
| Lm inlAm | 10^5 |     |

Liver

|     | CFUs |     |
|-----|------|-----|
| Lm  | 10^4 |     |
| Lm inlAm | 10^4 |     |

Figure 5. InlAm mediates targeting of villous M cells. The intestinal ileum was taken from E16P KI mice and wt mice orally inoculated by 10^9 Li-inlA and Li-inlAm at 5 hr post infection, respectively. The intestinal tissues were fixed and stained with WGA for goblet cells, NK1 16-2-4 monoclonal antibody for M cells, and for bacteria and nuclei. (A and B) The confocal Z-plane of an ileal villus from Li-inlAm infected wt mice demonstrates that Li-inlAm was able to target goblet cells (A) and villous M cells (B). Right panels show separated channels of boxed regions, showing the location of bacteria in villous epithelia. (C) The confocal Z-plane of an ileal villus from Li-inlA infected E16P KI mice shows that Li-inlA targeted goblet cells. (D) Relative location of bacteria in mice intestinal epithelia of villi is shown. The total number of Li-inlAm in wt mice intestinal villi epithelia was set to 100, 20 villi from two mice ileal loops were counted in each set. Scale bar, 20 μm. See also Figures S4, S5, S6, Movies S4, S5, S6, S7.

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Figure 6. Respective invasive potential of Lm and Lm-inlAm in orally inoculated E16P KI and wt mice. Mice were orally inoculated with 10^9 bacteria for 2 (n = 6, upper panel) or 4 (n = 6, lower panel) days. Bacterial loads in the ileum loops of small intestine, in spleens and in livers were shown. Statistical analysis was performed with the Mann-Whitney u test. See also Figure S7.

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Figure 7. Lm expressing InlA+ stimulate inflammatory response in the small intestine and compromise intestinal epithelial barrier integrity. The intestinal ileum was taken from E16P KI and wt mice orally inoculated by 10^10 Lm and Lm-inlA+ at 24 (A–C) or 48 (D–F) hr post infection, respectively. (A) Anti-Ly6G antibody staining indicates neutrophils (red, highlighted by the open arrowheads). Tissues were stained for Lm (green, highlighted by the arrows) and counterstained with WGA (grey) for goblet cells, respectively. Scale bar, 20 μm. (B) No obvious difference on neutrophil numbers was observed between wt and E16P KI mice infected by Lm, whereas orally Lm-inlA+ infection in wt mice induced neutrophil recruitment at 24 hr post infection. Statistical analysis was performed with the Mann-Whitney u test (n = 20 from 2 mice). (C) No obvious difference on bacterial numbers was observed between Lm and Lm-inlA+ in wt mice intestinal villi, whereas a significantly increased invasion of Lm in the villi was observed in E16P KI mice at 24 hr post infection. Statistical analysis was performed with the Mann-Whitney u test (n = 20 from 2 mice). (D and E) RNA
was extracted from the ileum loops of infected or PBS-treated mice 48 hr post infection (n = 4). Following reverse transcription reaction, gene expression was quantified by qPCR with normalization to the GAPDH transcript. Values are expressed as a mean + SD of the fold change relative to that in PBS-treated mice. No significant difference on IFN-γ (D) and IL-1β (E) expression was observed among PBS-treated, Lm and LmInlA-infected E16P KI mice. In contrast, Lm-inl A oral infection induced 5 to 10 fold increase of IFN-γ and IL-1β gene expression in intestinal tissue compared to Lm-infected and PBS-treated wt mice. Statistical analysis was performed with the unpaired Student’s t test. (F) Biotin (red) penetration into intestinal lamina propria was done to address intestinal barrier integrity during infection. Mice were sacrificed 2 days post infection. Biotin was injected into ileum loop followed by PBS wash and fixation. Tissues were stained for Lm (green, highlighted by the arrows) and counterstained with WGA (grey) for goblet cells, respectively. Biotin is located within lamina propria of the villi from Lm-inl A- infected mice but not Lm infected wt and E16P KI mice. Scale bar, 20 µm. See also Figure S8.

tn A in L. Listeria and Escherichia coli strains were respectively cultivated in BHI and LB at 37°C with shaking at 180 rpm. To deliver plasmids into L. E. coli S17-1 (colistin and nalidixic acid sensitive) cells were transformed with the plasmids followed by conjugation with L (colistin and nalidixic acid resistant). Mammalian cell lines used in this study were routinely cultivated at 37°C in 5% CO₂. Except for the culture medium for BHK21 which was supplemented with 5% fetal bovine serum, all the cell culture media were supplemented with 10% fetal bovine serum. Human epithelium LoVo cells were cultured in F12K nutrient GlutaMax medium. Mice epithelium Nme cells were cultured in DMEM GlutaMax medium supplemented with 10 µg/ml insulin. Mouse CT26 and guinea pig 104G1 cells were cultured in RPMI1640 GlutaMax medium supplemented with HEPEs buffer and sodium pyruvate. Human HeLa cells were cultured in MEM GlutaMax medium. Hamster BHK21 cells were cultured in MEM GlutaMax medium supplemented with tryptose phosphate buffer and HEPEs buffer. All the culture medium and related chemicals were purchased from Gibco (Invitrogen). Transient transfection of mammalian cells was performed with jetPRIME transfection kit (Polyplus Transfection). The scrambled (sc-37007) and mouse Ncad specific siRNAs (sc-35999) were purchased from Santa Cruz. For the transfection of siRNAs, mouse CT26 cells were seeded into the 24-well plates for 1 day and then transfected with scrambled siRNAs (25 nM) or mNcad-specific siRNAs (25 nM) followed by 1 day incubation and replacement of transfection medium with growth medium another 1 day of incubation before infection. For the transfection of plasmid DNAs, BHK21 cells were transiently transfected with pcDNA3 expression vector harboring the cDNAs of each cadherin (1 µg DNA for each well in a 24-well plate) followed by 2 days incubation before infection.

Construction of plasmids

The strategy to express inl A or inl A in L. Listeria and Escherichia coli strains were respectively cultivated in BHI and LB at 37°C with shaking at 180 rpm. To deliver plasmids into L. E. coli S17-1 (colistin and nalidixic acid sensitive) cells were transformed with the plasmids followed by conjugation with L (colistin and nalidixic acid resistant). Mammalian cell lines used in this study were routinely cultivated at 37°C in 5% CO₂. Except for the culture medium for BHK21 which was supplemented with 5% fetal bovine serum, all the cell culture media were supplemented with 10% fetal bovine serum. Human epithelium LoVo cells were cultured in F12K nutrient GlutaMax medium. Mice epithelium Nme cells were cultured in DMEM GlutaMax medium supplemented with 10 µg/ml insulin. Mouse CT26 and guinea pig 104G1 cells were cultured in RPMI1640 GlutaMax medium supplemented with HEPEs buffer and sodium pyruvate. Human HeLa cells were cultured in MEM GlutaMax medium. Hamster BHK21 cells were cultured in MEM GlutaMax medium supplemented with tryptose phosphate buffer and HEPEs buffer. All the culture medium and related chemicals were purchased from Gibco (Invitrogen). Transient transfection of mammalian cells was performed with jetPRIME transfection kit (Polyplus Transfection). The scrambled (sc-37007) and mouse Ncad specific siRNAs (sc-35999) were purchased from Santa Cruz. For the transfection of siRNAs, mouse CT26 cells were seeded into the 24-well plates for 1 day and then transfected with scrambled siRNAs (25 nM) or mNcad-specific siRNAs (25 nM) followed by 1 day incubation and replacement of transfection medium with growth medium another 1 day of incubation before infection. For the transfection of plasmid DNAs, BHK21 cells were transiently transfected with pcDNA3 expression vector harboring the cDNAs of each cadherin (1 µg DNA for each well in a 24-well plate) followed by 2 days incubation before infection.

Plasmids pcDNA3-mPcad and pcDNA3-mVEcad were constructed by inserting mPcad and mVEcad cDNAs into Novi-KpnI site of pcDNA3, respectively.

Invasion assay

Cell suspensions from confluent monolayers were seeded at a concentration of 5x10⁴ cells per well in 24-well tissue culture plates and grown for 40–48 hr in an antibiotics-free medium at 37°C. Lm and Li strains were grown to OD600 at 0.8 and 0.6 in BHI, respectively. Bacterial culture was then washed with PBS and diluted in cell culture medium without serum. Bacterial suspensions were added to the cells at a multiplicity of infection (MOI) of approximately 50 and incubated for 1 hr. Following wash with complete medium, 10 µg/ml of gentamicin was added to kill the extracellular bacteria for 1 hr. The cells were then washed by complete medium and PBS, and homogenized in PBS supplemented with 0.4% Triton X-100, followed by serial dilution and colony forming units (CFUs) counting. For cadiherin recruitment assay, the procedure was the same as the invasion assay except that the cell attachment buffer (HEPEs 20 mM, NaCl 150 mM, glucose 50 mM, MgCl₂ 1 mM, CaCl₂ 2 mM, MnCl₂ 1 mM, 0.1% BSA) was used for infection and PBS (Ca²⁺/ Mg²⁺) (Gibco) was applied to wash the non-attached bacteria stringently followed by fixation.

Animals

Eight to 10-week old C57BL/6 female mice (Janvier) and isogenic mEcad E16P KI female mice were food restricted overnight but allowed free access to water. Lm culture was prepared as described [6], and inoculated with a feeding needle intragastrically [44]. Mice were then immediately allowed free access to food and water. All the procedures were in agreement with the guidelines of the European Commission for the handling of laboratory animals, directive 86/609/EEC (http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm) and were approved by the Animal Care and Use Committee of the Institut Pasteur, as well as by the ethical committee of “Paris Centre et Sud” under the number 2010-0020.

Immunofluorescence labeling and immunoblotting

Preparation of tissue sections and whole mount tissues were as described [9, 25]. The following antibodies and fluorescent probes were used for immunostaining and Western blot: anti-hEcad clone HECID-1 mouse monoclonal antibody (Invitrogen), anti-mEcad clone ECCD-2 rat monoclonal antibody (Invitrogen), anti-β-actin clone AC-15 mouse monoclonal antibody (Sigma), anti-Ncad clone 32/N-cadherin mouse monoclonal antibody (BD), anti-Ncad clone GC-4 mouse monoclonal antibody (Sigma), anti-pan-cadherin clone CH-19 monoclonal antibody (Sigma), anti-M cell clone NKM 16-2-4 rat monoclonal antibody (Miltenyl Biotec), R6 anti-Li rabbit polyclonal antibody and R11 anti-Lm rabbit polyclonal antibody [45], Rat anti-mouse Ly-6G (BD), wheat germ agglutinin (WGA) conjugated with Alexa Fluor 647 [Jackson...
Figure 8. Revisited model of InlA™-expressing bacteria at the intestinal epithelium. In humanized mEcad-expressing E16P mouse, as well as in human, Lm targets accessible Ecad around intestinal goblet cells to cross intestinal epithelium, without inducing significant intestinal response.
The wt InlA of *Lm* does not interact with mEcad, thus limits the ability of *Lm* to cross wt mouse intestinal epithelium. Murinization of InlA enables *Lm-inlAm* to interact with mEcad and also Ncad of mouse. *Lm-inlAm* therefore invades not only the goblet cells but also the villous M cells expressing accessible Ncad in the intestinal epithelia of mice. Targeting of villous M cells, which is not observed in *Lm*-infected E16P KI mice and is not predicted to occur in humans, results in severe intestinal inflammation which induces polymorphonuclear neutrophil (PMN) infiltration, intestinal barrier damage and a delayed systemic dissemination in wt mice, all of which are not observed in human listeriosis and *Lm*-infected humanized mice. doi:10.1371/journal.ppat.1003381.g008

ImmunoResearch), Alexa Fluor 488 goat anti-rabbit (Invitrogen), Alexa Fluor 488 or Alexa Fluor 546 goat anti-mouse (Invitrogen), Alexa Fluor 487 donkey anti-rat (Jackson ImmunoResearch), Alexa Fluor 546 goat anti-rat (Invitrogen), Cy3-conjugated streptavidin (Jackson ImmunoResearch) and Hoechst 33342 (Invitrogen).

Biotin penetration experiment

Biotin was used as a molecule to address the integrity of intestinal epithelium as described previously [33]. Briefly, 2 mg/ml of EZ-link Sulfo-NHS-Biotin (Pierce) in PBS was slowly injected into the lumen of ileum loop via the open end adjacent to cecum immediately after removal of the entire ileum. After 3 min, the loop was opened followed by PBS wash and 4% paraformaldehyde fixation.

Intestinal tissue genes expression quantification

Four mice for each condition were sacrificed 2 days post infection. 1 cm-long of ileal loop of each animal was applied for RNA extraction. The RNA isolation, reverse transcription and quantitative real time PCR (qRT-PCR) were performed as described [46]. Primers used for qRT-PCR were pre-designed, validated RTqPCR primer pairs (SABioSciences, Qiagen) as follows: IFNG (IFN-γ, PPM03121A), IL1B (IL-1β, PPM03109F) and GAPDH (PMO2946E).

Statistical analysis

Values are expressed as mean ± SD. Statistical comparisons were made using the unpaired Student’s *t* test, Mann-Whitney *u* test or the *χ*² test as indicated. *p* values<0.05 were considered significant. Significant differences are marked with an asterisk for *p*<0.05, two asterisks for *p*<0.01, three asterisks for *p*<0.001 and four asterisks for *p*<0.0001.

Supporting Information

Figure S1 Murinization of InlA allows bacteria to recruit cadherin in Ecad-negative mouse cells, related to Figure 2. (A) Amino acids sequence alignment of first extracellular domains (EC1) of type I classical cadherins and mouse VE-cadherin, a type II classical cadherin. (B) Recruitment of mNcad in CT26 cells was performed as described in Figure 1. The coverslips were stained with the anti-L2 antibody and anti-Pan-cadherin (Pan-cad) antibody. Right panels show the boxed regions of separated channels and merge, demonstrating the recruitment of cadherin proteins specifically by InlA. Ncad antibodies, a specific marker on M cells surface. WGA was used to stain the mucus of goblet cells and cell membrane. Scale bar, 20 μm. (PDF)

Figure S2 Murinization of InlA promotes bacterial entry into Ecad-negative, Ncad-positive human and guinea pig cells, related to Figure 2. Human HeLa cells and guinea pig 104C1 cells are Ecad-negative and Ncad-positive cells. Cell invasion ability was evaluated by counting intracellular gentamicin resistant bacteria following 1 hr of infection (MOI 50) and 1 hr of gentamicin (10 μg/ml) incubation. No difference in bacterial entry is seen between *Lm* and its isogenic *inlA* null mutant (*LmΔinlA*), whereas the *Lm* harboring *inlAm* of which chromosomal inlA is replaced by *inlAm* (*Lm-inlAm*) promoted bacterial entry into both HeLa and 104C1 cells. Values are expressed as a mean ± SD (n = 3). Statistical analysis was performed with the unpaired Student’s *t* test. (PDF)

Figure S3 Ncad expression is detected on the apical pole of villous M cells in E16P KI mice, related to Figure 4. Intestinal tissue of wt mice was fixed and stained for luminal accessible Ncad with antibody against extracellular domain of Ncad (clone GC4) before tissue permeabilization, M cells with NKM 16-2-4 antibody, WGA and nuclei after tissue permeabilization. Projection of a 30 μm thick reconstructed intestinal villus (A) and one xy plane (B) are shown. Right panels show separated channels and merge of boxed regions in (B), showing Ncad on the apical side of NKM 16-2-4-positive cells. See also Movie S3. NKM 16-2-4 antibody is a monoclonal antibody raised against α(1,2) fucose moiety in absence of neighboring sialic acids, a specific marker on M cells surface. WGA was used to stain the mucus of goblet cells and cell membrane. Scale bar, 20 μm. (PDF)

Figure S4 *Lm-inlAm* target goblet cells and villous M cells in wt mice, related to Figure 5. The intestinal ileum was taken from wt or E16P KI mice orally inoculated by *10^10* *Lm* or *Lm-inlAm* at 5 hr post infection. The intestinal tissues were fixed. Vibratome sections were stained with WGA for goblet cells, NKM 16-2-4 monoclonal antibody for M cells, and for bacteria and nuclei. (A and B) The confocal Z-plane of an ileal villus from *Lm-inlAm* infected wt mice demonstrates that *Lm-inlAm* was able to target goblet cells (A, see also Figure S3A and Movie S3) and villous M cells (B, see also Figure S3B, Movie S4, and Movie S5). Right panels show separated channels and merge of boxed regions, showing the location of bacteria in villous epithelia. (C) The confocal Z-plane of an ileal villus from *Lm-inlAm* infected wt mice demonstrates that *Lm-inlAm* was able to target goblet cells (A, see also Figure S3A and Movie S3) and villous M cells (B, see also Figure S3B, Movie S4, and Movie S5). Right panels show separated channels and merge of boxed regions, showing the location of bacteria in villous epithelia. (D) Relative location of bacteria in mice intestinal epithelia of villi is shown. The total number of *Lm-inlAm* in wt mice intestinal villi epithelia was set to 100. 20 villi from two mice ileal loops were counted in each set. Scale bar, 20 μm. (PDF)

Figure S5 Intracellular location of bacteria targeting goblet and villous M cells, related to Figure 5. Orthogonal views of the infected cells in wt mice infected with *Lm-inlAm* (A and B, related to Figures S4A and B, respectively) and in E16P KI mice infected by *Lm* (C, related to Figure S4C) presented in Figure S5 were shown. These images demonstrate that the bacteria highlighted in the Figure S4 were intracellular. See also Movies S5, S6, and S7. (PDF)

Figure S6 *Lm-inlAm* attached to villous M cells and *Lm-inlAm* having reached the lamina propria underlying villous M cells, related to Figure 5. The intestinal ileum was taken from the wt mice orally inoculated by *10^10* *Lm* or *Lm-inlAm* at 5 hr post infection. The intestinal tissues were fixed. Vibratome sections were stained with WGA for goblet cells, NKM 16-2-4 monoclonal antibody for M cells, and for bacteria and nuclei. Results shown are two different confocal Z-planes of an ileal villus
Movie S3 Luminally accessible Ncad is expressed on the apical poles of villous M cells in E16P KI mice, related to Figure 4. Whole mount intestinal tissue of an E16P KI mouse was stained before permeabilization for accessible mNcad (green) and NKM 16-2-4 for M cells (red), and after permeabilization for nuclei (blue) and WGA for goblet cells (grey). Intestinal villus is oriented with the villus tip facing the viewer. The luminaly accessible apical surface of villous M cells is labeled with the anti-Ncad antibody. Images were acquired as a z stack by confocal microscopy and assembled as a three-dimensional reconstruction with Imaris software.

(MOV)

Movie S4 Li-inlA" targets both villous M cells and goblet cells in the intestinal villi upon oral inoculation of wt mice, related to Figure 5. Ileal loop of a wt mouse orally infected by Li-inlA" was taken 5 hr post infection, followed by fixation and staining for Li (green), M cells (red), goblet cells (grey) and nuclei (blue) after permeabilization. Images were acquired and assembled as described for Movie S1.

(MOV)

Movie S5 Lm-inlA" targets goblet cells in the intestinal villi upon oral inoculation of wt mice, related to Figure 5. Ileal loop of a wt mouse orally infected by Lm-inlA" was taken 5 hr post infection, followed by fixation. Vibratome section was stained for Lm-inlA" (green), M cells (red), goblet cells (grey) and nuclei (blue) after permeabilization. Images were acquired and assembled as described for Movie S1.

(MOV)

Movie S6 Lm-inlA" targets villous M cells in the intestinal villi upon oral inoculation of wt mice, related to Figure 5. Ileal loop of a wt mouse orally infected by Lm-inlA" was taken 5 hr post infection, followed by fixation. Vibratome section was stained for Lm-inlA" (green), M cells (red), goblet cells (grey) and nuclei (blue) after permeabilization. Images were acquired and assembled as described for Movie S1.

(MOV)

Movie S7 Lm targets goblet cells in the intestinal villi upon oral inoculation of E16P KI mice, related to Figure 5. Ileal loop of a wt mouse orally infected by Lm was taken 5 hr post infection, followed by fixation. Vibratome section was stained for Lm (green), M cells (red), goblet cells (grey) and nuclei (blue) after permeabilization. Images were acquired and assembled as described for Movie S1.

(MOV)

Table S1 Bacterial strains, plasmids and primers used in this study.

(DOC)

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Author Contributions

Conceived and designed the experiments: YHT OD HB ML. Performed the experiments: YHT OD HB. Analyzed the data: YHT OD HB ML. Wrote the paper: YHT OD HB ML.
References

1. Brown NF, Wickham ME, Coombes BK, Finlay BB (2006) Crossing the line: selection and evolution of virulence traits. PLoS Pathog 2: e92.

2. Lecuit M (2007) Human listeriosis and animal models. Microbes Infect 9: 1216–1223.

3. Buer J, Balling R (2003) Mice, microbes and models of infection. Nat Rev Genet 4: 195–205.

4. Lecuit M, Cossart P (2002) Genetically-modified-animal models for human infections: the Listeria paradigm. Trends Mol Med 8: 537–542.

5. Dorner M, Horwitz JA, Robbins JB, Barry WT, Feng Q, et al. (2011) A genetically humanized mouse model for hepatitis C virus infection. Nature 474: 208–211.

6. Lecuit M, Vandomme-Pourain S, Lefort J, Huerre M, Gounon P, et al. (2001) A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. Science 292: 1722–1725.

7. Ren RB, Costantini F, Gorgacz EJ, Lee JJ, Racaniello VR (2012) A murine model of murine poliovirus infection that mimics human disease. Science 335: 1655–1658.

8. Mathur R, Oh H, Zhang D, Park SG, Seo J, et al. (2012) A mouse model of human enterovirus 71. J Gen Virol 89: 633–639.

9. Disson O, Grayo S, Huillet E, Nikitas G, Langa-Vives F, et al. (2008) Conjugated action of two species-specific invasion proteins for fetoplacental infection in mice expressing a human poliovirus receptor: a new model for poliomyelitis. Cell 133: 353–362.

10. Marchetti M, Arico B, Burroni D, Figura N, Rappuoli R, et al. (1995) Bacterial invasion of epithelial cells: invasion processes. Appl Environ Microbiol 76: 3625–3636.

11. Santisang Y, Santisang K, Santisang P, Santisang S, Santisang W (2001) A novel M cell-specific carbohydrate-targeted mucosal vaccine effectively induces antigen-specific immune responses in mice expressing a human poliovirus receptor. PLoS Pathog 6: e1000976.

12. Bierne H, Travier L, Mahlakoiv T, Tailleux L, Subtil A, et al. (2012) Activation of type III interferon genes by pathogenic bacteria in infected epithelial cells and its modulation by E-cadherin expression. J Infect Dis 206: 1323–1331.

13. Arnold SJ (1992) Constraints on phenotypic evolution. Am Nat 140 Suppl 1: S79–S90.

14. Disson O, Nikitas G, Grayo S, Dussurget O, Cossart P, et al. (2009) Modeling the Receptor for Internalin, a Surface Protein Required for Entry of Listeria monocytogenes into Epithelial Cells. Cell 84: 923–932.

15. Geiger B, Volberg T, Ginsberg D, Bitzur S, Sabanay I, et al. (1990) Broad spectrum pan-cadherin antibodies, reactive with the C-terminal 24 amino acid residues of N-cadherin. J Cell Sci 97 Pt 6: 607–614.

16. Ono J, Yamada K, Higuchi Y, Tamada Y, Muto Y, et al. (2001) Development of a mouse model of Helicobacter pylori infection that mimics human disease. Science 267: 1655–1658.

17. Biezeino J, Bankwitz D, Huguen K, Has M, Prochazka I, et al. (2010) Adaptation of hepatitis C virus to mouse CD81 permits infection of mouse cells in the absence of human entry factors. PLoS Pathog 6: e1000900.

18. Woller T, Pasche B, Rochon M, Deppenmeier S, van den Heuvel J, et al. (2007) Extending the host range of Listeria monocytogenes by rational protein design. Cell 129: 891–902.

19. Lecuit M, Ohayon H, Gounon P, Seminary OD, Cossart P, et al. (2001) Internalin of Listeria monocytogenes: involvement in the intracellular infection of epithelial cells. Cell 104: 639–649.

20. Bierne H, Travier L, Mahlakoiv T, Subtil A, et al. (2012) Activation of type III interferon genes by pathogenic bacteria in infected epithelial cells and its modulation by E-cadherin expression. J Infect Dis 206: 1323–1331.

21. Phalipon A, Sansonetti PJ (2007) Shigellosis: a paradigm of host–pathogen interaction in the gut. Curr Opin Microbiol 10: 508–515.

22. Leung N, Turbide C, Balachandra B, Marcus V, Beauchemin N (2008) Dysregulated Wnt signaling in Ceacam1-/- mice. Oncogene 27: 4943–4953.

23. Gounon P, Le Guennec Y, Boyer P, Cossart P (1999) Intestinal tumor progression is promoted by decreased apoptosis and dysregulated Wnt signaling in Ceacam1-/- mice. Oncogene 18: 4373–4381.

24. Geiger B, Volberg T, Gounon P, Bierne H, Cossart P, et al. (1999) Broad spectrum pan-cadherin antibodies, reactive with the C-terminal 24 amino acid residues of N-cadherin. J Cell Sci 97 Pt 6: 607–614.

25. Nikitas G, Deschamps G, Nöel T, Cossart P, et al. (2010) Transcytosis of Listeria monocytogenes across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. J Exp Med 208: 2263–2277.

26. Disson O, Santisang Y, Cossart P, et al. (2009) Murinized InlA Receptors and Cell Tropism for murine Listeria monocytogenes. J Infect Dis 189: 2094–2100.

27. Bassil C, Duson D, Gouton JM, Arico B, Rappuoli R, et al. (2010) Transversion of human to murine N-cadherin and E-cadherin receptors for Listeria monocytogenes infection. J Infect Dis 200: 1156–1167.

28. Dejana E, Arico B, Meana R, Menta M, Ghi A, et al. (2007) A novel M cell-specific carbohydrate-targeted mucosal vaccine effectively induces antigen-specific immune responses. J Exp Med 204: 2789–2796.

29. Phan QT, Myers CL, Fu Y, Sheppard DC, Veevan MR, et al. (2007) A novel M cell-specific carbohydrate-targeted mucosal vaccine effectively induces antigen-specific immune responses in mice. J Infect Dis 195: 799–810.

30. Leung N, Turbide C, Balachandra B, Marcus V, Beauchemin N (2008) Dysregulated Wnt signaling in Ceacam1-/- mice. Oncogene 27: 4943–4953.

31. Geiger B, Volberg T, Gounon P, Bierne H, Beuchemin N (2008) Dysregulated Wnt signaling in Ceacam1-/- mice. Oncogene 27: 4943–4953.

32. Gounon P, Le Guennec Y, Boyer P, Cossart P (1999) Intestinal tumor progression is promoted by decreased apoptosis and dysregulated Wnt signaling in Ceacam1-/- mice. Oncogene 18: 4373–4381.

33. Gounon P, Le Guennec Y, Boyer P, Cossart P (1999) Intestinal tumor progression is promoted by decreased apoptosis and dysregulated Wnt signaling in Ceacam1-/- mice. Oncogene 18: 4373–4381.

34. Balestrino D, Hamon MA, Dortet L, Nahori MA, Pizarro-Cerda J, et al. (2010) Endocytic internalin A for enhanced infectivity of Listeria monocytogenes. Cell 143: 1001–1015.

35. Sansonetti PJ, Di Santo JP (2007) Debugging how bacteria manipulate the immune response. Immunity 26: 149–161.

36. Bierne H, Travier L, Mahlakoiv T, Tailleux L, Subtil A, et al. (2012) Activation of type III interferon genes by pathogenic bacteria in infected epithelial cells and mouse placentas. PLoS One 7: e39080.

37. Phalipon A, Sansonetti PJ (2007) Shigellosis: a paradigm of host–pathogen interaction in the gut. Curr Opin Microbiol 10: 508–515.