Recruitment of the Major Vault Protein by InlK: A *Listeria monocytogenes* Strategy to Avoid Autophagy

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Abstract

*L. monocytogenes* is a facultative intracellular bacterium responsible for listeriosis. It is able to invade, survive and replicate in phagocytic and non-phagocytic cells. The infectious process at the cellular level has been extensively studied and many virulence factors have been identified. Yet, the role of InlK, a member of the internalin family specific to *L. monocytogenes*, remains unknown. Here, we first show using deletion analysis and *in vitro* infection, that InlK is a *bona fide* virulence factor, poorly expressed *in vitro* and well expressed *in vivo*, and that it is anchored to the bacterial surface by sortase A. We then demonstrate by a yeast two hybrid screen using InlK as a bait, validated by pulldown experiments and immunofluorescence analysis that intracytosolic bacteria via an interaction with the protein InlK interact with the Major Vault Protein (MVP), the main component of cytoplasmic ribonucleoprotein particles named vaults. Although vaults have been implicated in several cellular processes, their role has remained elusive. Our analysis demonstrates that MVP recruitment disguises intracytosolic bacteria from autophagic recognition, leading to an increased survival rate of InlK over-expressing bacteria compared to InlK− bacteria. Together these results reveal that MVP is hijacked by *L. monocytogenes* in order to counteract the autophagy process, a finding that could have major implications in deciphering the cellular role of vault particles.

Introduction

*Listeria monocytogenes* is a Gram-positive bacterium responsible for listeriosis, a severe food-borne human infection with an overall mortality rate of 30% [1]. *L. monocytogenes* has evolved efficient strategies to survive in the intestine and cross the intestinal, blood-brain and placental barriers [2,3] leading to clinical features of the disease that include gastroenteritis, septicemia, central nervous system infections, and mother-to-child infections [4]. Inside the host, this facultative intracellular bacterium is able to invade phagocytic and non-phagocytic cells, replicate intracellularly, and spread directly from cell-to-cell, thereby escaping the immune response [3]. *L. monocytogenes* has thus emerged as a paradigm to study host-pathogen interactions and fundamental processes in cell biology [5]. For instance, the study of actin rearrangements upon entry and intracellular movements [6–9] is an example of how understanding a bacterial-induced process can yield insight into basic cellular processes. Namely, the listerial virulence factor ActA triggers the recruitment of Arp2/3 complex and Ena/VASP to mediate actin polymerization and propel the bacterium from one infected cell to another without exposure to the extra-cellular milieu [8,10]. Interestingly, as shown recently ActA also disguises the bacteria from autophagic recognition within the cytosol as ActA− bacteria becomes rapidly ubiquitinated and targeted to autophagy [9,11]. It is currently viewed that ubiquitin-associated bacteria recognized by the autophagy machinery are trapped by autophagosomal membrane for delivery into the lytic compartment where they undergo degradation by autolysosomes [11,12]. Interestingly, a variety of studies has noticed that autophagic markers can accumulate around intracytosolic *L. monocytogenes*, unless bacteria were forming actin tails [13,14]. Consequently, it has been hypothesized and shown that *L. monocytogenes* avoids ubiquitination and autophagic recognition by expressing ActA, and ActA mutants are efficiently targeted by autophagy [11]. While the role of ActA in autophagy is now established, the role that many other surface proteins play during *Listeria* infection remains fragmentary [15].

The vault particle is the largest cytoplasmic ribonucleoprotein complex known to date [16]. Originally identified as contaminants of clathrin-coated vesicles preparation, these complexes were named vault particles because of their barrel shaped morphology resembling the ceiling of cathedrals [17]. Mammalian vaults are composed of the highly conserved major vault protein (MVP) constituting more than 70% of the mass of the particle [16,18,19] which spontaneously forms vault particles without the need of other vault components [20]. The two other vault components are the telomerase associated protein (TEP-1) [21] and the vault poly(ADP)ribose polymerase (vPARP) [22–24]. Vault preparations...
Author Summary

L. monocytogenes is a food-borne pathogen responsible for listeriosis, a severe illness with a high mortality rate, which mainly affects immunocompromised patients and pregnant women. The bacterium is a facultative intracellular pathogen able to invade, survive and multiply in large variety of cells. Although the infectious process at the cellular level has been extensively studied, the role of InlK, a surface protein specific to L. monocytogenes, remains unknown. Here we established that L. monocytogenes use InlK to interact with a mammalian cytoplasmic protein named Major Vault Protein (MVP). Although MVP has been implicated in diverse cellular processes, its role remains elusive. Here we demonstrate that, inside the cell, L. monocytogenes is able, via InlK, to decorate its surface with MVP to escape autophagy, an innate immune defense system that protects the cell from invading pathogens. L. monocytogenes uses this camouflage strategy to efficiently survive inside cells.

have additionally been shown to contain several small untranslated RNAs [25,26]. Vaults exist in thousands of copies per cell and are widely expressed in all eukaryotic organisms, from Dictyostelium discoideum to mammals, except plants, Saccharomyces cerevisiae, Caenorhabditis elegans and Drosophila melanogaster [27]. Diverse roles have been proposed for MVP and/or vaults [27], including roles in drug resistance [28], cellular differentiation [29], innate immunity [30], virus infections [31], signaling cascades [28,32–35] and cell survival [33,36]. However, the precise cellular function(s) of MVP and vaults remains poorly understood. In addition, the MVP/~− mice are viable, healthy and show no obvious abnormalities [37,38].

The genome sequence of L. monocytogenes EGD-e has revealed the presence of 25 genes encoding proteins of the internalin family [39]. Proteins of this family, which are characterized by the presence of leucine-rich-repeats (LRRs), are mostly surface proteins [40]. Their binding to the bacterial surface is mediated by different anchoring domains, in particular the LPXTG motif which allows a sortase A mediated covalent attachment to the peptidoglycan [41]. The invasion protein, Internalin, is one such protein [42]. Comparative post-genomic studies have established that several members of the L. monocytogenes internalin family are absent in L. innocua, a closely related non-pathogenic species [40]. Lmo1290 is an internalin gene absent in L. innocua, herein referred to as inlK, which is expressed at very low levels in brain-heart-infusion medium [43,44] and induced during infection [43].

In this study we investigated the role of InlK in the infectious process. We first explored the expression of InlK and the virulence phenotype of the inlK deletion mutant. We then searched for potential host partners of InlK and identified MVP. We demonstrated that the InlK/MVP interaction occurs in the cytosol of infected cells at the bacterial surface. Moreover, our results reveal that MVP recruitment protects L. monocytogenes from autophagic recognition, leading to an increase in bacterial survival in infected cells.

Results

L. monocytogenes inlK encodes a virulence factor

The gene bmo1290 (= inlK) is 1797 bp long. It is located 331 bp downstream from gene bmo1289 which is followed by a transcriptional terminator. Lmo1290 is also followed by a transcriptional terminator upstream from the divergently transcribed oatA gene which encodes a peptidoglycan O-acetyltransferase (Figure 1A) [43]. The inlK gene is present in all 22 L. monocytogenes genomes sequenced to date and absent from the genomes of L. ivanovii and all non-pathogenic Listeria strains including L. innocua (Figure 1A), L. sedlensis, L. welshimeri and L. grayi, suggesting that InlK could be involved in Listeria virulence.

To assess the role of InlK in virulence, we generated an inlK deletion mutant (ΔinlK) in the strain EGD-e. The ΔinlK mutant grew as rapidly as the wild-type (WT) in broth medium and infected cells (macrophages and epithelial HeLa cells) (data not shown). The LD50 of the ΔinlK mutant after intravenous (i.v.) injection in BALB/c mice was 2.2 × 10^{3} CFU, compared with 1.7 × 10^{3} CFU for the WT strain. Inactivation of inlK resulted in complete survival of animals infected intravenously with 10^{4} bacteria (Figure 1B). In contrast, infection with the same number of WT bacteria led to 100% mortality. Moreover, the number of CFU recovered from spleens and livers of i.v. infected BALB/c mice after 24 h, 48 h, 72 h and 96 h of infection was significantly lower (~1 Log_{10} for the mutant compared to the WT (Figure 1C), and virulence of the mutant was fully restored by complementation (Figure 1C). Together, these results establish a role for InlK in the virulence of L. monocytogenes.

InlK is expressed in vivo

InlK is a 598 amino acid LPXTG surface protein predicted to be anchored to the peptidoglycan by sortase A (Figure 2A). To address whether L. monocytogenes produces InlK in vivo, we first generated an antibody against a purified recombinant InlK protein (Figure S1A) and used it to detect the protein at the bacterial surface by immunofluorescence. In agreement with previous whole genome transcriptomic results that demonstrated a low expression level of inlK in vitro [44], bacteria grown in brain-heart infusion (BHI) medium were not stained by the InlK antibody (Figure 2B), suggesting that InlK protein was poorly expressed on the surface or not produced. We then showed that InlK was not detected in bacterial total extracts (Figure 2C), also in agreement with previous data indicating that InlK is not present in the cell wall proteome of L. monocytogenes EGD-e grown in BHI medium [46]. Moreover, consistent with the fact that the two major regulators of virulence genes, PrfA and sigmaB, were not required for basal inlK transcription [44,47], the InlK protein level was also not detectable when bacteria were grown in charcoal supplemented medium or at low pH (data not shown).

To verify that the inlK open reading frame encoded a surface protein, inlK was expressed under the control of two constitutive promoters active in Listeria. We used either the promoter of the protease gene from Lactococcus lactis subsp. cremoris on the multicopy plasmid pPRT-inlK or the promoter P Hyper after integration on the chromosom of the plasmid pADc-inlK [48,49]. InlK antibodies efficiently labeled InlK on the surface of bacteria that constitutively expressed inlK (Figure 2B) and also detected the protein in bacterial total extracts (Figure 2C). This labeling was specific, as the InlK antibody did not label WT or inlK mutant bacteria grown in same conditions. Interestingly, when InlK was over-expressed by Listeria under the control of constitutive promoters, a polypeptide with a lower mass than expected was also detected by Western-blot (Figure 2C) indicating that the protein may be processed. Moreover, InlK was not detected by immunofluorescence at the surface of a ΔoatA sortase mutant over-expressing inlK (Figure 2B), but was then detected in the supernatant of the culture medium (Figure 2D). Taken together these results established that, when inlK is expressed, the protein is anchored at the bacterial surface in a sortase A-dependent manner.
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A

B

C

Liver

Spleen

Number of CFU

24h 48h 72h 96h

Number of CFU

24h 48h 72h 96h

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Recently, a whole genome transcriptomic analysis of *L. monocytogenes* during infection revealed that the gene *inlK* was better expressed *in vivo* compared to growth in BHI [43]. We thus investigated whether the InlK protein was indeed produced *in vivo* by testing for the presence of anti-InlK antibodies. Purified InlK was submitted to migration on polyacrylamide gel (Figure S1A) and blotted with two different rabbit anti-Listeria sera. As shown in Figure 2E, a rabbit anti-*L. monocytogenes* serum obtained after immunization with killed bacteria was not able to detect the purified InlK whereas the serum directed against live bacteria detected InlK. This signal was specific to InlK, as the antibodies did not label bovine serum albumine (BSA) used at the same concentration.

To confirm *in vivo* *inlK* expression, we constructed an expression reporter vector in which the expression of the bioluminescent operon *luxABCDE* was under the control of *inlK* promoter (*pPL2-P_{inlK-luxABCDE}*) (Figure 2E, a rabbit anti-Listeria sera). Together, these results confirm that InlK is expressed *in vivo*.

**InlK interacts with the Major Vault Protein**

To identify InlK interaction partners in the eukaryotic cell, we used InlK as a bait in a large-scale yeast two-hybrid screen and identified the Major Vault Protein (*MVP*) as a prey with a very high interaction score (Table S1). To confirm this interaction we performed a bacterial pull down assay and showed that GST-MVP purified protein bound to InlK over-expressing bacteria, but not to WT bacteria (Figure 3A). This interaction was specific as (i) the WT strain (which expresses InLA, InIB and InIH) did not bind MVP (data not shown), (ii) the overexpression of InlK (i.e. another internalin not expressed in BHI [49]) was not able to mediate bacterial binding to MVP, and (iii) InlK over-expressing bacteria were not able to bind another GST fusion protein, GST-ScarA. Finally, bacterial incubation with MVP-GFP transfected cells lysates confirmed the interaction between InlK and MVP (Figure 3B). This interaction occurred when InlK was either expressed on a multicopy plasmid, or integrated in the chromosome (Figure S2A).

This interaction between purified InlK and endogenous MVP was confirmed by co-immunoprecipitation assays (Figure 3C). Indeed, when purified InlK was incubated with HT29 cell lysate, it interacted with endogenous MVP and the two partners co-immunoprecipitated, as shown using an anti-MVP antibody (Figure 3C). Similar results were obtained with stable HEK293-HTP-InlK cells that were engineered to express InlK in their cytosol, under the control of a tetracyclin inducible promoter (Figure S2B).

The InlK/MVP interaction occurs in the cytosol at the bacterial surface and does not depend on actin polymerization

In agreement with a specific interaction between InlK and MVP, we observed that InlK over-expressing bacteria co-localized with MVP in MVP-GFP transfected HeLa cells whereas the *inlK* mutant or wild type bacteria that do not express InlK *in vitro* did not co-localize with MVP (Figure 3D). As MVP has been mainly described as a cytoplasmic protein [51,52] and InlK is targeted and anchored to the bacterial surface (Figure 2D), we hypothesized that the InlK/MVP interaction should occur in the cytosol of infected cells after lysis of the internalization vacuole. To test this hypothesis, we analyzed the localization of MVP recruiting bacteria. A differential immuno-staining protocol allowing extra- and intracellular *Listeria* to be distinguished showed that MVP was recruited to intracellular bacteria (Figure 4A). Irrespective of the time post-infection, ~20% of InlK over-expressing bacteria were observed to recruit MVP [24.3%±3.0%; 16.2±2.5; 18.2±1.6 and 18.5±4.8 (mean ± SEM from n = 3 experiments) at 1 h, 2 h, 4 h and 8 h post-infection respectively] (Figure 4A, right panel). To determine whether MVP was recruited to intracellular bacteria before or after lysis of the internalization vacuole, we used a marker of early times points after vacuole escape, YFP-CBD, a YFP fused phage protein known to bind *L. monocytogenes* peptidoglycan as soon as the vacuole membrane begins to lyse (Figure S3A) [53]. Cells were co-transfected with MVP-Tomato and YFP-CBD, fixed 4 h post-infection and immuno-stained for actin. As expected, bacteria that polymerized actin were efficiently labeled with YFP-CBD (Figure 4B, Figure S3B inset 2), confirming that YFP-CBD efficiently labels intracytosolic *Listeria*. Moreover, all MVP-positive bacteria were also labeled with YFP-CBD (Figure 4B, Figure S3B) revealing that MVP was recruited by intracytosolic bacteria after lysis of the internalization vacuole.

Interestingly, we did not observe the co-recruitment of MVP-GFP and endogenous actin to intracellular bacteria (Figure 4B, Figure S3B). Co-recruitment was also not observed in infected cells previously co-transfected with MVP-GFP and actin-CFP (Figure S3C). We therefore analysed the kinetics of MVP and actin recruitment by performing live-cell imaging. Cells were co-transfected with MVP-Tomato and actin-CFP, and infected with InlK expressing *L. monocytogenes*. Strikingly, MVP was recruited rapidly by InlK over-expressing bacteria and could then be replaced by actin (Figure 4C and Video S1), showing that MVP recruitment occurs before actin polymerization. We then verified that MVP recruitment occurred independently of actin polymerization using a *actA* mutant. Intracytosolic Δ*actI* over-expressing InlK were efficiently labeled with MVP (Figure S3D). This MVP recruitment was more efficient than for wild type bacteria. Indeed, the percentage of intracytosolic Δ*actI* over-expressing InlK having recruited MVP at 4 h post-infection was 83.2±12.7% (mean ± SEM from n = 3 experiments), compared to the 16.8±2.5% (mean ± SEM from n = 3 experiments) observed when using the InlK over-expressing strains that are able to polymerize actin via ActA. Together, these results suggested that ActA at least partially...
A

**InlK (598 AA)**

1  MSVKSNIVKGVCFAYAVPVSQTTPVFAQEFGPLKASQVNHVNPDS
2  YNGCIGGTYT-FIT-QMD
3  SRTYEGAMN---V7BDGFEVYSH
95  NFDDTFNTHH---FNTNGSSS
117  NLDDCNKII---YPSKL-DRS
138  CSNRTDDQCHSHENTDQSTKQ
165  ETTTSDAGYNG---AA---ETDMPQKTMP
188  EPLSAQPFQDG---VHDYRGCEDPF
210  RDTLYAVTSQ---GCKPKI--HKK
233  SGLTQNENQTLVYPFLMTDRTVNYDGVYFDFVKSSTAATSFYFTTTMNEQVQVN
290  QDDDPQMQDQDQDDDPQMQDQ
347  YFVTVDLSNLNZADSEKVTKTNIQEVRTEATFLSLDIHAKTDGGSTVTSDFADVDFNTPG
404  TTYVTQENAGLHQTSPVQTVNITIKAKTITADEKITYKVDTSKTEAEFLADIKAT
461  TNDGATAFADTFVTDRLSPGGKTVVTTLAENLQKALPQTVQMVIVEKTP1PDPPTT
518  PTFDPPTDPDPSTPVPWNPWKPVPFSYKIPSLTVNHKQKAAEGFSKAEPLRTDP
575  SLFVGACTPCILGSQWFSRK

B

Phase | α InlK | Overlay
--- | --- | ---
**WT** | | |
**ΔinlK** | | |
**WT + pAdc inlK** | | |
**ΔinlK + pPRT inlK** | | |
**ΔsrtA + pPRT inlK** | | |

C

**Total lysate**

**α InlK**

D

**Culture supernatant**

ΔsrtA + pPRT inlK

E

**500 ng**

- α anti-killed *Listeria*
- α anti-live *Listeria*
- Pre-immune serum
- α InlK

**200 ng**

- α InlK
- BSA
impairs MVP recruitment. As ActA protects bacteria from autophagy [11], these data also suggested that both InlK and ActA may protect bacteria from autophagy.

**MVP recruitment protects *Listeria* from autophagy**

To test if MVP recruitment could lead to autophagic escape, we used two well-established markers of autophagy, p62 (SQSTM1) and LC3 (Atg8) [54]. p62 has emerged as the prototypic adaptor involved in directing cytoplasmic substrates towards autophagic degradation [35], p62 interacts with ubiquitinated substrates via its ubiquitin-binding domain, and links them to the autophagosomal structural protein LC3. We infected MVP-transfected HeLa cells with InlK over-expressing bacteria for 4 h and after fixation, immuno-stained for endogenous p62 and actin. No co-localization could be observed between MVP and p62 (Figure 5A, Figure S4A) or MVP and LC3 (Figure 5B, Figure S4B). Interestingly, the vast majority of MVP-positive bacteria were completely devoid of anti-p62 labeling (95.7±2.0%; mean ± SEM from n = 3 experiments) but 4.9±2.0% (mean ± SEM from n = 3 experiments) were stained at one pole with MVP and at the other pole with p62. Similar results were obtained using GFP-LC3 (Figure 5, Figure S4B). As previously demonstrated [11], bacteria that had started to recruit actin were not labeled by p62 (Figure 5A) or GFP-LC3 (Figure 5B). Strikingly, when the MVP-positive bacteria that recruit actin were not labeled by p62 (Figure 5A) or GFP-LC3 (Figure 5B), the expression of InlK by the ΔactA strain restored the intracellular survival rate to the level of WT bacteria (Figure 6B), indicating that InlK could functionally replace ActA in its role in autophagy escape. Infection of MVP-transfected epithelial cells with ΔactA and ΔactA+InlK led to similar results (Figure 6C and 6D).

The intracellular survival of ΔactA and ΔactA+InlK was then analysed in RAW 264.7 macrophages treated with control or MVP siRNA (Figure 6E). As previously observed (Figure 6B), the ΔactA+InlK strain replicated faster than the ΔactA strain in control cells (Figure 6E). Strikingly, in MVP-depleted cells, the ΔactA+InlK strain did not replicate faster than the ΔactA strain (Figure 6F), confirming the role of InlK/MVP interaction in survival rate. Taken together, these data show that the specific recruitment of MVP to the bacterial surface via InlK leads to a better survival of *L. monocytogenes*.

**Discussion**

*L. monocytogenes* has emerged as a paradigm to study host-pathogen interactions and fundamental processes in cell biology [5,59]. However, the role of many proteins expressed on the bacterial surface during *Listeria* infection remains fragmentary [15]. In this study we report that InlK, a *L. monocytogenes* surface protein of the internalin family, plays a critical role in *Listeria* virulence. We show that InlK is anchored to the listerial surface through its LPXTG peptidoglycan anchoring signal by sortase A and is produced during *in vivo* infection, whereas it cannot be detected on bacteria grown in BHI medium [44] or within the cytosol of tissue-cultured cells. This *in vivo* specific expression profile was previously described for other virulence factors of *L. monocytogenes*, e.g. the internalin InlJ, that behaves as an adhesin [49] and recently LntA, a secreted bacterial protein involved in chromatin remodeling and type III interferon response [60]. Furthermore, our results confirm and extend our recently published transcriptomic analysis of *L. monocytogenes* [43] which identified inlK as a gene highly activated during *in vivo* infection and that may play a role in the infectious process. Together, our results demonstrate that InlK is a so far undescribed virulence factor of *L. monocytogenes*.

To enter, survive and spread from cell-to-cell, *L. monocytogenes* has been shown to interact with several host partners. We revealed here that MVP is a specific cellular interactor of InlK. The highly conserved MVP protein constitutes more than 70% of the mass of

**Figure 2. InlK is expressed in vivo.** A. InlK amino acid sequence. The signal sequence is underlined and the different regions of leucine rich repeats (LRRs) are outlined. The consensus pentapeptide LPXTG at the C-terminal end is boxed. B. Detection by immunofluorescence microscopy of InlK over-expressing in *L. monocytogenes* EGD-e (WT), ΔinlK, WT+ΔactA-ΔinlK, ΔactA+inlK and the EGD-e mutant over-expressing inlK (ΔactA+pPRT-inlK) grown in BHI medium using the rabbit polyclonal anti-InlK antibody. InlK was detected at the surface of InlK over-expressing bacteria (WT+pPRT-inlK and ΔinlK+pPRT-inlK), whereas it was undetectable at the surface WT bacteria or at the surface of the ΔactA mutant over-expressing inlK. C. Detection of InlK by Western blot on total lysates of *L. monocytogenes* EGD-e (WT), ΔinlK and ΔactA+pPRT-inlK grown in BHI using the rabbit polyclonal anti-InlK antibody. Decreased concentrations of recombinant purified InlK were used as a positive control. D. Detection of secreted InlK in the supernatant of ΔactA mutants over-expressing InlK. Western blotting was carried out on trichloroacetic acid precipitates of ΔactA and ΔactA+pPRT-inlK culture (OD_{600}=1) supernatants using the rabbit polyclonal anti-InlK antibody. doi:10.1371/journal.ppat.1002168.g002
L. monocytogenes Avoids Autophagy Via InlK

A

\[ \text{WT}^+ \text{pPRT empty} \quad \Delta \text{inlK}^+ \text{pPRT empty} \quad \Delta \text{inlK}^+ \text{pPRT inlK} \quad \text{WT}^+ \text{pPRT inlK} \]

\[ \text{input purified GST-MVP} \]

\[ \alpha \text{MVP} \]

\[ \alpha \text{InlK} \]

\[ \text{inlK} \]

\[ \alpha \text{GST} \]

\[ \alpha \text{InlK} \]

\[ \text{input purified GST-ScarA} \]

B

\[ \text{WT}^+ \text{pPRT empty} \quad \Delta \text{inlK}^+ \text{pPRT empty} \quad \Delta \text{inlK}^+ \text{pPRT inlK} \quad \text{WT}^+ \text{pPRT inlK} \]

\[ \text{input MVP-GFP transfected cell lysate} \]

\[ \alpha \text{MVP} \]

\[ \alpha \text{InlK} \]

\[ \text{inlK} \]

C

\[ \text{HT29 lysate} \quad - \quad + \quad \text{Purified InlK} \quad - \quad + \quad \text{Lysis Buffer} \]

\[ \text{WB : } \alpha \text{MVP} \]

\[ \text{WB : } \alpha \text{InlK} \]

\[ \text{WB : } \alpha \text{InlK} \]

D

\[ \text{MVP-GFP} \quad \text{Listeria} \quad \text{Merged} \]

\[ \text{WT} \]

\[ \Delta \text{inlK} \]

\[ \Delta \text{inlK}^+ \text{pPRT inlK} \]
the largest cytoplasmic ribonucleoprotein (RNP) complex known, i.e., vault particles [16,18,19]. Since its first description in 1986 [61], several putative functions have been attributed to this RNP complex. Data that link the vault complex to various functions have suggested roles in multidrug resistance [28,62], transport [63], signaling [28,32–34] and apoptotic resistance [33,36] or innate immunity [63]. However, no compelling evidence for a cellular role was reported unequivocally and MVP was mainly considered as a scaffold protein. Nevertheless, vaults were previously found to be implicated in g-herpesvirus (Epstein-Barr and Kaposi’s sarcoma virus) [31,64] and Pseudomonas aeruginosa infectious processes [30]. During Epstein-Barr or Kaposi’s sarcoma virus infection, the expression of vault RNAs (vRNAs) was shown to be specifically up-regulated in human lymphocytes [31,64]. However, the function of this overexpression was not assessed. In addition, not only vRNA but also MVP was reported to be upregulated during viral infection by human T-cell lymphotropic virus type I (HTLV-I) infection [65]. In the case of bacteria, MVP was implicated in host resistance to P. aeruginosa lung infection [30]. Indeed, a rapid recruitment of MVP to lipid rafts was observed when human lung epithelial cells were infected with P. aeruginosa. This recruitment was dependent on bacterial binding to the cystic fibrosis transmembrane conductance regulator CFTR. Moreover, no evidence of direct binding between MVP and bacteria was observed.

As with a variety of intracellular microbes, intracytosolic L. monocytogenes are recognized by autophagy, a cell-autonomous effector mechanism of innate immunity that protects the cytosol against bacterial invasion [66]. Perrin et al. first demonstrated that cytosolic L. monocytogenes occasionally colocalized with ubiquitin in infected cells, and this association was more frequent in case of the ∆actA strain [13,14]. More recently, Yoshikawa et al. demonstrated that the recruitment of VASP, Arp2/3 complex and actin via ActA protect bacteria from ubiquitination and autophagic recognition [11]. Here we reveal that L. monocytogenes has a second strategy to escape autophagy in the absence of ActA (Figure 7). Indeed, no significant difference could be observed between the intracellular survival rate of WT and WT+InlK bacteria in infected RAW 264.7 macrophages (Figure 6B), suggesting that when ActA is expressed it is sufficient for Listeria to escape from autophagy. In contrast, in absence of ActA, InlK protects against autophagy. Together, our results show that the bacteria are able, via InlK, to decorate their surface with MVP in order to escape from autophagy (Figure 7). It will be thus of the highest importance to decipher in which cells InlK is expressed in vivo and when the InlK/MVP interaction takes place during infection. These data will be critical to unravel the role of InlK in the pathophysiology of Listeria infection. It will also be of great interest to further study the link between actin polymerization, MVP, autophagy, and pathogen dissemination.

Materials and Methods

Bacterial strains, growth conditions and reagents

Listeria strains (Table S2) were grown in brain-heart infusion (BHI) medium (Difco; BD) and Escherichia coli were grown in Luria-Bertani Medium (LB) medium (Difco; BD). When required, chloramphenicol and erythromycin were used at final concentrations of 7 μg/ml and 5 μg/ml respectively for L. monocytogenes and kanamycin, erythromycin and chloramphenicol were used at final concentration of 50 μg/ml, 150 μg/ml and 33 μg/ml, respectively for E. coli.

Generation of EGD-e ∆inlK mutant strain and inlK over-expressing strains

Generation of ∆inlK mutant strain. Two ~700 pb fragments flanking inlK gene were PCR amplified from EGD-e chromosomal DNA. The primers used for the inlK 5’ flanking fragment were A (5’-TTG GAT CTT TAG ATT TCA CAA AAG-3’ and B (5’-TAA CAC GGG TCA GTC ATT ATC CTC TCC ACT C-3’), and the primers used for the 3’ fragment were C (5’-GAA AAC GCC TAA AAA ACT ATC CGC CCA G-3’) and D (5’-TTG GTC CAT GGT TAA GCA GTA TTG CTG GTC-3’). After restriction of the amplified 5’ and 3’ fragments with BamHI and MluI, and MluI and NoI respectively, 5’ and 3’ fragments were coligated in the thermosensitive plasmid pMAD [67] digested by BamHI and NoI, yielding the pMAD-∆inlK plasmid. The sequence was verified by sequencing. This plasmid was electroporated into L. monocytogenes EGD-e. Independent colonies were used for allelic exchange in L. monocytogenes wild-type EGD-e, which was performed as previously described [49], generating a ∆inlK isogenic deletion mutant (Table S2). Deletion of the entire inlK gene was confirmed by PCR amplification and sequencing.

Generation of InlK over-expressing strains. To express InlK in L. monocytogenes the pPRT- and pADc- derivative plasmids were constructed as described below. In the pPRT-inlK plasmid, inlK was expressed under the control of the promoter region of the protease gene from Lactococcus lactis subsp. cremoris, which is active in Listeria [49]. This is a multicopy plasmid which expresses an erythromycin resistance gene used for cloning selection. The pADc-inlK plasmid generated as previously described by Balestrino et al. [48] was derived from the integrative pPL2 plasmid, which inserts in the Listeria chromosome at the tRNAArg-atlBB site, thereby avoiding the requirement for antibiotic pressure to maintain the plasmid and preventing heterogeneity of InlK expression due to variation in the plasmid copy number.
Figure 4. InlK/MVP interaction occurs in the cytosol, before actin polymerization. A. Detection of MVP recruitment at the surface of intracellular InlK over-expressing bacteria. HeLa cells were transfected with MVP-GFP (red), infected with InlK expressing Listeria (ΔinlK+pPRT-inlK) for 4 h, fixed for fluorescence light microscopy. Intra- (only green) and extracellular (cyan = green+blue) bacteria were differentially stained with anti-Listeria antibody (cf Material and Methods). Inset regions are magnified. Arrows indicate another intracellular bacterium which recruit MVP-GFP. The scale bar represents 1 μm. The right panel represents the quantification of the intracellular bacteria that recruit MVP (mean%±SEM%) shown in the left panel. Statistical analyses were performed on the results of 3 independent experiments using the Student’s t test. No significant difference was
found between the 4 time points. B. Detection of MVP recruitment at the surface of intracytosolic InlK over-expressing bacteria. HeLa cells were transfected with MVP-tomato (red) and YFP-CBD (green), infected with InlK over-expressing *Listeria* (ΔinlK-pPRT-inlK) for 4 h, fixed for fluorescence light microscopy and stained with phalloidin (blue). MVP positive bacteria were also labeled with YFP-CBD revealing that MVP was recruited by intracytosolic bacteria after the lysis of the internalization vacuole. Inset regions are magnified. The scale bar represents 1 μm. C. Kinetics of MVP and actin recruitment at the surface of InlK over-expressing bacteria. HeLa cells were transfected with MVP-tomato (red) and actin-GFP (green), infected with InlK over-expressing *Listeria* (ΔinlK-pPRT-inlK) for 4 h, and prepared for real-time video microscopy. Image series were collected every 15 min for 2 h. The left part shows an MVP positive bacterium that never recruits actin. The right part shows MVP replacement by actin around the bacterium. No colocalization of MVP-Tomato and actin-GFP was detected. Time is indicated along the Y axis. The entire image sequence can be viewed as Video S1.

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**Cell lines and infection**

HeLa cells (human epithelial cervix carcinoma; ATCC CCL2), JEG3 cells (human placenta chorionicarcoma, ATCC HTB-36) and RAW 267.4 murine macrophages (ATCC TIB-71) were grown as recommended by ATCC (Manassas, VA). Cells were infected with exponentially growing *Listeria* strains such that the multiplicity of infection was 50 bacteria per cell (MOI50) for epithelial cell lines and MOI10 for RAW 267.4 macrophages. After 1 h of infection for epithelial cell lines and 15 min for RAW 267.4 macrophages, cells were washed and treated with 25 μg/ml of gentamicin. Incubation times were as indicated. All experiments were performed in serum-free medium. Then, cells were washed three times with PBS 1X (Difco, BD) and lysed by adding 50 μl of 0.1% Triton X-100. The number of viable bacteria released from cells was assessed by plating serial dilutions of bacteria on agar plates.

**siRNA experiments**

2.5×10⁵ RAW 267.4 macrophages per well were plated in 12 wells plates and incubated at 37°C in 10% CO₂. 24 h after plating, cells were treated with 80 nM of either a pool of anti-mouse MVP siRNA (ON-TARGETplus SMART pool L-049201-01-005 Mouse MVP, Dharmacon) or control siRNA (ON-TARGETplus Non-targeting siRNA:1, Dharmacon), using Lipofectamine 2000 (Invitrogen) as recommended by the manufacturer. The following day, the medium was changed and the cells were incubated in complete medium for another 24 hours. Infections were performed as above-mentioned and the efficiency of siRNA knock-down was assessed by performing Western-blot on total cell lysates in each experiment (Figure 6E).

**InlK purification**

The *inlK* coding sequence (aa 27–568) was amplified using primers *lmo1290-Fw: 5’- GAG TCG GAT CCG GTA TTT GCT pPRT-3’* and *lmo1290-Rev: 5’- GAG TCG TGC GAG TCG GAT CCG GTA TTT GCT pPRT-3’,* and subjected to double digestion with *BamHI* and *SalI* enzymes. The ligation product was electroporated in *E.coli* XL-1 Blue and positive clones were selected on 50 μg/ml supplemented BHI and sequenced (BUG 2812). For purification, *E.coli BL21 (DE3) (Invitrogen)* were chemically transformed with the purified His₅-InlK-His₅ expressing pET28b plasmid. Bacteria were grown in 50 μg/ml supplemented LB until OD₆₀₀ 0.6 and IPTG was added at the final concentration 1 mM for 4 additional hours. Bacteria were lysed using a French press and the supernatant was recovered. His₅-InlK-His₅ purification was performed using TALON His-Tag Purification Resins (Clontech). Increased concentration of imidazole (0–200 mM) in Tris-HCl 20 mM, NaCl 0.5 M (pH = 8) were used for purification and elution of InlK. The purified protein was dialysed against Tris-HCl 20 mM, NaCl 0.5 M (pH = 8) buffer and concentrated using AmiconUltra centrifugal filter (Millipore).

**Antibodies and reagents**

The primary antibodies used in this study were anti-actin mouse monoclonal (mAb) (AC-15; Sigma-Aldrich), anti-LRP mAb (MVP was also named LRP for Lung resistance protein) (Ref:610152; BD Biosciences), anti-p62 mAb (Ref:610832, BD Biosciences), anti-ubiquitin mAb (FK-2, Affiniti), anti-Atg8 (LC3) rabbit polyclonal (pAb) (Novus Biologicals, Ref:NB100-2331), anti-killed *L. monocytogenes* pAb (R11), anti-live *L. monocytogenes* pAb (R177). Monoclonal antipeptide antibody that recognizes ActA (A16) was obtained as previously described [68]. An anti-InlK polyclonal rabbit antibody (R190) was generated against His₅-InlK-His₅ recombinant protein (aa 27–568) deleted from its signal peptide and pep tidoglycan-anchoring sequence and affinity-purified on a ECH Sepharose 4B column (GE Healthcare) coupled with 2.5 mg His₅-InlK-His₅ recombinant protein expressed from pET28b-InlK plasmid as described above. The polyclonal pre-immune serum of R190 (pre-immune R190) was recovered from rabbits before they were s.c injected with purified InlK. The secondary antibodies were Alexa Fluor 488- and 546-conjugated goat anti-mouse and anti-rabbit, respectively (Molecular Probes) and HRP-conjugated goat anti-mouse and goat anti-rabbit (AbCys). Alexa fluor 647-conjugated phallolidin was purchased from Molecular Probes; DAPI from Roche Applied Sciences; and the Amersham ECL Plus kit from Ge Healthcare.

The GST-tagged purified recombinant MVP protein was purchased from Abnova (Ref:H00009961-P01).

**Immunofluorescence microscopy**

Cells were fixed in 4% paraformaldehyde (PFA) in 1X PBS for 20 minutes at room temperature. Cells were then rinsed in 1X PBS before incubation in blocking solution (0.3% BSA, 50 mM NH₄Cl in PBS, pH 7.4) containing 0.05% saponin. Cells were then incubated with the primary antibodies diluted in the blocking solution for 30 min at room temperature, rinsed 3 times in 1X PBS and further incubated for 30 minutes with the secondary antibodies diluted in blocking solution. Where needed, fluorescent phallolidin was added with the secondary antibodies to label actin. Cells were then rinsed 5 times in 1X PBS and mounted on glass coverslip using Fluoromount mounting medium (EMS, PA). The differential immuno-staining between extra- and intracellular *Listeria* was previously described [69]. Samples were analysed either with a Zeiss Axiosvert 135 epifluorescence microscope connected to a CCD camera or with a Zeiss LSM510 confocal microscope (Carl Zeiss, Germany). Images were acquired with a 100X oil immersion objective and images were processed using MetaMorph (Universal Imaging Corp.).

**Plasmids**

The MVP-GFP plasmid that encodes EGFP fused to MVP C-terminus has been previously described [70]. To construct MVP-CFP (BUG 2908) and MVP-Tomato (BUG 2909), the MVP coding sequence was isolated from the MVP-GFP (BUG 2907) plasmid by double enzyme digestion (*HindIII* and *BamHI*) and
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Figure 5. MVP impairs the recruitment of autophagy markers. A. Impaired recruitment of p62 to MVP positive Listeria. HeLa cells were transfected with MVP-GFP (green), infected with InlK over-expressing Listeria (∆inlK+PRT-inlK) for 4 h, fixed for fluorescence light microscopy, and stained with phalloidin (blue) and anti-p62 antibody (red). Inset regions are magnified. Arrows indicate independent bacteria. The scale bar represents 1 μm. The vast majority of MVP-positive bacteria were completely devoid of anti-p62 labeling (95.1 ± 2.0%; mean ± SEM from n = 3 experiments) but 4.9 ± 2.0% of cells were stained with phalloidin (blue) and anti-p62 antibody (red). Inset regions are magnified. Arrows indicate independent bacteria. The scale bar represents 1 μm. B. Impaired recruitment of GFP-LC3 on MVP positive Listeria. HeLa cells were transfected with MVP-tomato (red) and GFP-LC3 (green), infected with InlK over-expressing Listeria (∆inlK+PRT-inlK) for 4 h, fixed for fluorescence light microscopy, and stained with phalloidin (blue) and anti-p62 antibody (red). Inset regions are magnified. Arrows indicate independent bacteria. The scale bar represents 50 μM, pH 7.5, NaCl 150 mM, EDTA 2 mM, NP40 0.2%.

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 ligated in pECFP-N1 and ptdTomato-N1. Briefly, ptdTomato-N1 was constructed by replacing EGFP, from pEGFP-N1 vector (Invitrogen), by tdTomato, from ptdTomato-LcA vector (BUG 2420) [69]. Plasmid encoding CBD-YFP (BUG 2305) [53], actin-GFP (BUG 2421), actin-CFP (BUG 2155) and GFP-LC3 (BUG 3046) [71] were described elsewhere. Cells transfections were performed with FuGENE HD (Roche) as recommended by the manufacturer.

Bacterial pull down assay

To test the binding of bacteria to GST-MVP, L. monocytogenes strains were grown in BHI to an OD600 of 1.0, and 1 ml of each culture was taken for each reaction. Bacterial cells were washed twice in buffer with 20 mM HEPES pH 7.5, 150 mM NaCl, resuspended in 1 ml of binding buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, and 0.2% BSA), and incubated at room temperature on a rotating wheel for 30 min. GST-MVP recombinant protein was added to a final concentration of 1 μg/ml and samples were incubated with rotation for an additional hour. Samples were centrifuged and pellets were washed three times in 20 mM HEPES pH 7.5, 300 mM NaCl, 0.05% Tween 20 and three times in buffer lacking Tween 20. The final bacterial pellets were resuspended in 20 microliters of 2X sample buffer, boiled for 10 min, and stored at −20°C before migration on 8% SDS-PAGE gels and Western blotting.

To analyse the binding of bacteria to transfected MVP-GFP HeLa cell lysates, HeLa cells were grown on 75 cm² flask, then transfected with MVP-GFP plasmid 24 h prior to the experiment. Cells were lysed at 4°C for 10 min in a 1 ml of lysis buffer (Tris-HCl 50 mM, pH 7.5, NaCl 150 mM, EDTA 2 mM, NP40 1%, AEBSF 1 mM, Na2VO4 3 mM). Cells were scraped and the lysates were incubated with rotation for 10 min, and stored at −20°C before migration on 8% SDS/PAGE gels and Western blotting.

Bacterial pull down assay

For yeasts, plasmids containing the inlK gene were expressed from the constitutive promoter ADE2. Cells were transformed using standard methods and grown on minimal medium containing casamino acids and either 3% glucose or 2% galactose. Western blots of purified proteins were probed with rabbit polyclonal antibody against InlK and antiserum against actin. The images shown are representative of three independent experiments.

Western blot. The immunoblots shown are representative of three independent experiments. Right panel: Quantification of the relative LC3-II level (mean ± SEM) shown in the left panel. Statistical analyses were performed on the results of 3 independent experiments using the Student’s t test. P values of <0.05 were considered statistically different.

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Murine infection experiments

All experiments were performed according to Institut Pasteur guidelines for laboratory animal husbandry. For determination of LD50, groups of 8-week-old BALB/c female mice (Charles River Laboratory) were injected i.v. with increasing concentrations of *L. monocytogenes* WT strain or ΔinlK mutant. LD50 were determined by the probit method at 10 days after inoculation.

Bacterial growth in mice was determined by injecting 6- to 8-week-old female BALB/c mice intravenously with a sublethal bacterial inoculum, 10^4 CFU. After 24, 48 72 and 96 h of infection, liver and spleen were dissected in sterile conditions and the numbers of CFU were determined by plating serial dilutions of organ (liver and spleen) homogenates on BHI agar medium.

Ethics statement

All animals were handled in strict accordance with good animal practice as defined by the relevant national and local animal welfare bodies, and all animal work was approved by the Institut Pasteur.

Figure 6. MVP-dependent escape from autophagy leads to increased *Listeria* survival. A. InlK and ActA expression in *Listeria* strains used for survival assays. Total lysates of *L. monocytogenes* EGD-(pADc-GFP), EGD-(pADc-inlK), ΔactA-(pADc-GFP) and ΔactA-(pADc-inlK) grown in BHI were immunoblotted using anti-ActA and anti-InlK antibodies. B. Intracellular survival of EGD-(pADc-GFP), EGD-(pADc-inlK), ΔactA-(pADc-GFP) and ΔactA-(pADc-inlK) in RAW 26.74 macrophages. Statistical analyses were performed on the results of 3 independent experiments using the Student’s t test. P values of <0.05 were considered statistically different and are labeled here as *. C. Intracellular survival of ΔactA-(pADc-GFP) and ΔactA-(pADc-inlK) in MVP-GFP transfected Jeg3 cells. Statistical analyses were performed on the results of 3 independent experiments using the Student’s t test. P values of <0.05 were considered statistically different and are labeled here as *. D. Intracellular survival of ΔactA-(pADc-GFP) and ΔactA-(pADc-inlK) in MVP-GFP transfected HeLa cells. Statistical analyses were performed on the results of 3 independent experiments using the Student’s t test. P values of <0.05 were considered statistically different and are labeled here as *.

Figure 7. Model for escape of autophagic recognition for *L. monocytogenes* expressing InlK. During intracellular growth, cytoplasmic bacteria are able to escape from autophagy process using two independent virulence factors, ActA and InlK. On the one hand, the recruitment of VASP, Arp2/3 complex and actin via ActA masks the bacteria from ubiquitination and autophagic recognition. On the other hand, MVP recruitment via InlK is also able to protect bacteria from ubiquitination and autophagic recognition. In that way, depending on ActA and InlK expression, four possibilities could be distinguished: (1) When neither ActA nor InlK are expressed, the bacterial ubiquitination is followed by p62 and LC3 recruitment, leading to autophagosome formation around the bacterium. (2) When ActA is expressed (e.g. wild-type bacterium (WT) grown in BHI before cell infection) it is sufficient for *Listeria* to escape from autophagy. (3) In contrast, in the absence of ActA, InlK efficiently protects bacteria against autophagy recognition via MVP recruitment. (4) Finally, when ActA and InlK are co-expressed by the bacterium, InlK rapidly recruits MVP at the surface of the bacterium. Then, in some instance, ActA replaces InlK leading to a switch of the bacteria disguised from MVP to actin. The model is partially based on the results of Yoshikawa et al [11].

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Pasteur animal experimentation committee which comply with European regulations (directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes).

Supporting Information

Figure S1 In vitro, in cellulo and in vivo expression of inlK. A. Coomassie staining of purified InlK recombinant protein and bovine serum albumine (BSA). B. In cellulo and in vitro expression of inlK revealed by bioluminescence. Left panel: RAW 264.7 macrophages and HeLa epithelial cells were infected for 4 h with wild-type L. monocytogenes EGD-e that contain a bioluminescent reporter of either inlK promoter [EGD-e-(pPL2-Padk-luxABCD)] or hly promoter [EGD-e-(pPL2-Ppadk-luxABCD)], and submitted to photon detection with IVIS 100 (Xenogen/Caliper) system. Right panel: EGD-e-(pPL2-Ppadk-luxABCD) and EGD-e-(pPL2-Ppadk-luxABCD) were grown in BHI to OD600 1.0 and submitted to photon detection with IVIS 100 (Xenogen/Caliper) system. C. In vivo expression of inlK revealed by bioluminescence. Left panel: Five BALB/c mice were i.v. infected with either EGD-e-(pPL2-Ppadk-luxABCD) or EGD-e-(pPL2-Ppadk-luxABCD). Each 24 h mice were anesthetized and submitted to photon detection with IVIS 100 (Xenogen/Caliper) system. Right panel: Quantification of the CFU number recovered from livers and spleens of infected mice, 72 h post infection. NS = No significant difference. (TIF)

Figure S2 InlK interacts with MVP. A. Bacterial pull-down of MVP-GFP from transfected HeLa cell lysates with the L. monocytogenes strains EGD ΔactA-(pADc-GFP), EGD ΔactA-(pADc-inlK) and EGD-e ΔinlK-(pPRT-inlK). MVP-GFP bound to InlK over-expressing bacteria but not to other bacteria. B. Co-immunoprecipitation of InlK and endogenous MVP in stable over-expressing bacteria but not to other bacteria. B. Co-inlK and InlK. The scale bar represents 1 μm. D. Detection of MVP recruitment at the surface of intracytosolic ΔactA and InlK over-expressing ΔactA Listeria. HeLa cells were co-transfected with MVP-tomato (red) and CBD-YFP (green), infected with ΔactA or ΔactA-(pADc-inlK) for 4 h, and fixed for fluorescence light microscopy. Inset regions are magnified. The scale bar represents 1 μm. The percentage of intracytosolic ΔactA over-expressing InlK having recruited MVP at 4 h post-infection was 88.3±12.7% versus no recruitment for ΔactA. (TIF)

Figure S4 MVP and autophagy markers do not co-localize. A. Impaired recruitment of p62 to MVP positive Listeria. HeLa cells were transfected with MVP-GFP (green), infected with InlK over-expressing Listeria (ΔinlK+pPRT inlK) (left panel) or ΔinlK Listeria (right panel) for 4 h, fixed for fluorescence light microscopy, and stained with phalloidin (blue) and anti-p62 antibody (red). Inset regions are magnified. Arrows indicate independent bacteria. The scale bar represents 1 μm. The vast majority of MVP-positive bacteria were completely devoid of anti-p62 labeling (95.1±2.0%; mean ± SEM from n = 3 experiments) but 4.9±2.0% (mean ± SEM from n = 3 experiments) were stained at one pole with MVP and at the other pole with p62. B. Polar recruitment of GFP-LC3 to MVP positive Listeria. HeLa cells were transfected with MVP-tomato (red) and GFP-LC3 (green), infected with InlK over-expressing Listeria (ΔinlK+pPRT inlK) for 4 h, fixed for fluorescence light microscopy. Four different bacteria are shown. The scale bar represents 1 μm. C. Quantification of MVP and LC3 recruitment at the surface of ΔactA overexpressing Listeria (mean ± SEM%). Quantifications correspond to the data represented in Figure S5D. The percentages MVP+/LC3+ bacteria, MVP+/LC3-, MVP-/LC3+ and MVP-/LC3- were 88.6±12.8%, 0.0±0.0%, 8.2±9.3% and 3.1±3.5% respectively. Statistical analyses were performed on the results of 3 independent experiments using the Student’s t-test. (TIF)

Table S1 L. monocytogenes strains used in this study. (DOC)

Table S2 Results of the yeast two-hybrid screening. The L. monocytogenes wild-type [72] and the ΔactA [73] were previously published. (DOC)

Video S1 Kinetics of MVP and actin recruitment at the surface of InlK over-expressing bacteria. HeLa cells were transfected with MVP-tomato (red) and actin-GFP (green), infected with InlK over-expressing Listeria (ΔinlK+pPRT inlK) for 4 h, and prepared for real-time video microscopy. Image series were collected every 15 min for 2 h. (AVI)

Video S2 Kinetics of autophagy escape for MVP positive Listeria. Jeg3 cells were transfected with MVP-tomato (red) and GFP-LC3 (green), infected with InlK over-expressing Listeria (ΔinlK+pPRT inlK) for 4 h, and prepared for real-time video microscopy. Image series were collected every 5 min for 2 h. The video shows that the GFP-LC3 membranous aggregate detaches from the MVP positive Listeria. (AVI)

Video S3 Kinetics of autophagy escape for MVP positive Listeria. Jeg3 cells were transfected with MVP-tomato (red) and GFP-LC3 (green), infected with InlK over-expressing Listeria (ΔinlK+pPRT inlK) for 4 h, and prepared for real-time video microscopy. Image series were collected every 5 min for 2 h. The
video shows that the GFP-LC3 membranous aggregate on MVP positive bacteria does not lead to an autophagosome formation, whereas those bacteria efficiently divided. (AVI)

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Author Contributions

Conceived and designed the experiments: LD SM PC. Performed the experiments: LD ASL EG MAN OD. Analyzed the data: LD SM ASL EG MAN OD PC. Contributed reagents/materials/analysis tools: LD SM ASL EG MAN OD EACW PC. Wrote the paper: LD SM PC.
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