AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity

InlP, a New Virulence Factor with Strong Placental Tropism

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Intrauterine infection is a major detriment for maternal-child health and occurs despite local mechanisms that protect the maternal-fetal interface from a wide variety of pathogens. The bacterial pathogen *Listeria monocytogenes* causes spontaneous abortion, stillbirth, and preterm labor in humans and serves as a model for placental pathogenesis. Given the unique immunological environment of the maternal-fetal interface, we hypothesized that virulence determinants with placental tropism are required for infection of this tissue. We performed a genomic screen in pregnant guinea pigs that led to the identification of 201 listerial genes important for infection of the placenta but not maternal liver. Among these genes was *lmrg1778* (*lmo2470*), here named *inlP*, predicted to encode a secreted protein that belongs to the internalin family. InlP is conserved in virulent *L. monocytogenes* strains but absent in *Listeria* species that are nonpathogenic for humans. The intracellular life cycle of *L. monocytogenes* deficient in *inlP* (Δ*inlP*) was not impaired. In guinea pigs and mice, InlP increased the placental bacterial burden by a factor of 3 $\log_{10}$ while having only a minor role in other maternal organs. Furthermore, the Δ*inlP* strain was attenuated in intracellular growth in primary human placental organ cultures and trophoblasts. InlP is a novel virulence factor for listeriosis with a strong tropism for the placenta. This virulence factor represents a tool for the development of new modalities to prevent and treat infection-related pregnancy complications.

The immunological environment of the maternal-fetal interface is unique because protection of the fetus from pathogens has to be balanced with tolerance of the fetus by the maternal immune system (1, 2). How this is accomplished is one of the major enigmas of mammalian reproduction. Contrary to the long-standing hypothesis that the pregnant mother is immunocompromised (3), recent evidence suggests that the maternal immune system is intricately regulated during pregnancy, and the placenta is well guarded against infection (4–6). A few predominantly intracellular microbes are able to infect the placenta and cause pregnancy complications such as preterm labor, fetal damage, and death (5, 7). Given the unique immunological environment of the maternal-fetal interface and the inability of many pathogens to colonize the placenta, we hypothesized that specific virulence determinants are required for microbes to survive and replicate in this tissue.

Listeria monocytogenes is a facultative intracellular bacterial pathogen that causes spontaneous abortion, preterm labor, and stillbirth in humans and other mammals (8, 9). There are ~1,600 human cases in the United States per year, and about one-third of these cases are pregnancy associated (10). *L. monocytogenes* is also extremely amenable to experimental analysis and therefore has been exploited over the past 5 decades to understand host-pathogen interactions of intracellular microbes (11, 12). *L. monocytogenes* can infect a wide variety of phagocytic and nonphagocytic cells. Cell wall surface proteins that belong to the internalin (Inl) family of virulence factors promote bacterial adherence and internalization into nonphagocytic host cells via binding to receptors on the host cell membrane (13). After internalization, the bacterium's intracellular life cycle is facilitated by key virulence determinants that are expressed under the control of the transcriptional regulator PrfA (14): vacuolar escape is mediated largely by listeriolysin O (LLO), with contributions from two phospholipases (PlcA and PlcB) and a metalloproteinase (Mpl). Once in the cytoplasm, ActA orchestrates actin tail formation that allows the bacteria to migrate into cell wall protrusions, which are ingested by neighboring cells, where the life cycle begins anew. Importantly, all pathogens that are able to infect the maternal-fetal interface via the hematogenous route have intracellular life cycles (5, 7). Thus, we chose to exploit *L. monocytogenes* for two reasons: its importance for human health and its utility as a model for intracellular pathogenesis.

The internalin family contains several important virulence factors (13). Twenty-five members of the internalin family have been identified in *L. monocytogenes*. All members contain leucine-rich repeats (LRRs), and most are anchored to the bacterial cell wall. The best-characterized internalins are InlA and InlB, which play a critical role in crossing the intestinal barrier (15–17). However, their role in crossing the maternal-fetal barrier has been controversial (18–20). Nine cell wall-bound internalins, in addition to InlA and InlB, have been evaluated, and some of these internalins contribute to the adhesion to and invasion of various cell types and/or virulence in the nonpregnant mouse model (21–24). Four members of the internalin family lack any known cell wall anchor domains. The prototype of this subgroup, InlC, is secreted (25) and was recently shown to modulate innate host immune re-

Received 21 July 2016 **Returned for modification** 22 August 2016
Accepted 2 October 2016

Accepted manuscript posted online 10 October 2016

Citation Faralla C, Rizzuto GA, Lowe DE, Kim B, Cooke C, Shiow LR, Bakardjiev AI. 2016. InlP, a new virulence factor with strong placental tropism. Infect Immun 84:3584–3596. doi:10.1128/IAI.00625-16.

Editor: N. E. Freitag, University of Illinois at Chicago

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Supplemental material for this article may be found at http://dx.doi.org/10.1128/IAI.00625-16.

sponses (26). Furthermore, InlC binds to a cytosolic mammalian adaptor protein, which affects cell-to-cell spread (27). To date, the functions of the other secreted internalins are unknown.

Among all the small-animal models, the maternal-fetal interface of guinea pigs resembles that of humans most closely (28). Therefore, we performed a genomic screen in pregnant guinea pigs to identify tissue-specific *L. monocytogenes* virulence determinants *in vivo*. We used transposon site hybridization, a negative-selection screen based on transposon mutagenesis and microarray technology, which led to identification of bacterial genes that are required for survival of *Mycobacterium tuberculosis* in murine liver and spleen (29), chronic infection with *Salmonella enterica* serovar Typhimurium in mice (30), virulence of *Francisella novicida* (31), and host specificity of *Legionella pneumophila* (32).

Among the genes identified as being important for infection of the placenta was *lmrg1778* (*lmo2470*), here named *inlP*, which is predicted to encode a protein of previously unknown function that belongs to the secreted internalins (13). Our data show that InlP strongly promotes placental infection while having only a minor role in infection of other maternal organs. Discovery of its mechanism of action will unravel virulence strategies required for colonization of the maternal-fetal interface.

MATERIALS AND METHODS

Ethics statement. For human subjects, this study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Institutional Review Board (IRB) at the University of California, San Francisco (UCSF), where all experiments were performed (IRB number 11-05530). All patients provided written informed consent for the collection of samples and subsequent analysis. For vertebrate animals, this study was carried out in strict accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health (33). All protocols were reviewed and approved by the Animal Care and Use Committee (IACUC) at the University of California, San Francisco (IACUC number AN079731-03A).

Bacterial strains. *L. monocytogenes* strains used in this study are 10403S (erythromycin susceptible) (34) and DP-L3903 (erythromycin resistant) (35). The transposon mutant library was created as described previously (36) and contained ~30,000 colonies. Bacterial inocula for infection of guinea pigs (18) and human organ cultures (37) were prepared as described previously.

Bacterial mutant strains generated for this study were constructed by using the temperature-sensitive plasmid pKSV7 as previously described (38). Briefly, ~500-bp DNA fragments containing the sequences flanking the target genes were amplified. DNA fragments were designed with HindIII/KpnI (5′-CCAATTATCAGGTTTCACATAGA AAGCTTCTAC-3′/5′-GTATATTTTTCAATCTATTTATGGTACCATGAATAATAG-3′) or KpnI/EcoRI (5′-GTATAATCACAATTATGCTACTGGAGGGGTACCC TCTTAT G-3′/5′-CATTATCACGGAGCAAAAGCAGGAATTCAATTA GCGCACG-3′) restriction sites (sequences are underlined). KpnI sites were used for the ligation of the two DNA fragments, and HindIII and EcoRI sites were used for the integration of the fragments into pKSV7, followed by electroporation into 10403S cells. Bacteria were grown at 42°C (restrictive temperature) in the presence of chloramphenicol (7.5 μg/ml). This led to the generation of a merodiploid intermediate. To excise the respective wild-type (WT) gene and cure the plasmid, strains were grown at 30°C (permissive temperature) without chloramphenicol. *L. monocytogenes* revertants (chloramphenicol susceptible) were tested for the deletion of target genes by PCR. Complementation of deletion strains was performed as previously described, using the pPL2 site-specific integration vector (39). DNA fragments were designed with BamHI/SalI restriction sites (sequences are underlined) (5′-CCGCTCCGGATCCAAG CATCGTTAAATCAAACG-3′ and 5′-GCTGGAAGTCGACAAACTCT GAACTTCCAG-3′).

Microarray hybridizations. Extraction of genomic DNA (gDNA), enzymatic digestion with AluI and MseI, T7 transcription, and preparation of samples for microarray hybridization were performed as described previously (30). Briefly, gDNA was purified from bacterial cultures by using the Epicentre Gram-positive DNA purification kit (Epicentre Biotechnologies, Madison, WI), replacing lysozyme with mutanolysin (5 U/μl; Sigma-Aldrich, St. Louis, MO). Each gDNA sample was divided in two and incubated separately with AluI or MseI (New England BioLabs, Ipswich, MA). Two micrograms of digested gDNA was used as the template for *in vitro* transcription with the AmpliScribe T7-Flash transcription kit (Epicentre Biotechnologies) according to the manufacturer's protocol, except that all reaction volumes and reagent concentrations were doubled, and the reaction was allowed to proceed for 16 h. RNA was purified with the MEGAclear kit (Ambion, Austin, TX), and 2 μg was used for a reverse transcriptase (RT) step using SuperScript III with random hexamers (Invitrogen, Grand Island, NY) as primers. Amino-allyl dUTP (Sigma-Aldrich) was incorporated during the RT reaction, followed by CyDye (Amersham Biosciences, Little Chalfont, United Kingdom) conjugation. All samples were hybridized against a common reference in which gDNA from a separate culture of the library grown overnight was used as the template for the *in vitro* transcription reactions. The reference was conjugated to Cy3, whereas all the samples were conjugated to Cy5. Oligonucleotides (70-mers) for the *L. monocytogenes* microarray (version 2) were obtained from the J. Craig Venter Institute (http://www.jcvi.org/cms/home/), and microarrays were printed at the Center for Advanced Technology at UCSF (http://cat.ucsf.edu/) (40). A total of 6,247 oligomers were spotted onto each array. Oligonucleotide sequences were based on EGDe (41) (2,843 oligonucleotides) and three food isolates, F6854 (916 oligonucleotides), H7858 (704 oligonucleotides), and F2365 (1,884 oligonucleotides) (42). Hybridizations were performed as described previously (30).

Microarray data analysis. Microarrays were gridded by using SpotReader software (Niles Scientific) and GenePix Pro 6 (Molecular Devices) and analyzed by using Acuity 4 software (Molecular Devices). The highest-quality spots meeting extra-stringent criteria were identified. These highest-quality spots were used to calculate normalization factors such that the median Cy5/Cy3 ratio was brought to 1. These factors were then applied to the entire data set after removal of low-quality spots. Each experimental sample was zero transformed to the input/input control. To identify transposon mutants whose abundance changed *in vivo*, microarray data were filtered to include only genes present in all organs and where at least 40% of animals had a signal over the background. The values were then $\log_2$ transformed and median centered. Significance analysis of microarrays (SAM) was then used to determine which genes were significantly overrepresented or relatively absent in organs, with significance being a false discovery rate (FDR) of <1%. Significant genes were then exported for further analysis. Statistically overabundant or absent genes in the liver and placenta were clustered by using a Euclidean distance similarity metric and a complete linkage clustering algorithm in Cluster3 (43). Heat maps were then generated by using JavaTree (44).

Eukaryotic cells. JEG-3 (choriocarcinoma cell line), J774 (mouse macrophage-like cell line), and MDCK (Madin-Darby canine kidney) cells were purchased from ATCC (https://www.atcc.org/) and propagated according to the instructions of ATCC. Human first-trimester placental fibroblasts and human trophoblast progenitor cells (hTPCs) were a generous gift from Susan Fisher and were propagated and differentiated as previously described (45, 46). Mixed glial cell cultures were generated from newborn mice and plated onto poly-D-lysine (Millipore)-coated plates as previously described (47). Intracellular growth curves were performed as previously described (18). Cells were plated and analyzed based on optimal growth conditions for each cell type and/or the preferences of the investigator performing the experiment: JEG-3 and J774 cells were evaluated as monolayers on glass coverslips, primary human first-trimester placental fibroblasts were analyzed as monolayers in 24-well tissue

culture plates, undifferentiated hTPCs were grown on 0.5% gelatin (Sigma)-coated 6-well plates and were 80% confluent at the time of infection, and differentiated hTPCs were grown on Matrigel-coated transwell filters and formed regularly interspersed clusters of cells at the time of inoculation. Primary mixed murine glial cell cultures were grown in 24-well plates (Corning) and analyzed as monolayers. MDCK cells were seeded onto 12-mm polycarbonate transwell inserts with 0.4-μm pores (Corning) and grown for 72 h to a density of ~1.5×10^6 cells/well to form a polarized epithelial monolayer at the time of infection. Cell-to-cell spread was determined as described previously (27). Polarized MDCK cultures were infected with *L. monocytogenes* wild-type or Δ*inlP* strain at a multiplicity of infection (MOI) of 140 bacteria per cell. At 8 h postinfection, cells were fixed in fresh 4% paraformaldehyde, rinsed in phosphate-buffered saline (PBS), blocked in 0.7% fish skin gelatin (Sigma), and permeabilized with 0.2% Triton in PBS. Primary staining was performed with rat anti-ZO-1 clone R40.76 (1:300; Santa Cruz Biotechnology) and polyclonal rabbit *Listeria* O antiserum (1:500; BD Biosciences). Secondary staining was done with CF405M phalloidin (1:40; Biotium, Hayward, CA), Alexa Fluor 488 goat anti-rat (1:500; Thermo Fisher Scientific), and Alexa Fluor 568 goat anti-rabbit (1:500; Thermo Fisher Scientific). Samples were mounted in Vectashield mounting medium (Vector Laboratories) and imaged with a Yokagawa CSU22 spinning-disk confocal microscope (Yokagawa Electrical Corporation, Sugarland TX). Images were analyzed by using FIJI software (48).

Vertebrate animals. (i) Guinea pigs. For screens, pregnant female Hartley outbred guinea pigs (gestational age, 25 days) were purchased from Elm Hill Breeding Labs (Chelmsford, MA) and injected intravenously (i.v.) with 1×10^9 CFU of *L. monocytogenes* as previously described (18). Placenta and maternal liver were removed at 24 h postinfection (p.i.). For additional explanations regarding dose and time point, see the legend of Fig. S2 in the supplemental material. Tissues were homogenized in PBS, plated onto brain heart infusion (BHI) agar plates containing 2 μg/ml erythromycin, and incubated overnight at 37°C. Approximately 10,000 CFU from each organ were scraped off the agar plates into PBS, the bacteria were pelleted, and the pellets were resuspended in 5 ml BHI broth–40% glycerol. The initial input inoculum was also plated and collected to account for the effect that this step may have on the complexity of the library. One-milliliter aliquots were frozen at −80°C. For strain-specific i.v. infections, pregnant guinea pigs and nonpregnant female guinea pigs (aged 3 to 4 weeks) were injected i.v. with 5×10^5 CFU. Maternal liver, spleen, and placenta were processed at 24 and 72 h p.i. as described above for CFU enumeration. Placentas harvested at 24 h p.i. were symmetrically halved. One half was used for CFU enumeration, and the other half was fixed in fresh 10% neutral buffered formalin for 24 h and paraffin embedded by using standard techniques. At least two full-thickness sections that included the maternal uterus, implantation site, and placental labyrinth were stained with hematoxylin and eosin (H&E) and evaluated in a blind fashion by a pathologist (G.A.R.). Immunohistochemistry (IHC) using polyclonal rabbit *Listeria* antisera (BD Difco) was performed by using standard techniques. For strain-specific oral infections, nonpregnant female guinea pigs (aged 3 to 4 weeks) were orally inoculated with 1×10^8 CFU as previously described (49). Liver, spleen, mesenteric lymph nodes, and the distal 30 cm of the small intestine were processed at 7 days p.i. as described above for CFU enumeration.

(ii) Mice. Male and female C57BL/6J mice aged 6 to 8 weeks were purchased from the Jackson Laboratory. After mating, the timing of pregnancy was evaluated by visualization of a copulation plug (embryonic day 0.5), and the mice were used 9 to 10 days thereafter (embryonic days 9.5 to 10.5). Nonpregnant and pregnant females were injected i.v. with 10^4 to 10^5 CFU and euthanized at the indicated time points (24 to 72 h p.i.). Placenta, maternal liver, and spleen were homogenized in PBS and plated onto BHI agar plates, and numbers of bacteria per organ were determined.

Human tissues. Human placental and decidual organ cultures were prepared and infected as previously described (37, 50). For competitive-index experiments, cultures were infected with a 1:1 ratio of wild-type to specific mutant strains with differential erythromycin susceptibilities, as described previously (51). For microscopy, organ cultures were fixed and stained as previously described (37). Primary antibodies used were mouse anti-HCG-61 clone SC-51606 (1:500; Santa Cruz) and polyclonal rabbit *Listeria* O antiserum (1:500; BD Biosciences, San Jose, CA). Secondary antibodies used were Alexa Fluor 594 goat anti-mouse or Alexa Fluor 568 goat anti-mouse (1:500; Thermo Fisher Scientific) and Alexa-Fluor 488 goat anti-rabbit (1:500; Thermo Fisher Scientific), nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) (Affymetrix, Santa Clara, CA), and sections were mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA). Stitched high-power images were acquired on a Nikon Ti-E epifluorescence microscope with a DS-Ri2 camera and NIS-Elements 4.30 software (Nikon Corporation, Tokyo, Japan) or with a Keyence BZ-X700 fluorescence microscope and BZ-X Analyzer software (Keyence Corporation of America, Chicago, IL). The area and perimeter of the villous stroma were measured by using FIJI software, and *L. monocytogenes* bacteria were counted with the Manual Cell Counter plug-in. Brightness and contrast were adjusted with Photoshop (Adobe Systems).

Analysis of Lmrg1778 conservation in *Listeria* strains. Homologs of the protein sequence of Lmrg1778 were found by using NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and by searching for highly aligned proteins in the *Listeria* genus only. Homologous sequences were aligned with CLC Main Workbench 6.7.1 (Qiagen, Alameda, CA) to generate a neighbor-joined phylogram. The Newick file of the phylogram was exported, and ETE Toolkit v3.0 was then used to visualize the phylogram (52).

Statistics. See above for analysis of microarray data. The Mann-Whitney U test was used to compare bacterial burdens, and a log rank (Mantel-Cox) test was used to compare percentages of infected placentas.

RESULTS

Negative-selection screen for identification of virulence determinants *in vivo*. We performed a negative-selection screen to identify *L. monocytogenes* genes that contribute to organ tropism *in vivo* (see Fig. S1 in the supplemental material). We used a *mariner* transposon mutant library (36) for i.v. inoculation of pregnant guinea pigs with 10^9 CFU. A high dose was chosen because placental colonization is restricted (51, 53), and we wanted to decrease the effect of the bottleneck as much as possible. At 24 h p.i., a time point when the animals still appeared healthy, we compared input pools to output pools by using microarray technology. The input pool consisted of *L. monocytogenes* transposon mutants grown in BHI broth. The output pools were composed of *L. monocytogenes* transposon mutants isolated from maternal liver ($n = 4$) or placenta ($n = 8$). Genes that were relatively absent in the output pools in comparison to the input pools were considered potentially important for infection *in vivo*, because bacterial mutants with transposon insertions in these genes were less fit to grow and survive in liver or placenta than in BHI medium. The numbers of genes in this category were 128 and 240 for liver and placenta, respectively (see Fig. S2 and Table S1 in the supplemental material). On the other hand, overabundant genes in the output pools may convey a fitness disadvantage for growth in BHI medium. In this category, we identified 183 genes in the liver but none in the placenta.

We focused on the genes that were relatively absent because we wanted to identify genes that convey an advantage for fitness *in vivo*. One hundred twenty-eight genes in the liver (4.5% of the genome) were highly reproducible across biological replicates. Among these were key virulence determinants of the core *prfA* regulon (54), specifically the four genes that encode the proteins that facilitate vacuolar escape: *hly*, *plcA*, *plcB*, and *mpl*. We did not

identify *actA*, probably because a loss of its function results in a virulence defect at time points later than 24 h (35). We identified additional genes that have been shown to be important for virulence in the murine model of listeriosis, e.g., the fibronectin binding protein *fbpA* (55) and the enzyme responsible for D-alanine esterification of lipoteichoic and teichoic acids (*dltD*) (56). In summary, the negative selection of genes encoding previously identified listerial pathogenicity determinants in liver versus BHI broth validated our screen.

In comparison to maternal liver, bacterial seeding of the placenta is relatively restricted (51, 53), which leads to greater variability in the pool of transposon mutants that colonize the placenta at the time of i.v. inoculation. Thus, a relative absence or overabundance of specific genes may reflect the ratio of transposon mutant strains that seeded the placenta at the time of inoculation rather than advantages or disadvantages in bacterial fitness in the placenta. To evaluate the effect of the bottleneck in the placenta, we took advantage of the many mutants that are not required for growth *in vivo*. Our analysis found that there were 1,935 genes with transposon insertions shared between the liver, placenta, and BHI broth. This represents ~60% of the coding genes in *L. monocytogenes* and confirms that there is a bottleneck in the placenta. However, we found that only 240 genes were significantly absent in the placenta versus BHI broth. Since passage through a bottleneck is a random process, the finding that 240 genes (8.4% of the genome) were relatively absent in eight biological replicates is most likely due to negative selection.

To identify virulence factors with placental tropism, we compared the genes that were relatively absent in the placenta versus BHI medium to the genes that were not necessary for survival in the liver (see Fig. S3 in the supplemental material). This group contained 201 genes, which fell into 33 broad categories based on gene ontology (Fig. 1; see also Table S1 in the supplemental material). The 3 largest categories encompassed genes with functions related to oxidation reduction and the regulation of biological processes, followed by 10 categories with genes involved in metabolic processes. A smaller percentage of relevant genes were involved in cellular motility and flagellum organization.

InlP is highly conserved in *L. monocytogenes*. Among the genes specifically important for placental infection was *lmrg1778* (*lmo2470*), here named *inlP*, which is predicted to encode a protein (388 amino acids [aa]) with five or six LRRs. The presence of a signal peptide but the lack of a predicted cell wall-anchoring domain places InlP in the category of secreted internalins, which has four known members (13). The prototype of this subgroup, InlC, is secreted, modifies innate host immune responses (26), and boosts cell-to-cell spread (27) via binding to cytosolic host proteins. The other three secreted internalins share little homology to InlC, and their functions for virulence *in vivo* were previously unknown. Phylogenetic analysis of InlP identified closely related homologs as being overrepresented in the human pathogen *L. monocytogenes* and absent in strains not pathogenic for humans (see Fig. S4 in the supplemental material).

Therefore, we decided to investigate the role of InlP in the pathogenesis of listeriosis. We generated an isogenic mutant strain of *L. monocytogenes* strain 10403S (wild type) deficient in *inlP* (Δ*inlP*). The phenotype of the Δ*inlP* strain did not differ from that of the wild type in standard *in vitro* assays, including intracellular growth and cell-to-cell spread (see Fig. S5 in the supplemental material). Next, we infected nonpregnant mice with either the wild-type or the Δ*inlP* strain. Twenty-four hours after i.v. inoculation with 10^4 CFU, the bacterial burdens in liver and spleen did not differ in wild-type- versus Δ*inlP* mutant-infected animals (see Fig. S6A and S6B in the supplemental material). When the inoculum was increased to 7×10^4 CFU, the Δ*inlP* mutant was slightly attenuated in spleen (0.5 $\log_{10}$) and liver (0.4 $\log_{10}$) (Fig. 2A and B), a difference that was restored by isogenic complementation of the Δ*inlP* mutant with *inlP* (see Fig. S6C in the supplemental material). The attenuation of the Δ*inlP* mutant was more pronounced at 72 h p.i., as evidenced by 0.7 and 1.3 log virulence defects in spleen and liver, respectively (Fig. 2A and B) as well as a difference in survival (see Fig. S6D in the supplemental material). The wild-type and Δ*inlP* strains did not differ in their ability to colonize the nonpregnant uterus at 24 h p.i., suggesting that InlP does not affect trafficking. However, the Δ*inlP* strain was attenuated in virulence in the uterus by 1 $\log_{10}$ at 72 h p.i. (Fig. 2C). We concluded that InlP is a novel virulence factor for systemic infection in mice.

InlP strongly promotes placental infection *in vivo*. To test the role of InlP during pregnancy, we inoculated pregnant mice i.v. on day 10.5 of gestation with 10^4 or 5×10^4 CFU of the wild-type or Δ*inlP* strain. At 48 h p.i., the Δ*inlP* mutant was attenuated in maternal spleen (1 $\log_{10}$) and liver (0.8 to 1.7 $\log_{10}$) (Fig. 2D and E). In contrast to the liver and spleen, bacterial colonization of the placenta is restricted, leading to a much higher variability in the bacterial burden among the placentas from each animal. To demonstrate this variability, we randomly picked four wild-type-infected pregnant mice and showed CFU in their placentas in individual plots (see Fig. S6E and S6F in the supplemental material). Gross examination of gravid uteri from mice infected with 1×10^4 CFU showed widespread hemorrhages in wild-type- but not Δ*inlP* mutant-infected fetoplacental units (see Fig. S6G in the supplemental material). Mice given Δ*inlP* bacteria had fewer infected placentas than did mice given the wild type, 52% (13/25) versus 94% (34/36) for infection with 1×10^4 CFU and 81% (54/66) versus 100% (62/62) for infection with 5×10^4 CFU. These differences reached statistical significance for both doses ($P < 0.005$). The median number of CFU per gram was reduced by 3 $\log_{10}$ in the placentas of animals infected with the Δ*inlP* strain in comparison to the wild type regardless of the infectious dose (Fig. 2F).

Next, we evaluated the phenotype of the Δ*inlP* strain in guinea pigs. First, we infected nonpregnant animals i.v. with 5×10^5 CFU or orally with 1×10^8 CFU of the wild-type or Δ*inlP* strain. We did not observe a difference between the wild-type and Δ*inlP* strains in liver, spleen, small intestine, and mesenteric lymph nodes except for the spleen 72 h after i.v. inoculation, when the bacterial burden of the Δ*inlP* strain was 5-fold higher than that of the wild-type strain (Fig. 3A to F). Next, we infected pregnant guinea pigs i.v. on day 32 of gestation with 5×10^5 CFU of the wild-type or Δ*inlP* strain. At 72 h p.i., the wild-type and Δ*inlP* strains were equally virulent in maternal spleen, and the Δ*inlP* strain was attenuated by 0.5 $\log_{10}$ in maternal liver ($P < 0.05$) (Fig. 3G and H). The percentages of infected placentas were 100% (12/12) and 67% (20/30) for the wild-type and Δ*inlP* strains, respectively ($P < 0.01$), and the median numbers of CFU per gram were 3 $\log_{10}$ lower in Δ*inlP* mutant-infected than in wild-type-infected placentas ($P < 0.0001$) (Fig. 3I). In conclusion, data from both rodent models suggest that InlP strongly (1,000-fold) promotes pla-

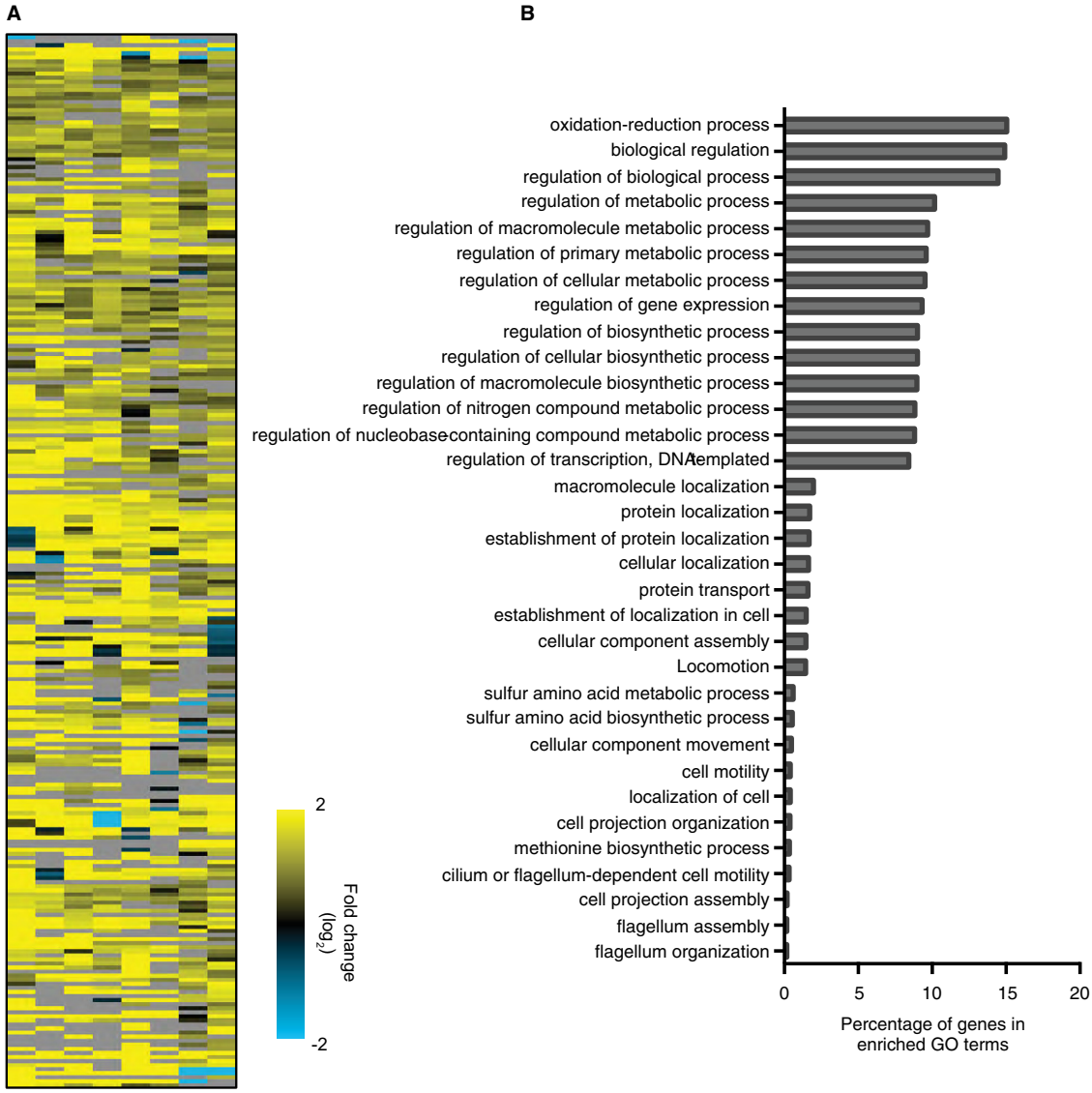

FIG 1 Negative selection of *L. monocytogenes* mutants *in vivo*. Pregnant guinea pigs were injected i.v. with 10^9 CFU of the transposon mutant library. Bacteria surviving at 24 h p.i. in maternal liver ($n = 4$) and placenta ($n = 8$) were collected. The transposon mutants present in each sample were identified by microarray analysis. Data for the genes negatively selected in placenta only are represented. (A) Heat map of genes relatively absent in placenta versus BHI broth that are also not necessary for survival in the liver. Yellow represents a relative absence of the signal in the placenta compared with the liver, and blue represents a relative overabundance of the signal in the placenta compared with the liver. (B) Role category diagram representing the distribution of genes relatively absent in placenta, shown in panel A, according to gene ontology (GO) categories.

cental infection while having only minor effects on other tissues.

Histological examination of the maternal-fetal interface in guinea pigs. During pregnancy, fetal trophoblasts invade the decidua (uterine implantation site) and remodel the maternal arteries. Consequently, maternal blood flows into the placenta, where nutrient and gas exchanges between mother and fetus occur. We and others previously demonstrated that infection of the maternal-fetal interface begins in decidua and invasive trophoblasts (37, 57). To further investigate the role of InlP, we microscopically evaluated the placenta and decidua of guinea pigs infected with the wild-type or Δ*inlP* strain (Fig. 4A). The median bacterial burdens in these tissues were 1.6×10^6 CFU/g (range, 1.9×10^4 to 6.3×10^7 CFU/g) and 3.9×10^4 CFU/g (range, 1×10^2 to 7.9×10^7 CFU/g) for the wild-type and Δ*inlP* strains, respectively (CFU data are included in Fig. 3). Sections of the maternal-fetal interface were stained with H&E or by IHC with antisera against *L. monocytogenes* (2 sections per placenta). Focal collections of acute inflammatory cells, predominantly neutrophils, with various degrees of necrotic debris were identified in the placenta (Fig. 4B) and within the walls of the decidual vasculature (Fig. 4D). IHC revealed large aggregates of *L. monocytogenes* in most of the lesions (Fig. 4C). The microscopic appearances of the lesions appeared similar for both strains, but the median numbers of inflammatory lesions per tissue (placenta and decidua) were 3 and 0 for wild-type- and Δ*inlP* mutant-infected animals, respectively (Fig. 4F). This difference did not reach statistical significance ($P = 0.11$) but was consistent with the observed difference in bacterial burdens

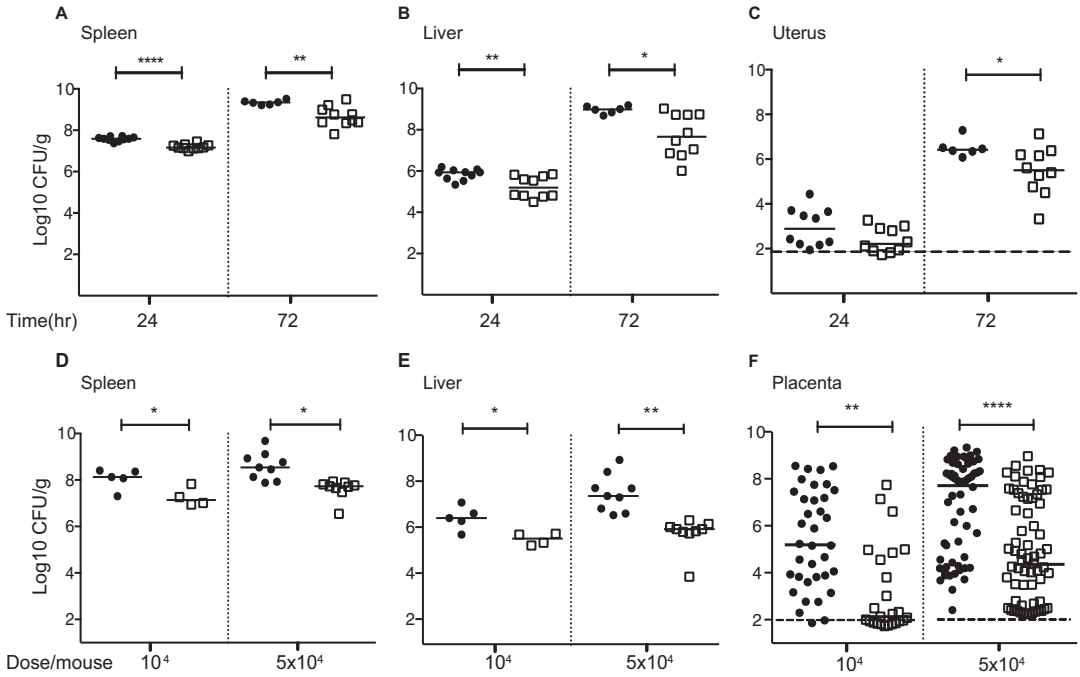

FIG 2 InlP is a virulence determinant in mice. Shown are data for intravenous infection of nonpregnant (A to C) and pregnant (D to F) mice with the wild-type (black circles) or Δ*inlP* (empty squares) strain. (A to C) CFU per gram in spleen (A), liver (B), and uterus (C) 24 and 72 h p.i. with 7×10^4 CFU. (D to F) CFU per gram in spleen (D), liver (E), and placenta 48 h p.i. with 10^4 CFU or 5×10^4 CFU. Total numbers of placentas were 36 for wild-type and 25 for Δ*inlP* mutant infection with an inoculum of 10^4 CFU and 62 for wild-type and 66 for Δ*inlP* mutant infection with an inoculum of 5×10^4 CFU. Mann-Whitney U test was used for statistical analysis (*, $P < 0.05$; **, $P < 0.005$; ****, $P < 0.0001$). Horizontal bars indicate medians, and horizontal dashed lines indicate the detection limit.

between the two strains in the placenta, which was statistically significant (Fig. 3I). However, the most striking difference between the wild type and the Δ*inlP* mutant was the degree of decidual necrosis, which was scored on a scale from 1 (healthy to minimal) to 4 (severe) (Fig. 4G and H; see also Table S2 in the supplemental material). The median decidual necrosis scores were 4 and 2 for wild-type- and Δ*inlP* mutant-infected deciduas, respectively ($P = 0.007$) (Fig. 4I).

Role of InlP in infection of primary human placental organ cultures. To further investigate the role of InlP in infection of the human maternal-fetal interface, we utilized primary human organ cultures. Since infection begins in the decidua (5), we first compared wild-type and Δ*inlP* mutant infections in decidual organ cultures, which showed no statistically significant difference between these two strains (see Fig. S7A in the supplemental material).

Next, we analyzed infection of placental organ cultures (37, 58). These organ cultures are derived from first-trimester placentas and vary in shape and size. Therefore, we used the competitive-index assay to determine the role of InlP in infection of primary human placental tissues. We infected placental organ cultures with the Δ*inlP* and wild-type strains at a 1:1 ratio, with each strain having differential susceptibilities to erythromycin (35). Equal colonization rates at 6 h p.i. suggested that InlP does not have a functional role in host cell invasion (Fig. 5A). Over time, the intracellular growth of the Δ*inlP* mutant decreased, resulting in a >1-$\log_{10}$ attenuation at 72 h p.i. ($P < 0.0001$) (Fig. 5C). It is possible that InlP secreted by wild-type bacteria can compensate for the lack of InlP in the Δ*inlP* strain in these experiments. In this case, the observed virulence defect of the Δ*inlP* strain in comparison to wild-type *L. monocytogenes* might be an underestimation. Isogenic complementation of the Δ*inlP* strain with *inlP* restored the wild-type phenotype (Fig. 5A to C).

For comparison, we coinfected primary human placental organ cultures with wild-type *L. monocytogenes* and *L. monocytogenes* strains deficient in known key virulence factors at a 1:1 ratio. All of the deletion mutants were significantly attenuated (Fig. 5A to C). An *L. monocytogenes* strain deficient in InlA was 1-$\log_{10}$ attenuated at 6, 24, and 72 h p.i., consistent with a defect in invasion and normal intracellular growth. The attenuation of mutants with an impaired intracellular life cycle was more pronounced at later time points: *L. monocytogenes* strains defective in vacuolar escape due to a lack of LLO (Δ*hly*) or PlcAB were 3-fold attenuated at 6 h p.i., a difference that increased to 1 to 2 $\log_{10}$ at 24 h and 72 h p.i.; the *L. monocytogenes* strain deficient in ActA was equally as virulent as the wild type at 6 h p.i. and was attenuated by 1 $\log_{10}$ and 2 $\log_{10}$ at 24 and 72 h p.i., respectively. Thus, the degree of attenuation of Δ*inlP* mutant infection in comparison to wild-type infection of primary human placental tissue is within the range of attenuation of previously known key virulence determinants.

Next, we examined these placental organ cultures by immunofluorescence microscopy. As previously described, *L. monocytogenes* infection starts in extravillous trophoblasts (EVTs), spreads along subsyncytial trophoblasts, and reaches the villous stroma by 72 h p.i. (37). This pattern of invasion and spread was observed with the wild-type and Δ*inlP* strains (Fig. 6A and B). However, the intracellular growth and/or spread of the Δ*inlP* mutant was impaired, as demonstrated by the significantly lower numbers of

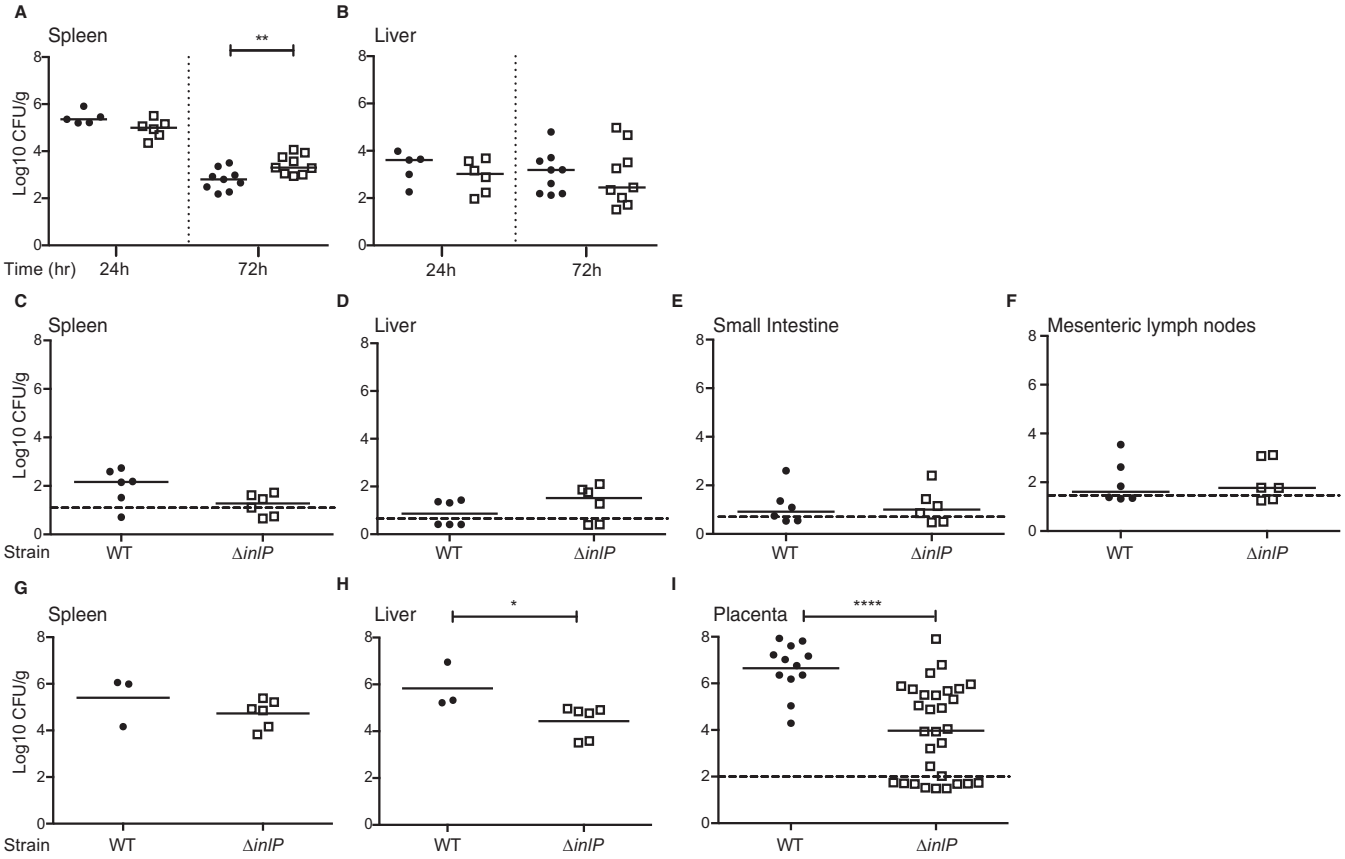

FIG 3 InlP is a virulence determinant with strong placental tropism in pregnant guinea pigs. Nonpregnant (A to F) and pregnant (G to I) guinea pigs were infected i.v. (A, B, and G to I) with 5×10^5 CFU or orally (C to F) with 1×10^8 CFU of the wild-type (black circles) or Δ*inlP* (empty squares) strain. (A and B) CFU per gram in spleen (A) and liver (B) of nonpregnant guinea pigs 24 and 72 h after i.v. inoculation. (C to F) CFU per gram in spleen (C), liver (D), small intestine (E), and mesenteric lymph nodes (F) of nonpregnant guinea pigs 7 days after oral inoculation. (G to I) CFU per gram in spleen (G), liver (H), and placenta (I) of pregnant guinea pigs 72 h after i.v. inoculation. The total numbers of placentas are 12 for wild-type-infected and 30 for Δ*inlP* mutant-infected animals. Data for each group represent the results of at least 2 independent experiments. Mann-Whitney U test was used for statistical analysis (*, $P < 0.05$; **, $P < 0.005$; ****, $P < 0.0001$). The detection limit varies between organs and is between 2 and 100 CFU/g per organ (horizontal dashed lines). Horizontal bars indicate medians.

Δ*inlP* bacteria than of wild-type bacteria per area or perimeter of villous stroma (Fig. 6C and D). In contrast, the Δ*inlP* mutant was not attenuated for growth and spread during infection of isolated primary human placental fibroblasts (Fig. 6E).

Since escaping the growth restriction in EVTs is the first hurdle that *L. monocytogenes* has to overcome when colonizing the placenta (59), we tested whether InlP promotes intracellular growth in EVTs. For this purpose, we utilized primary human trophoblast progenitor cells (hTPCs), which can be differentiated *in vitro* to resemble EVTs (45). Although wild-type *L. monocytogenes* grows well in undifferentiated hTPCs, its growth is reduced in differentiated hTPCs (see Fig. S7B in the supplemental material), consistent with previously reported observations in human organ cultures showing that less-differentiated mononuclear trophoblasts are more permissive for bacterial growth than EVTs (59). In undifferentiated hTPCs, the Δ*inlP* strain behaved like the wild-type strain (see Fig. S7C in the supplemental material). In contrast, infection of differentiated hTPCs with Δ*inlP* and wild-type bacteria at a 1:1 ratio revealed reduced intracellular growth of the Δ*inlP* strain in comparison to the wild type (Fig. 6F), suggesting that InlP contributes to overcoming the EVT barrier.

Finally, we tested the role of InlP in infection of primary murine mixed glial cell cultures, which were used previously to evaluate target cells for *L. monocytogenes* infection of the central nervous system (60). Intracellular growth curves for mixed glial cells showed no difference between the wild-type and Δ*inlP* strains (see Fig. S7D in the supplemental material).

DISCUSSION

The results of this study demonstrate that InlP strongly promotes placental infection in two pregnant rodent models. The systemic defects observed in nonpregnant mice validate InlP as a novel virulence factor and suggest that InlP could be important for bacterial virulence in other tissues as well. In primary human placental organ cultures, the Δ*inlP* strain was significantly attenuated in competitive-index experiments against wild-type *L. monocytogenes*. This degree of attenuation is comparable to that of an *L. monocytogenes* strain deficient in LLO, one of the most attenuated strains *in vivo*, due to its impaired intracellular life cycle (51, 61). In summary, the highlight of this study is the discovery of InlP, a novel virulence factor for listeriosis with strong placental tropism.

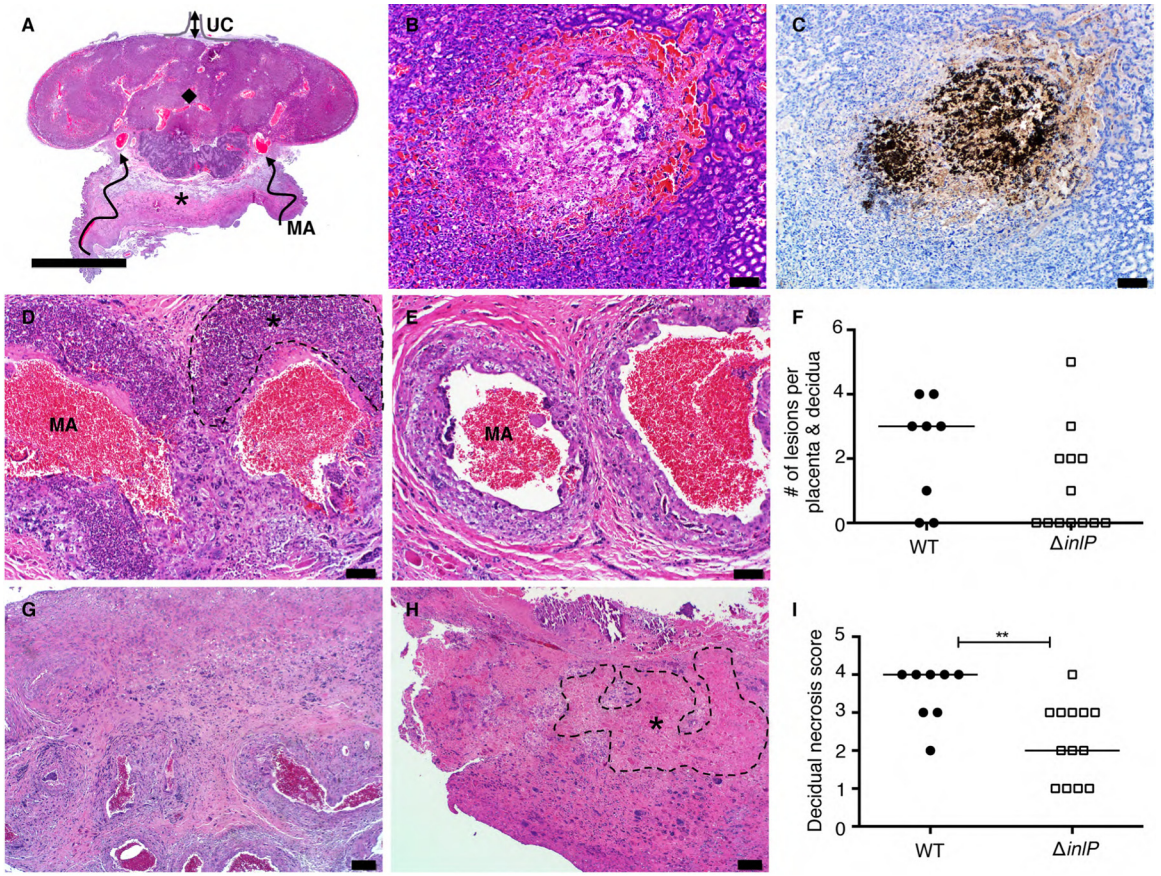

FIG 4 Histological evaluation of the maternal-fetal interface of guinea pigs infected with wild type ($n = 8$) or Δ*inlP* mutant ($n = 13$) for 24 h. (A) Whole-tissue section showing the anatomy of the maternal-fetal interface, including placenta (black diamond), decidua (*), maternal arteries (MA), and umbilical cord (UC). (B) H&E staining of a representative placental lesion composed of neutrophils, mononuclear cells, and necrotic debris. (C) IHC of the lesion shown in panel B demonstrating abundant *L. monocytogenes* bacteria. (D and E) Inflammatory lesions (*) in walls of maternal decidual arterioles (MA labels the lumen) (D) versus unaffected arterioles (MA labels the lumen) (E). (F) Numbers of lesions per placenta and decidua for wild-type (WT) (black circles) versus Δ*inlP* mutant (empty squares) infection. (G and H) Representative images of decidual necrosis with a score of 1 (G) versus a score of 4 (H). The contiguous zone of coagulative necrosis is >2 mm long (*). (I) Decidual necrosis scores for wild-type (black circles) versus Δ*inlP* mutant (empty squares) infection. Decidual necrosis was scored as follows: 1 for normal to minimal, 2 for mild, 3 for moderate, and 4 for severe (see Table S2 in the supplemental material for a detailed description of scoring). Bars, 6 mm (A), 100 μm (B to E), and 200 μm (G and H). Mann-Whitney U test was used for statistical analysis (**, $P = 0.007$). Horizontal bars in panels F and I indicate medians.

Most likely, there are other genes among the 201 hits of our screen that confer bacterial fitness with a tropism for the placenta. One of the largest groups of genes was related to oxidation reduction processes and includes, e.g., *mrsA* and *fri*. MrsA (methionine *S*-sulfoxide reductase) repairs proteins that have been oxidatively damaged by the formation of methionine sulfoxides (62), and Fri (ferritin) protects DNA from oxidative damage (63). Interestingly, mammalian development occurs in physiological hypoxia (64), and the placenta produces many reactive oxygen species (65). Thus, placental pathogens that are able to withstand oxidative stress can adapt to the placental niche. The results of our screen also included genes that encode the highly conserved flagellar export apparatus (66). This was surprising because most *L. monocytogenes* strains do not produce flagella at 37°C (67). However, 2% of *L. monocytogenes* 10403S bacteria express flagella when grown at 37°C (68), and among 100 human clinical isolates, 20 strains had flagellar motility at 37°C (69). Although flagellin is not essential for systemic listeriosis in mice (70), perhaps it contributes to placental virulence.

InlP is conserved in *L. monocytogenes*. *Listeria* strains without homology to InlP are not associated with human disease, including *L. innocua* (41) and *L. ivanovii* (71). We did not find any other prokaryotes that contain sequences with homology to InlP, suggesting that this virulence factor is specific for the pathogenesis of listeriosis. InlP appears to be particularly important for placental infection compared to infection of liver, spleen, nonpregnant uterus, small intestine, and mesenteric lymph nodes *in vivo* and primary murine mixed glial cell cultures *in vitro*. There is typically a large amount of variability in placental infections even among all the placentas from one animal (see Fig. S6E and S6F in the supplemental material) (19, 72). The variability in placental CFU is due to the bottleneck in placental colonization, which does not seem to differ between wild-type and Δ*inlP* strains. However, the net bacterial burden of the Δ*inlP* strain in the placenta is 3 $\log_{10}$ lower than that in wild-type-infected placentas. The bacterial burden is a composite of bacterial replication, elimination, and trafficking. Typically, the net bacterial burden reaches stationary phase before beginning to decrease. It is likely that bacteria in

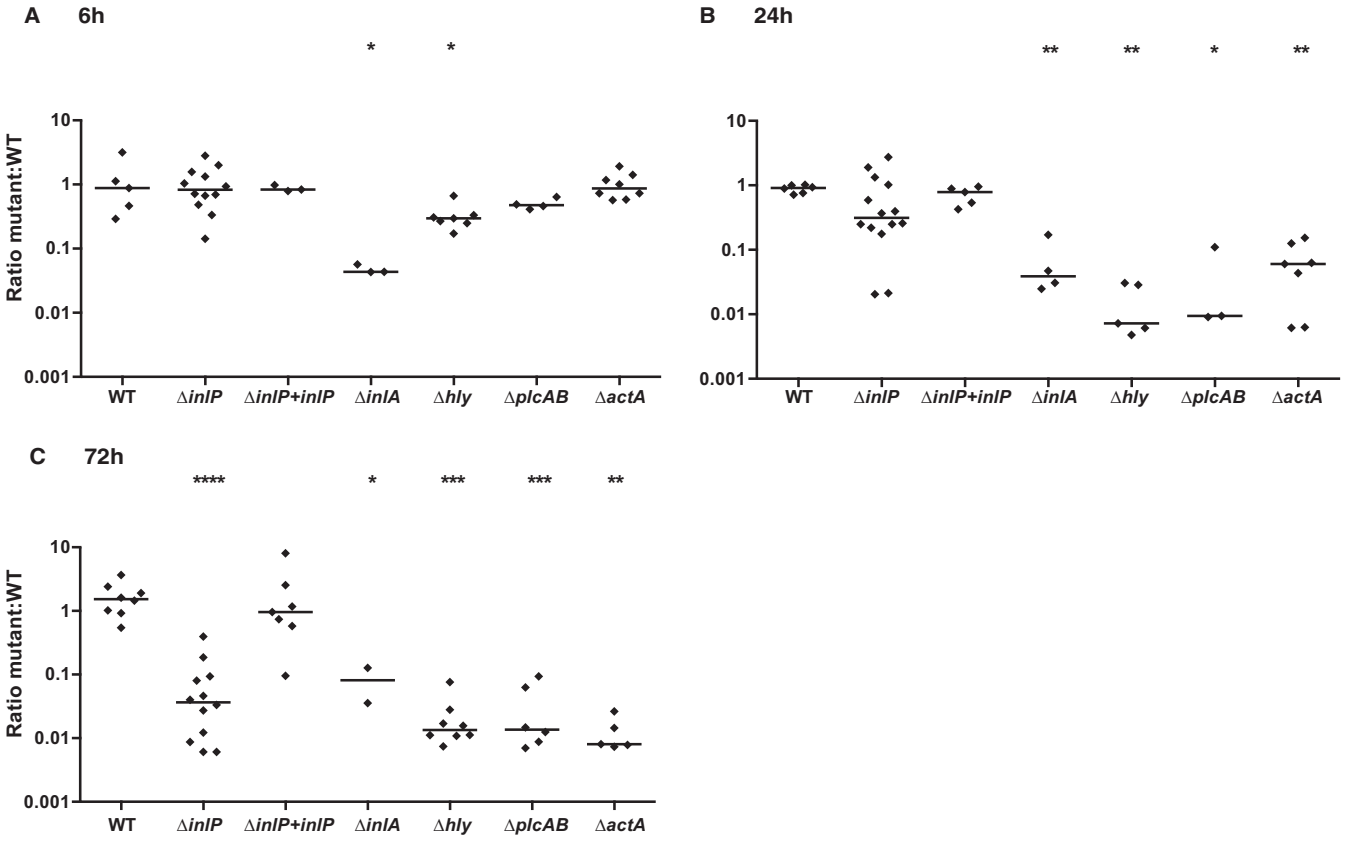

FIG 5 Role of virulence factors in human placenta. Primary human placental organ cultures were infected with a 1:1 ratio of WT (erythromycin resistant) and WT (erythromycin susceptible) or the following mutant strains (all erythromycin susceptible): Δ*inlP*, Δ*inlP* mutant complemented with *inlP*, Δ*inlA*, Δ*hly*, Δ*plcAB*, and Δ*actA*. The ratio of erythromycin-resistant to erythromycin-susceptible CFU was determined at 6 h p.i. (A), 24 h p.i. (B), and 72 h p.i. (C). Mann-Whitney U test was used for statistical analysis (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$; ****, $P < 0.0001$). Horizontal bars indicate medians.

highly infected placentas have reached stationary phase, and even wild-type bacteria cannot continue to grow any further due to limits in the nutrients available and/or immune responses that increase bacterial elimination.

InlP belongs to the internalin family, which includes InlA and InlB. Evaluation of the roles of InlA and InlB in placental infection *in vivo* has been complicated by the species specificity of the receptor interactions: both internalins bind to human and gerbil E-cadherin and Met, but InlA and InlB have a reduced affinity for murine E-cadherin (73) and guinea pig cMet (74), respectively. InlA plays no role in placental infection of guinea pigs (18), perhaps due to the lack of an interaction between InlB and guinea pig Met (75). However, in three pregnant rodent models with functional InlA- and InlB-host cell receptor interactions, the role of InlA and InlB in placental infection, if present, is small: placental infection of mice injected with *L. monocytogenes* expressing InlA engineered to interact with murine E-cadherin does not differ from wild-type infection (19). Infection of pregnant gerbils or transgenic mice expressing human E-cadherin with *L. monocytogenes* bacteria deficient in InlA, InlB, or both revealed an ~0.5-log virulence defect in the placenta in comparison to wild type (20). We previously infected primary human placental organ cultures with Δ*inlA*, Δ*inlB*, or Δ*inlAB* bacteria (see Fig. 4B in reference 37), which showed significantly decreased placental invasion of the Δ*inlA* strain in comparison to wild type. We did not observe an effect of the Δ*inlB* mutant on invasion of human placental organ cultures, and the Δ*inlAB* double-deletion mutant did not differ significantly from the Δ*inlA* mutant. Thus, in this study, we compared the roles of InlA and InlP in infection of placental organ cultures in a competitive-index assay (Fig. 5).

Our results from placental cell and organ culture models do not support a significant role for InlP in host cell invasion because CFU at early time points were similar for the wild-type and Δ*inlP* strains, while the Δ*inlA* strain was attenuated 2 $\log_{10}$ in invasion of human trophoblasts that express E-cadherin *in vitro* as well as primary human placental organ cultures (Fig. 5A) (18, 76). In contrast, InlP promotes bacterial pathogenesis at stages downstream of host cell invasion, as evidenced by the reduced intracellular growth in differentiated human trophoblasts and human placental organ cultures. It is possible that InlP and InlA may have synergistic or additive effects for infection in the placenta, and further studies will be performed to evaluate this hypothesis.

What is the reason for the observed discrepancy in InlA's importance for trophoblast invasion *in vitro* and placental infection *in vivo*? In addition to internalin-mediated host cell invasion, *L. monocytogenes* can spread from cell to cell via actin-based motility. Interestingly, previously reported studies in mice and guinea pigs indicate that most of the bacterial burden *in vivo* is inside host cells (53, 77). These studies suggest that cell-to-cell spread is an impor-

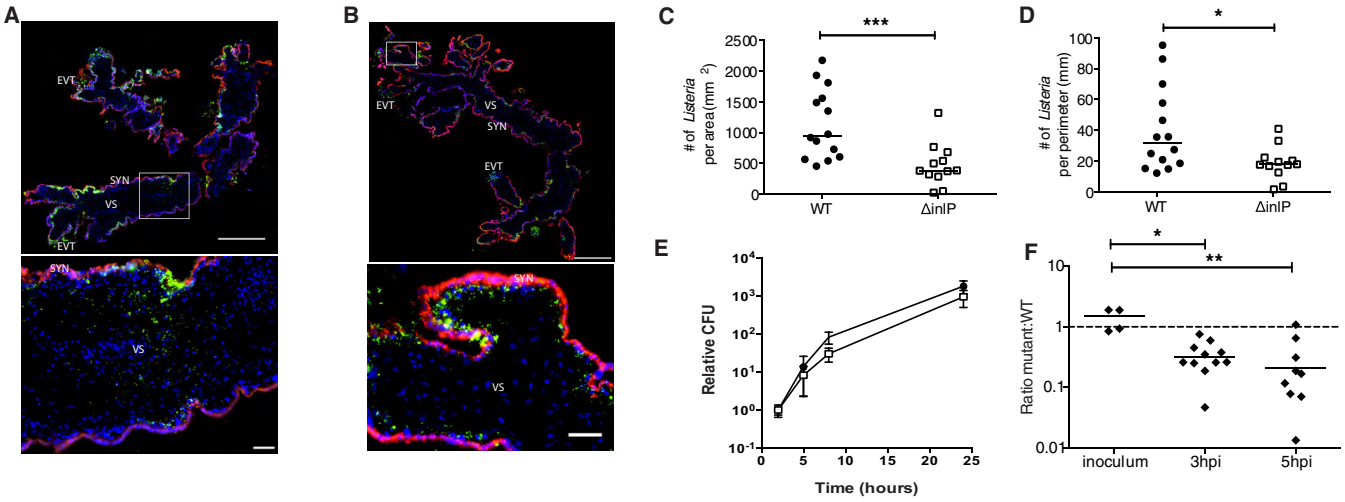

FIG 6 InlP is important for virulence in human placenta. (A to D) Human placental organ cultures from 3 donors were analyzed 72 h p.i. with either wild type (WT) or Δ*inlP* mutant. (A) Representative image of a WT-infected placental organ culture stained for *L. monocytogenes* (green), βHCG (human chorionic gonadotropin) (to stain syncytiotrophoblasts) (red), and DAPI (blue). The rectangle in the top image denotes the location of the high-power image at the bottom. SYN, syncytiotrophoblast; EVT, extravillous trophoblast; VS, villous stroma. (B) Representative image of a placental organ culture infected with Δ*inlP* mutant and stained as described above for panel A. (C and D) Numbers of *L. monocytogenes* bacteria present per area (C) and perimeter (D) of villous stroma quantified by immunofluorescence microscopy for 14 WT- and 12 Δ*inlP* mutant-infected organ cultures. (E) Intracellular growth of the WT (black circles) and Δ*inlP* mutant (empty squares) in primary human placental fibroblasts as determined by a gentamicin protection assay (averages of data from two independent experiments performed in triplicate). Error bars indicate standard errors of the means. (F) Competitive index in differentiated primary human trophoblast progenitor cells infected with erythromycin-resistant WT and erythromycin-susceptible Δ*inlP* mutant bacteria at a 1:1 ratio. Mann-Whitney U test was used for statistical analysis of data in panels C to E, and a Kruskal-Wallis test with multiple comparisons was used for analysis of data in panel F (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$). Horizontal bars indicate medians. The dashed line represents a ratio of 1. Bars, 500 μm (low-power images) and 50 μm (high-power insets).

tant mechanism for placental infection *in vivo* and can compensate for a lack of InlA–E-cadherin and/or InlB-cMet interactions.

Like InlC, InlP belongs to the small subgroup of secreted internalins. Thus, potential functional roles can be inferred for InlP in comparison to InlC. Interestingly, there is evidence that InlC interacts directly with IκB kinase α (IKKα) and modifies innate host cell responses to infection (26). Thus, an intriguing possible functional role for InlP is in interference with specific host defense pathways via direct binding to host cell proteins, which in turn could lead to decreased intracellular bacterial replication or survival. InlC also binds to Tuba, a cytosolic mammalian adaptor protein, to promote bacterial cell-to-cell spread (27). We previously observed that *L. monocytogenes* grows to a high density in subsyncytial trophoblasts, whereas only very few bacteria reach the villous stroma, and suggested that the basal membrane represents another barrier against listerial spread into deeper layers of the placenta (37). In placental organ cultures infected with the wild-type or Δ*inlP* strain, the appearance of infected subsyncytial trophoblasts did not differ. Both strains heavily infected subsyncytial trophoblasts, but the number of bacteria that reached the villous stroma was lower for Δ*inlP* mutant infection than for wild-type infection. While it is still possible that InlP facilitates cell-to-cell spread in subsyncytial trophoblasts, we did not observe such a role for InlP in MDCK cells (see Fig. S5D and S5E in the supplemental material). Thus, we favor the hypothesis that InlP is involved in promoting bacterial spread across the basement membrane. Once *L. monocytogenes* has reached the villous stroma, it can grow in fetal placental trophoblasts whether InlP is present or not (Fig. 6E). Further studies to evaluate the mechanistic roles of InlP are currently in progress.

There are several possibilities for the observed organ tropism of InlP. One possibility is organ-specific expression of host protein binding partners. On the other hand, evidence is mounting that the metabolic environment of the host cell can regulate the expression and/or activity of bacterial virulence determinants (78). Thus, an alternate possibility is that InlP is regulated by host signals that are differentially expressed in maternal liver and spleen versus placenta.

Our detailed histological examination of the maternal-fetal interface of infected guinea pig placentas at 24 h p.i. demonstrated inflammatory lesions with abundant bacteria and neutrophils, consistent with data from previous studies in pregnant mice (57). The appearances of inflammatory lesions were similar in Δ*inlP* and wild-type infections. It is possible that evaluation by flow cytometry or at additional time points would uncover distinct differences in immune cell composition. On the other hand, the finding that the Δ*inlP* strain is attenuated in human placental organ cultures argues that the effects of InlP, at least in part, occur independently of the influx of maternal immune cells. The most striking histological difference between Δ*inlP* and wild-type infections was the degree of decidual necrosis. Tissue necrosis *in vivo* correlates with a widespread infiltration of neutrophils that are recruited to contain bacterial infection (79). Consistent with this, we observed bacterial foci, neutrophil infiltration, and tissue necrosis in the decidua (Fig. 4). This does not occur in an *ex vivo* system such as decidual organ cultures. Thus, the lack of neutrophil recruitment and tissue necrosis in decidual organ cultures (see Fig. S7A in the supplemental material) may have affected the bacterial burden. It is interesting to note that previous reports by Redline and Lu described decidual necrosis in pseudopregnant mice after intrauterine inoculation with *L. monocytogenes* as well (57). Furthermore, decidual senescence and necrosis have been

associated with pregnancy complications, including preterm labor (80, 81), one of the clinical manifestations of pregnancy-associated listeriosis. Despite the high rates of morbidity and mortality associated with preterm birth, the underlying molecular mechanisms of preterm labor remain largely undefined (82).

In summary, we have discovered a novel virulence determinant for *L. monocytogenes* that is predicted to belong to the secreted internalins. Although InlP contributes to systemic virulence in mice, the phenotype is strongest in the placenta in two rodent models. InlP represents a novel tool that we can use to understand host-pathogen interactions at the maternal-fetal interface and may serve as a springboard for the development of new modalities to prevent and treat infection-related pregnancy complications such as preterm labor.

ACKNOWLEDGMENTS

We are grateful to Sandi Wong and Fay Pon for technical assistance; Anita Sil, Mark Voorhies, and David Weiss for helpful discussions; Susan Fisher, Tippi Mackenzie, Shaeri Mukherjee, Adrian Erlebacher, Joanne Engel, and Stephen Gitelman for critical reading of the manuscript; and Cedric Brimacombe for useful comments. hTPCs were a generous gift from Susan Fisher. We acknowledge Mark Weinstein and the San Francisco General Hospital Pathology Department for expert assistance with immunohistochemistry.

This work was supported by National Institutes of Health grant R01AI084928 and Burroughs Wellcome Fund grant 41259 to A.I.B. G.A.R. is supported by grant F32AI108195, a Society for Pediatric Pathology Young Investigator Research grant, and the University of California Partnerships for Faculty Diversity President's Postdoctoral Fellowship. C.C. received support from grant T32AI060537. D.E.L. received support from grants T32AI060537 and F32AI120676.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

FUNDING INFORMATION

This work, including the efforts of Anna I. Bakardjiev, was funded by HHS | National Institutes of Health (NIH) (R01AI084928). This work, including the efforts of Gabrielle A. Rizzuto, was funded by HHS | National Institutes of Health (NIH) (F32AI108195). This work, including the efforts of David E. Lowe and Cara Cooke, was funded by HHS | National Institutes of Health (NIH) (T32AI060537). This work, including the efforts of David E. Lowe, was funded by HHS | National Institutes of Health (NIH) (F32AI120676). This work, including the efforts of Anna I. Bakardjiev, was funded by Burroughs Wellcome Fund (BWF) (41259).

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