Growth of *Listeria monocytogenes* in the Guinea Pig Placenta and Role of Cell-to-Cell Spread in Fetal Infection

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Listeria monocytogenes **causes foodborne outbreaks that lead to infection in human and other mammalian fetuses. To elucidate the molecular and cellular mechanisms involved in transplacental transmission, we characterized placental-fetal infection in pregnant guinea pigs inoculated with wild-type *(wt)* or mutant *L. monocytogenes* strains. The *wt* strain increased in number in the placenta by >1000-fold during the first 24 h after inoculation—an increase that was unparalleled in other maternal organs. The ActA$^-$ mutant, which is impaired in cell-to-cell spread and attenuated in maternal organs, increased in the placenta by a similar amount, although, in fetal infection, the number of ActA$^-$ mutant bacteria was 100-fold lower, compared with that of the *wt* strain. Furthermore, a mutant impaired in vacuolar escape was rapidly eliminated from maternal organs but persisted in the placenta. We concluded that cell-to-cell spread facilitates maternal-to-fetal transmission. Furthermore, the placenta provides a protective niche for growth of *L. monocytogenes.***

Listeria monocytogenes is a ubiquitous, rapidly growing, facultative, intracellular, gram-positive bacterium. Infection of humans and animals has been traced to contaminated foods and can lead to serious, often fatal disease. In humans, disease is most common in pregnant women, newborns, and immunocompromised individuals [1]. Pregnant women have an estimated 17-fold increased incidence of disease [2], compared with that in other individuals, and usually develop a nonspecific febrile illness, which can lead to a placental-fetal infection that results in spontaneous abortion, premature labor, stillbirth, or neonatal sepsis and meningitis [3, 4]. Despite significant fetal and neonatal morbidity and mortality due to vertical transmission of *L. monocytogenes,* little is known about its underlying molecular and cellular mechanisms. We previously developed a pregnant guinea pig model of listeriosis that mimics human disease [5]. The structural similarities between the guinea pig placenta and the human placenta make our model an attractive one for elucidation of the roles that important virulence factors play in vivo [6].

The biological processes of intracellular growth and the bacterial determinants of the pathogenicity of *L. monocytogenes* were extensively studied during the past decade [7, 8]. Bacterial cell-wall surface proteins called internalins (Inls) promote bacterial adherence and internalization into nonphagocytic host cells [9]. InlA and InlB are the best characterized Inls, and they bind to E-cadherin and c-Met-tyrosine kinase, respectively [10, 11]. After internalization, the bacterium escapes from the vacuole into the cytoplasm. This escape is largely mediated by a pore-forming virulence factor called listeriolysin O (LLO) [7, 8]. Once it is in the cytoplasm,

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the bacterium multiplies rapidly. ActA, a bacterial surface protein, induces polymerization of host cell actin filaments, which enable *L. monocytogenes* to migrate to the host cell periphery and into cell-wall protrusions. These pseudopodia are engulfed and ingested by neighboring cells, in which the life cycle begins anew [12–14]. Therefore, *L. monocytogenes* can infect cells by 2 different mechanisms: direct invasion and cell-to-cell spread.

The roles that Inls play in virulence have yet to be fully explained. Although InlB is known to play a role in hepatic infection [15, 16], the mouse model, which has been used for decades in the study of listerial pathogenesis, is not a good model for use in the evaluation of the role of InlA, because there is a single amino-acid substitution in murine E-cadherin, compared with human E-cadherin. This structural difference significantly decreases the affinity of InlA to murine E-cadherin [17, 18]. Human and guinea pig E-cadherin interact equally well with InlA, and there is evidence that InlA plays a role in the crossing of the intestinal barrier in guinea pigs, humans, and transgenic mice that express human E-cadherin in the intestinal epithelium [19, 20].

The placental-fetal barrier in the monohemochorial placenta of humans and guinea pigs consists of a single layer of fetally derived trophoblasts [6, 21]. We and others have shown elsewhere that the invasion of primary human trophoblasts in vitro is mediated by InlA [5, 22]. Furthermore, Lecuit et al. have shown that infection of syncytiotrophoblasts in human placental explants with a *L. monocytogenes* strain deficient in InlA reduced the number of bacteria by 10-fold, compared with that in infection with wild-type *(wt) L. monocytogenes* [22]. In humans, epidemiological data have shown that 100% of the *L. monocytogenes* strains isolated in pregnancy-associated and focal infections, 98% of the strains isolated in central nervous system (CNS) infections, and 93% of the strains isolated in bloodstream infections expressed full-length InlA. In contrast, only 65% of the strains isolated from food products expressed full-length InlA [20]. These data are consistent with experimental evidence suggesting that InlA plays a role in the crossing of the intestinal barrier [19]. Because the crossing of the intestinal barrier is a prerequisite for placental infection in naturally occurring listeriosis, it cannot be concluded from the available epidemiological data that InlA mediates the crossing of the placental-fetal barrier in humans. Furthermore, we have shown elsewhere that InlA is not important for the crossing of the placental-fetal barrier in the pregnant guinea pig model of listeriosis [5].

We therefore decided to examine the role that cell-to-cell spread plays in trophoblast infection and vertical transmission. The ActA$^-$ mutant, a *L. monocytogenes* strain deficient in ActA, is impaired in its ability to spread from cell to cell. In vitro studies have shown that a single J774 cell initially infected with a single ActA$^-$ bacterium harbors 250–500 bacteria at 8 h after infection [23]. Subsequent bacterial growth results in lysis of the host cell and necrotic death. In an animal model of infection, cell lysis would result in the exposure of extracellular bacteria to host defense mechanisms—specifically neutrophils, macrophages, and the humoral immune response. Indeed, cell-to-cell spread has been shown to be important for virulence. In the murine model of listeriosis, an ActA$^-$ mutant strain of *L. monocytogenes* is 1000-fold less virulent than is the *wt* strain [23]. In the present study, we compare placental-fetal infection with the ActA$^-$ mutant and *wt L. monocytogenes* in the pregnant guinea pig model of listeriosis. We show that cell-to-cell spread facilitates maternal-to-fetal transmission. Although it is attenuated in maternal organs, the ActA$^-$ mutant seems to grow well in the placenta. Furthermore, a *L. monocytogenes* strain deficient in vascuolar escape is rapidly eliminated in maternal organs but is able to persist, and even to grow, in the placenta. Our results demonstrate that the mammalian placenta provides a unique protective environment for *L. monocytogenes.*

MATERIALS AND METHODS

Bacterial strains and growth conditions. *L. monocytogenes* 10403S (erythromycin-sensitive *wt*) [24] and the following strains derived from it were used: DP-L3903 (erythromycin-resistant *wt*) [25], DP-L3078 (ActA$^-$) [26], DP-L2161 (erythromycin-sensitive LLO$^-$) [27], and DP-L4694 (erythromycin-resistant ActA$^-$). DP-L4694 was generated from strain DP-L3078 by transduction of the gene for erythromycin resistance from strain DP-L3903 by use of phage U153, as described elsewhere [25, 28, 29]. All strains were propagated in brain-heart infusion (BHI) agar and broth (Becton Dickinson). For guinea pig studies, bacteria were grown in BHI broth to log phase, washed once with PBS, and resuspended in fresh BHI. Aliquots were frozen and stored at –80°C. On the day of infection, aliquots were thawed, diluted to a concentration of 1:5 in fresh BHI, and grown to log phase.

Guinea pig studies. All guinea pigs were housed and handled in accordance with federal and institutional guidelines. The animal use committee at the University of California, Berkeley, approved the animal use protocol. Pregnant female Hartley outbred guinea pigs between days 25 and 45 of gestation were purchased from Simonsen Laboratories or Elm Hill Breeding Labs. Pregnant females were injected with *L. monocytogenes* into their foot veins between days 42 and 52 of gestation. Guinea pigs were premedicated with a subcutaneous injection of 0.05 mg/kg atropine (Phoenix Scientific) and then anesthetized with isoflurane (Baxter Healthcare Corporation) before inoculation with either 1×10^8 or 1×10^9 cfu of bacteria. The dose of 1×10^9 cfu was used when histologic examination was planned. Such a high dose was necessary for sufficient numbers of bacteria to be detected by immunohistochemistry. Because this dose caused clinical illness in the guinea pigs at

24 h after inoculation, a dose of 1×10^8 cfu was used for experiments with later time points—in particular, the competitive index analysis. Guinea pigs were euthanized at specified time points after inoculation, and the organs were harvested and homogenized in 0.2% NP-40 (Biosciences). Serial dilutions were plated on Luria-Bertani (LB) agar plates (Becton Dickinson) and were incubated overnight at 37°C. The number of bacteria per organ was counted.

A competitive index analysis was performed as described elsewhere [25]. Briefly, the erythromycin-sensitive *wt* 10403S strain was compared with the erythromycin-resistant ActA$^-$ strain DP-L4694. The strains were mixed at a 1:1 ratio. Guinea pigs were injected with a total infectious dose of 1×10^8 cfu of bacteria. Guinea pigs were euthanized at 33 h after inoculation, and the organs were harvested and homogenized in 0.2% NP-40. Serial dilutions were plated on LB agar plates and on BHI agar plates containing erythromycin (Sigma) at a concentration of 2 μg/mL. The plates were incubated overnight at 37°C, and erythromycin-sensitive and erythromycin-resistant colonies were counted. The competitive index was determined by calculation of the ratio of erythromycin-resistant to erythromycin-sensitive colonies.

Histologic analysis. Tissue was fixed in 10% buffered formalin phosphate (Fisher Scientific) and processed by routine methods to provide paraffin wax sections (4 μm), which were stained with hematoxylin-eosin. Immunohistochemistry was performed at the California Animal Health and Food Safety Laboratory in Davis, California, using a rabbit anti-*Listeria* primary antibody (Difco Laboratories) and a peroxidase detection kit (Vector Laboratories). Examination of stained placental sections was performed by microscopy, and examiners were blinded to the identity of the infecting strains.

RESULTS

Early events during* L. monocytogenes *infection in the pregnant guinea pig. We intravenously (iv) inoculated pregnant guinea pigs between days 42 and 52 of gestation with 1×10^9 cfu of the *wt* strain. This gestational age corresponds with the late second/early third trimester of pregnancy. For histologic analysis, a sufficient number of bacteria had to be present in the placenta to allow for localization of organisms during the early stages of infection, specifically the first 24 h after inoculation. Thus, it was necessary to use a relatively high inoculum, which typically causes lethal disease in 2–3 days.

At 30 min after inoculation, the number of bacteria in the placenta was between 1000-fold and 10,000-fold lower than that in the maternal liver and spleen and between 10-fold and 100-fold lower than that in the maternal lungs and kidneys (figure 1). We concluded that *L. monocytogenes* seeds the placenta initially with fewer bacteria than it does any of the other maternal organs. During the subsequent 24 h after inoculation, we observed a >1000-fold increase in the number of *L. monocytogenes* in the placenta—an increase unparalleled in any other maternal organ we tested (figure 1). This increase in the number of bacteria in the placenta could be due to bacterial growth or influx. Influx could occur in the form of extracellular bacteria or infected maternal cells—specifically, macrophages. If the increase was solely due to bacterial growth, the predicted generation time of *L. monocytogenes* in the guinea pig placenta at 6–10 h after inoculation would be ~70 min (figure 1*A*).

L. monocytogenes *impaired in cell-to-cell spread.* We iv inoculated pregnant guinea pigs with 1×10^9 cfu of the ActA$^-$ mutant. In maternal organs, the ActA$^-$ mutant was attenuated, compared with the *wt* strain (figure 1*B*–*E*). The ActA$^-$ mutant was either unable to grow (in maternal lungs [figure 1*D*] and kidneys [figure 1*E*]) or began to decrease in number at 10 h after inoculation (in maternal liver [figure 1*B*] and spleen [1*C*]). In the placenta, in contrast, the ActA$^-$ mutant increased in number by ~1000-fold during the first 24 h after inoculation, which was similar to the growth of the *wt* strain (figure 1*A*).

Histologic examination of placental sections. Histologic examination was performed on placental sections taken from guinea pigs at 0.5, 6, 10, and 24 h after inoculation with 1×10^9 cfu of either the *wt* strain or the ActA$^-$ mutant. Fewer than 5 bacteria per placental section were observed at 0.5, 6, and 10 h after inoculation. At these early time points, no difference in the number of bacteria was detected between guinea pigs inoculated with the *wt* strain and those inoculated with the ActA$^-$ mutant. Placental lesions consisting of neutrophils, macrophages, and necrotic trophoblasts were observed in both groups of guinea pigs at 24 h after inoculation. Lesions were most frequent and severe in the ascending and radial maternal arterial main lacunae. These vascular structures are lined by endovascular trophoblasts and are the primary arterial blood supply to the placenta from the maternal circulation. Bacteria were present in variable numbers and appeared to be extracellular, inside of neutrophils and macrophages, and occasionally inside of trophoblasts (figure 2). In one-half of the guinea pigs infected with the ActA$^-$ mutant, we found, in the labyrinth region, small clusters of trophoblasts that were filled with bacteria (figure 2*B*). This visually demonstrates the effect that impaired cell-to-cell spread has and shows that *L. monocytogenes* grows well inside of trophoblasts in vivo.

***Cell-to-cell spread and transmission of* L. monocytogenes.** We compared transmission of *L. monocytogenes* from mother to fetus in pregnant guinea pigs iv inoculated with 1×10^9 cfu of either the *wt* strain or the ActA$^-$ mutant. At 10 h after inoculation, fetal infection with *L. monocytogenes* was not detectable (data not shown). At 24 h after inoculation, infected livers were found in all of the fetuses in pregnant guinea pigs inoculated with the *wt* strain but in only 3 of the fetuses in pregnant guinea pigs inoculated with the ActA$^-$ mutant (figure

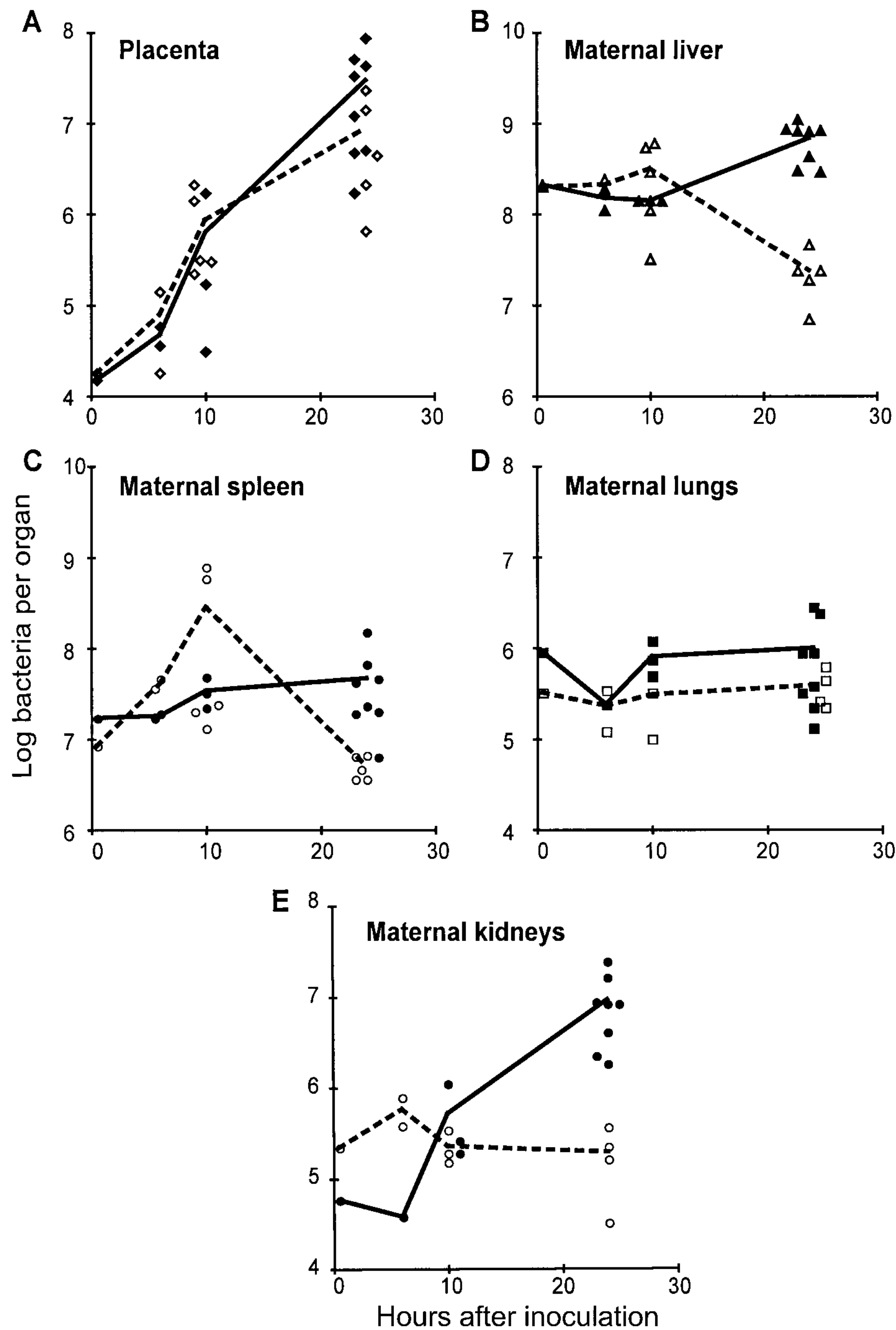

Figure 1. Listeriosis in pregnant guinea pigs. The number of bacteria per placenta *(A),* maternal liver *(B),* maternal spleen *(C),* maternal lungs *(D),* and maternal kidneys *(E)* was counted at 0.5, 6, 10, and 24 h after intravenous inoculation of pregnant guinea pigs with 1×10^9 cfu of wild-type *(wt; black circles)* or $ActA^-$ *(white circles) Listeria monocytogenes.* Solid and broken lines connect mean values of *wt* and $ActA^-$ strains, respectively.

3*A*). To further quantify the virulence defect of the $ActA^-$ mutant in fetal infection, we performed a competitive index analysis with a 1:1 ratio of the erythromycin-sensitive *wt* strain to the erythromycin-resistant $ActA^-$ mutant. At 33 h after inoculation, we observed a 100-fold decrease in virulence of the $ActA^-$ mutant in fetal liver (figure 3*B*). In contrast, we observed a <10-fold decrease in virulence of the $ActA^-$ mutant in placenta, maternal liver, and maternal spleen. This indicates that cell-to-cell spread contributes to the crossing of the placental-fetal barrier.

L. monocytogenes *impaired in vacuolar escape.* To evaluate whether the placental environment is particularly per-

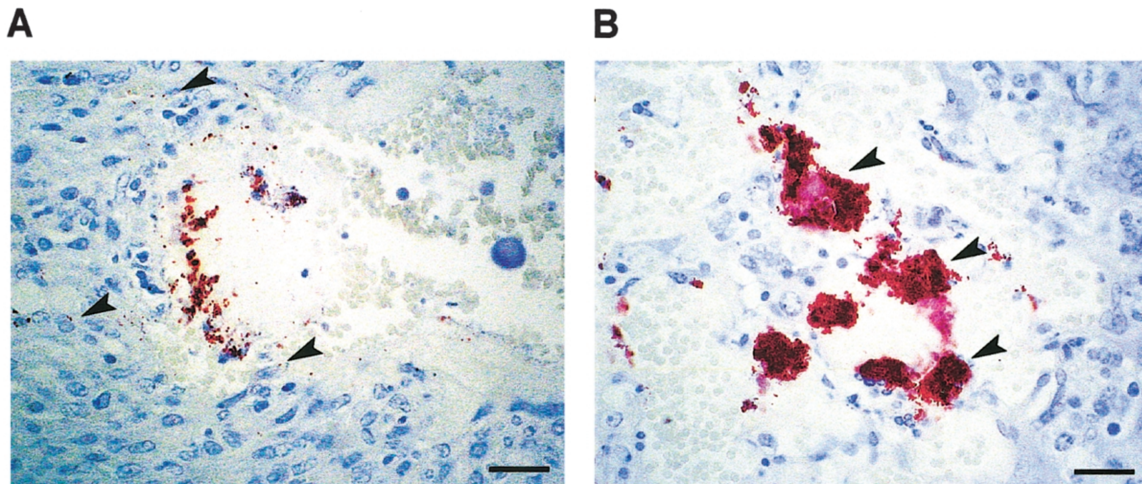

Figure 2. Immunohistochemical examination of guinea pig placental sections. Pregnant guinea pigs were intravenously inoculated with 1×10^9 cfu of wild-type *(wt; A)* or ActA$^-$ *(B) Listeria monocytogenes.* In guinea pigs infected with the *wt* strain *(A),* abundant intracellular and extracellular bacteria were present in the inflammatory infiltrate, and a small number of bacteria appeared to be inside of trophoblasts *(arrowheads).* In comparison, in guinea pigs infected with the ActA$^-$ mutant *(B),* a large number of bacteria filled trophoblasts *(arrowheads).* Bar, 26 μm.

missive for listerial growth, we iv inoculated pregnant guinea pigs with 1×10^9 cfu of the LLO$^-$ mutant. This strain is impaired in its ability to escape the vacuole and is absolutely avirulent in the murine model of listeriosis [30]. At 24–48 h after inoculation, the LLO$^-$ mutant was eliminated rapidly from maternal organs, including liver, spleen, lungs, and kidneys (figure 4*A*). In contrast, the LLO$^-$ mutant was able to persist in the placenta and even exhibited a slight increase in number (figure 4*B*). Most important, these results indicate that *L. monocytogenes* cannot be effectively eliminated from the placental environment.

DISCUSSION

The results of the present study show that *L. monocytogenes* does not preferentially seed the guinea pig placenta in an iv model of inoculation. However, there was a >1000-fold increase in the number of bacteria in the placenta during the first 24 h after inoculation. Such an increase was not observed in any other maternal organ. In addition, *L. monocytogenes* strains that were impaired in their ability to spread from cell to cell or to escape the vacuole were able to grow or persist in the placenta, despite being attenuated in maternal organs. Although cell-to-cell spread is not essential for placental infection, we observed a decrease in the number of bacteria in fetal infection with the ActA$^-$ mutant, compared with infection with the *wt* strain.

In the present study, we focused on the role that cell-to-cell spread plays in vertical transmission. This seems relevant, because *L. monocytogenes* is a facultative, intracellular organism, and its intracellular lifestyle is important for virulence. In the natural setting of foodborne listeriosis, *L. monocytogenes* disseminates after it crosses the intestinal barrier. Dissemination most likely occurs via the hematogenous route. Indeed, maternal bacteremia is documented in ~50% of all cases of listeriosis during pregnancy in humans [3, 31]. *L. monocytogenes* could disseminate extracellularly or inside host cells—possibly infected maternal macrophages. Infected monocytes have been implicated in the crossing of the blood-brain barrier and the development of CNS infection with *L. monocytogenes* [32–34]. A similar mechanism has also been proposed for the dissemination of *Salmonella typhimurium* [35], another foodborne pathogen that can survive in mononuclear phagocytic cells and cause invasive systemic disease. Another example is dissemination of the intracellular parasite *Toxoplasma gondii,* which has been suggested to occur via infected immature dendritic cells [36]. Furthermore, the interaction of *Plasmodium falciparum*–infected red blood cells expressing erythrocyte membrane protein 1 with chondroitin sulfate A, as well as with hyaluronic acid, leads to the sequestration of infected erythrocytes in the placenta and has been shown to be important for the pathogenesis of placental malaria [37, 38]. Thus, cell-to-cell spread from infected maternal macrophages to trophoblasts might be a more relevant mechanism for trophoblast infection than is direct invasion by extracellular bacteria. However, we found that cell-to-cell spread was not essential for placental infection. A disadvantage of our pregnant guinea pig model is that we infected the animals by iv inoculation, which led to an initial seeding of the placenta by extracellular bacteria. We used this route because oral infection of pregnant guinea pigs with 1×10^9 cfu of the *wt* strain did not lead to placental infection (data not shown).

In our pregnant guinea pig model, we observed a 100-fold decrease in the number of bacteria in fetal infection with the

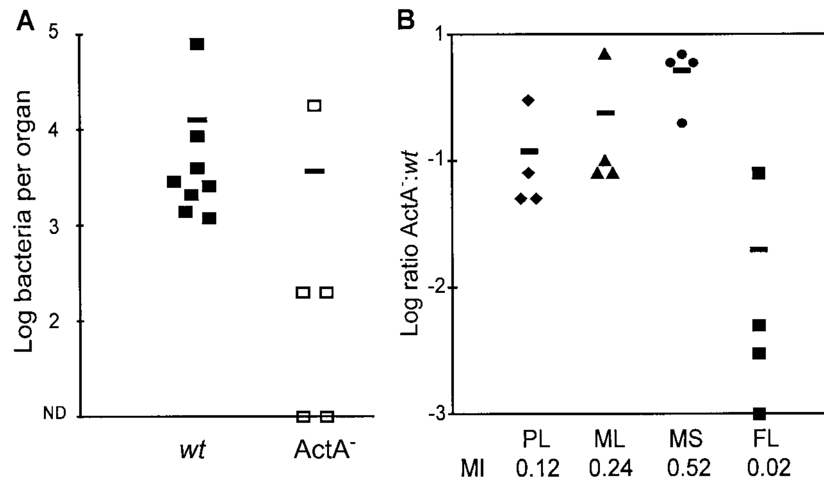

Figure 3. Fetal infection with wild-type *(wt)* or ActA$^-$ *Listeria monocytogenes* strains. *A,* The number of bacteria per fetal liver was counted at 24 h after intravenous (iv) inoculation of pregnant guinea pigs with 1×10^9 cfu of *wt (black rectangles)* or ActA$^-$ *(white rectangles) L. monocytogenes.* ND represents <100 bacteria per organ, which is below the level of detection. Black bars represent mean values. *B,* Competitive index analysis of ActA$^-$ and *wt* strains. Pregnant guinea pigs were iv inoculated with a 1:1 mixture of the 2 strains at a total dose of 1×10^8 cfu of bacteria. Ratios between erythromycin-resistant (ActA$^-$) and erythromycin-sensitive *(wt)* colonies were calculated for placenta (PL), maternal liver (ML), maternal spleen (MS), and fetal liver (FL). Black bars represent mean indices (MI).

ActA$^-$ mutant, compared with that in fetal infection with the *wt* strain. These results suggest that cell-to-cell spread plays a role in vertical transmission. A potential explanation for this finding is the large size of the syncytiotrophoblasts, compared with that of macrophages. In pregnant guinea pigs infected with the *wt* strain, the trophoblasts contained only a small number of bacteria. In contrast, the ActA$^-$ mutant appeared to reach high densities inside the trophoblasts before being able to infect neighboring cells (figure 2). This could decrease the efficiency of spread to fetal tissues.

Once the placenta was infected, we found a >1000-fold increase in the number of bacteria, which could be due to growth or influx of bacteria. The generation time of *L. monocytogenes* in a variety of guinea pig, murine, and human cell lines and primary cells is ~60 min [30]. In comparison, if the increase of *L. monocytogenes* in the guinea pig placenta is solely due to bacterial growth, the generation time would be ~70 min. This would lead to the conclusion that *L. monocytogenes* proliferates virtually unrestricted in the placental environment. In addition, in the placenta, we observed an increase in the number of LLO$^-$

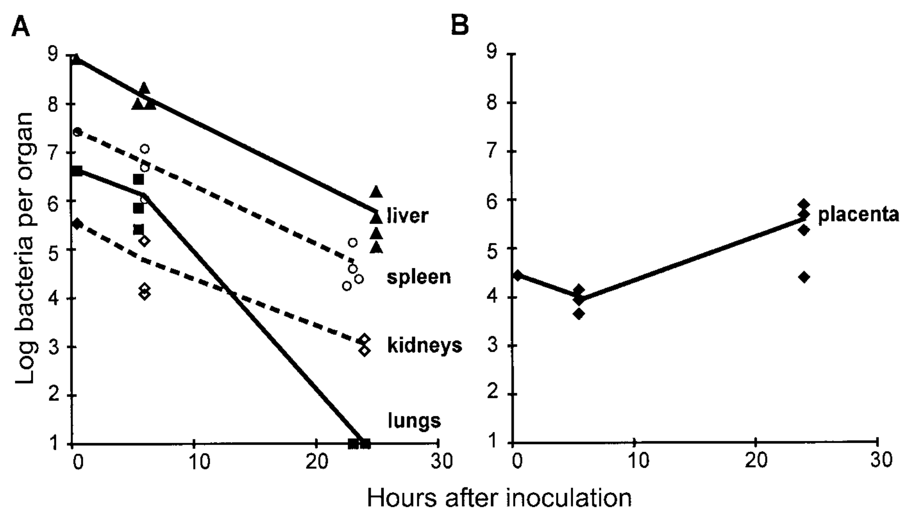

Figure 4. Phenotype of the listeriolysin O$^-$ (LLO$^-$) mutant strain of *Listeria monocytogenes* in pregnant guinea pigs. The number of bacteria per maternal liver, spleen, kidneys, lungs *(A),* and placenta *(B)* was counted at 0.5, 6, and 24 h after intravenous inoculation of pregnant guinea pigs with 1×10^9 cfu of the LLO$^-$ mutant. Solid and broken lines connect mean values.

mutant, which could be due to extracellular or intracellular bacterial growth. The latter could occur if the LLO$^-$ mutant could escape from the phagolysosome because of the activity of phospholipases, which contribute to vacuolar escape in some cell types [39]. Consistent with this hypothesis is evidence that the LLO$^-$ mutant escapes to a limited degree from the vacuole in the human choriocarcinoma cell line BeWo (data not shown). Our observations that *L. monocytogenes* mutants that are attenuated in maternal organs are able to increase in number or persist in the placenta suggest that growth, at least, contributes to the observed increase in the number of bacteria in the placenta.

The unique immunological condition of pregnancy is a possible explanation for why the placenta provides a protective environment for the growth of *L. monocytogenes.* The maternal immune system faces the double task of preventing the rejection of the semi-allogeneic fetus and protecting the mother and the fetus against infection. How these tasks are accomplished simultaneously remains to be fully explained. Suppression of maternal T cell–mediated immunity—for example, by induction of apoptosis in T cells via secretion of HLA-G and Fas ligand [40, 41] and by starvation of T cells for the essential amino acid tryptophan via production of indoleamine 2,3-dioxygenase [42]—most likely contributes to maternal immunological tolerance of the fetus. Changes in maternal cell-mediated immunity may increase the importance of the innate immune response for the defense against infection [43]—especially against intracellular pathogens. Indeed, cells of the innate immune response are strongly represented at the maternal-fetal interface. The predominant population of leukocytes in the hemochorial placenta of humans and rodents consists of non–antigen-specific NK cells and macrophages [44–46]. The interaction between macrophages and NK cells is critical during the early stages of listeriosis [47, 48]. However, the roles that uterine macrophages and NK cells play in protection against vertical transmission of *L. monocytogenes* are unknown.

In human tissues, uterine NK cells comprise 30%–40% of the total cells in first trimester decidua [44]. Notably, interleukin-15–deficient mice, which are depleted of NK cells, are not more susceptible to placental infection with *L. monocytogenes* than are *wt* mice [49], which suggests a negligible role for NK cells in the defense against *L. monocytogenes,* at least in murine placental tissues.

Macrophages act as primary host cells for *L. monocytogenes* and critical effector cells that are essential for the defense against listeriosis [47]. Colony-stimulating factor 1 (CSF-1) is the major regulator of cells of the mononuclear phagocytic lineage [50, 51]. CSF-1 is synthesized to very high concentrations at the maternal-fetal interface by the uterine epithelium [52–54]. In mouse peritoneal macrophages, CSF-1 stimulates macrophage phagocytic activity and intracellular growth of *L. monocytogenes* but not bactericidal activity [55, 56]. High concentrations of CSF-1 in the placenta could, therefore, shift the role of macrophages away from that of immune effector cells and toward that of primary host cells for *L. monocytogenes.* Contrary to this explanation are the results of a study on the effect that CSF-1 has on placental-fetal infection in a pregnant mouse model of listeriosis [57, 58]. The osteopetrotic mouse *(Csf1^{op}/Csf1^{op}),* which carries a null mutation in the CSF-1 gene, had higher levels of *L. monocytogenes* in placental-fetal tissues than did the heterozygous control mouse (+/*Csf1^{op}*), because of a failure to recruit neutrophils to the placenta [58]. However, results in a pregnant mouse model of listeriosis might not readily reflect the situation in the pregnant guinea pig model. For unknown reasons, the placental-fetal unit of guinea pigs—and humans—is much more susceptible to infection than is that of mice. Differences in susceptibility are apparent for infection with a variety of pathogens, such as cytomegalovirus [59, 60] and *T. gondii* [61, 62], in addition to *L. monocytogenes.* Furthermore, the time course of infection may be another important factor. The present study focused on the first 24 h after inoculation, whereas increased susceptibility of the *Csf1^{op}/Csf1^{op}* mouse to placental infection started to become apparent at 24 h and was significant at 72 h after inoculation.

In summary, we show evidence that the placenta provides a protective niche for *L. monocytogenes* and that cell-to-cell spread facilitates transplacental fetal infection. Regardless of how the bacteria travel to the placenta, the results of the present study clearly show that the placenta is uniquely permissive of listerial growth.

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References

1. Schlech WF III. Epidemiology and clinical manifestations of *Listeria monocytogenes* infection. In: Fischetti VA, ed. Gram-positive pathogens. Washington, DC: American Society for Microbiology Press, **2000**: 473–9.
2. Southwick FS, Purich DL. Intracellular pathogenesis of listeriosis. N Engl J Med **1996**; 334:770–6.
3. Mylonakis E, Paliou M, Hohmann EL, Calderwood SB, Wing EJ. Listeriosis during pregnancy: a case series and review of 222 cases. Medicine (Baltimore) **2002**; 81:260–9.
4. Teberg AJ, Yonekura ML, Salminen C, Pavlova Z. Clinical manifestations of epidemic neonatal listeriosis. Pediatr Infect Dis J **1987**; 6: 817–20.
5. Bakardjiev AI, Stacy BA, Fisher SJ, Portnoy DA. Listeriosis in the pregnant guinea pig: a model of vertical transmission. Infect Immun **2004**; 72:489–97.

6. Leiser R, Kaufmann P. Placental structure: in a comparative aspect. Exp Clin Endocrinol **1994**; 102:122–34.
7. Dussurget O, Pizarro-Cerda J, Cossart P. Molecular determinants of *Listeria monocytogenes* virulence. Annu Rev Microbiol **2004**; 58:587–610.
8. Portnoy DA, Auerbuch V, Glomski IJ. The cell biology of *Listeria monocytogenes* infection: the intersection of bacterial pathogenesis and cell-mediated immunity. J Cell Biol **2002**; 158:409–14.
9. Cabanes D, Dehoux P, Dussurget O, Frangeul L, Cossart P. Surface proteins and the pathogenic potential of *Listeria monocytogenes.* Trends Microbiol **2002**; 10:238–45.
10. Mengaud J, Ohayon H, Gounon P, Mege RM, Cossart P. E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells. Cell **1996**; 84:923–32.
11. Shen Y, Naujokas M, Park M, Ireton K. InIB-dependent internalization of *Listeria* is mediated by the Met receptor tyrosine kinase. Cell **2000**; 103:501–10.
12. Gruenheid S, Finlay BB. Microbial pathogenesis and cytoskeletal function. Nature **2003**; 422:775–81.
13. Welch MD, Mullins RD. Cellular control of actin nucleation. Annu Rev Cell Dev Biol **2002**; 18:247–88.
14. Goldberg MB. Actin-based motility of intracellular microbial pathogens. Microbiol Mol Biol Rev **2001**; 65:595–626.
15. Brockstedt DG, Giedlin MA, Leong ML, et al. *Listeria*-based cancer vaccines that segregate immunogenicity from toxicity. Proc Natl Acad Sci USA **2004**; 101:13832–7.
16. Gaillard JL, Jaubert F, Berche P. The *inlAB* locus mediates the entry of *Listeria monocytogenes* into hepatocytes in vivo. J Exp Med **1996**; 183:359–69.
17. Lecuit M, Dramsi S, Gottardi C, Fedor-Chaiken M, Gumbiner B, Cossart P. A single amino acid in E-cadherin responsible for host specificity towards the human pathogen *Listeria monocytogenes.* EMBO J **1999**; 18:3956–63.
18. Schubert WD, Urbanke C, Ziehm T, et al. Structure of internalin, a major invasion protein of *Listeria monocytogenes,* in complex with its human receptor E-cadherin. Cell **2002**; 111:825–36.
19. Lecuit M, Vandormael-Pournin S, Lefort J, et al. A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. Science **2001**; 292:1722–5.
20. Jacquet C, Doumith M, Gordon JI, Martin PM, Cossart P, Lecuit M. A molecular marker for evaluating the pathogenic potential of food-borne *Listeria monocytogenes.* J Infect Dis **2004**; 189:2094–100.
21. Kaufmann P, Davidoff M. The guinea-pig placenta. Adv Anat Embryol Cell Biol **1977**; 53:5–91.
22. Lecuit M, Nelson DM, Smith SD, et al. Targeting and crossing of the human maternofetal barrier by *Listeria monocytogenes:* role of internalin interaction with trophoblast E-cadherin. Proc Natl Acad Sci USA **2004**; 101:6152–7.
23. Brundage RA, Smith GA, Camilli A, Theriot JA, Portnoy DA. Expression and phosphorylation of the *Listeria monocytogenes* ActA protein in mammalian cells. Proc Natl Acad Sci USA **1993**; 90:11890–4.
24. Bishop DK, Hinrichs DJ. Adoptive transfer of immunity to *Listeria monocytogenes:* the influence of in vitro stimulation on lymphocyte subset requirements. J Immunol **1987**; 139:2005–9.
25. Auerbuch V, Lenz LL, Portnoy DA. Development of a competitive index assay to evaluate the virulence of *Listeria monocytogenes actA* mutants during primary and secondary infection of mice. Infect Immun **2001**; 69:5953–7.
26. Skoble J, Portnoy DA, Welch MD. Three regions within ActA promote Arp2/3 complex-mediated actin nucleation and *Listeria monocytogenes* motility. J Cell Biol **2000**; 150:527–38.
27. Jones S, Portnoy DA. Characterization of *Listeria monocytogenes* pathogenesis in a strain expressing perfringolysin O in place of listeriolysin O. Infect Immun **1994**; 62:5608–13.
28. Hodgson DA. Generalized transduction of serotype 1/2 and serotype 4b strains of *Listeria monocytogenes.* Mol Microbiol **2000**; 35:312–23.
29. Glomski IJ, Decatur AL, Portnoy DA. *Listeria monocytogenes* mutants that fail to compartmentalize listerolysin O activity are cytotoxic, avirulent, and unable to evade host extracellular defenses. Infect Immun **2003**; 71:6754–65.
30. Portnoy DA, Jacks PS, Hinrichs DJ. Role of hemolysin for the intracellular growth of *Listeria monocytogenes.* J Exp Med **1988**; 167:1459–71.
31. Siegman-Igra Y, Levin R, Weinberger M, et al. *Listeria monocytogenes* infection in Israel and review of cases worldwide. Emerg Infect Dis **2002**; 8:305–10.
32. Drevets DA, Jelinek TA, Freitag NE. *Listeria monocytogenes*-infected phagocytes can initiate central nervous system infection in mice. Infect Immun **2001**; 69:1344–50.
33. Drevets DA. Dissemination of *Listeria monocytogenes* by infected phagocytes. Infect Immun **1999**; 67:3512–7.
34. Drevets DA, Leenen PJ, Greenfield RA. Invasion of the central nervous system by intracellular bacteria. Clin Microbiol Rev **2004**; 17:323–47.
35. Vazquez-Torres A, Jones-Carson J, Baumler AJ, et al. Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. Nature **1999**; 401:804–8.
36. McKee AS, Dzierszinski F, Boes M, Roos DS, Pearce EJ. Functional inactivation of immature dendritic cells by the intracellular parasite *Toxoplasma gondii.* J Immunol **2004**; 173:2632–40.
37. Fried M, Duffy PE. Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. Science **1996**; 272:1502–4.
38. Beeson JG, Rogerson SJ, Cooke BM, et al. Adhesion of *Plasmodium falciparum*-infected erythrocytes to hyaluronic acid in placental malaria. Nat Med **2000**; 6:86–90.
39. Marquis H, Doshi V, Portnoy DA. The broad-range phospholipase C and a metalloprotease mediate listeriolysin O-independent escape of *Listeria monocytogenes* from a primary vacuole in human epithelial cells. Infect Immun **1995**; 63:4531–4.
40. Fournel S, Aguerre-Girr M, Huc X, et al. Cutting edge: soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis in activated CD8+ cells by interacting with CD8. J Immunol **2000**; 164:6100–4.
41. Makrigiannakis A, Zoumakis E, Kalantaridou S, et al. Corticotropin-releasing hormone promotes blastocyst implantation and early maternal tolerance. Nat Immunol **2001**; 2:1018–24.
42. Munn DH, Zhou M, Attwood JT, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science **1998**; 281:1191–3.
43. Sacks G, Sargent I, Redman C. An innate view of human pregnancy. Immunol Today **1999**; 20:114–8.
44. Hunt JS, Petroff MG, Burnett TG. Uterine leukocytes: key players in pregnancy. Semin Cell Dev Biol **2000**; 11:127–37.
45. Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol **2002**; 2:656–63.
46. Trundley A, Moffett A. Human uterine leukocytes and pregnancy. Tissue Antigens **2004**; 63:1–12.
47. Edelson BT, Unanue ER. Immunity to *Listeria* infection. Curr Opin Immunol **2000**; 12:425–31.
48. Unanue ER. Inter-relationship among macrophages, natural killer cells and neutrophils in early stages of *Listeria* resistance. Curr Opin Immunol **1997**; 9:35–43.
49. Barber EM, Pollard JW. The uterine NK cell population requires IL-15 but these cells are not required for pregnancy nor the resolution of a *Listeria monocytogenes* infection. J Immunol **2003**; 171:37–46.
50. Stanley ER, Guilbert LJ, Tushinski RJ, Bartelmez SH. CSF-1—a mononuclear phagocyte lineage-specific hemopoietic growth factor. J Cell Biochem **1983**; 21:151–9.
51. Webb SE, Pollard JW, Jones GE. Direct observation and quantification of macrophage chemoattraction to the growth factor CSF-1. J Cell Sci **1996**; 109:793–803.
52. Bartocci A, Pollard JW, Stanley ER. Regulation of colony-stimulating factor 1 during pregnancy. J Exp Med **1986**; 164:956–61.
53. Pollard JW, Bartocci A, Arceci R, Orlofsky A, Ladner MB, Stanley ER. Apparent role of the macrophage growth factor, CSF-1, in placental development. Nature **1987**; 330:484–6.
54. Pollard JW. Role of colony-stimulating factor-1 in reproduction and development. Mol Reprod Dev **1997**; 46:54–60; discussion 60–1.
55. Cheers C, Hill M, Haigh AM, Stanley ER. Stimulation of macrophage

phagocytic but not bactericidal activity by colony-stimulating factor 1. Infect Immun **1989**; 57:1512–6.
56. Denis M, Gregg EO. Identification of cytokines which enhance (CSF-1, IL-3) or restrict (IFN-gamma) growth of intramacrophage *Listeria monocytogenes*. Immunol Lett **1991**; 27:237–42.
57. Guleria I, Pollard JW. Aberrant macrophage and neutrophil population dynamics and impaired Th1 response to *Listeria monocytogenes* in colony-stimulating factor 1-deficient mice. Infect Immun **2001**; 69:1795–807.
58. Guleria I, Pollard JW. The trophoblast is a component of the innate immune system during pregnancy. Nat Med **2000**; 6:589–93.
59. Medearis DN. Mouse cytomegalovirus infection III. Attempts to produce intrauterine infections. Am J Hyg **1964**; 80:113–20.
60. Schleiss MR. Animal models of congenital cytomegalovirus infection: an overview of progress in the characterization of guinea pig cytomegalovirus (GPCMV). J Clin Virol **2002**; 25(Suppl 2):S37–49.
61. Wright I. Transmission of *Toxoplasma gondii* across the guinea-pig placenta. Lab Anim **1972**; 6:169–80.
62. Haumont M, Delhaye L, Garcia L, et al. Protective immunity against congenital toxoplasmosis with recombinant SAG1 protein in a guinea pig model. Infect Immun **2000**; 68:4948–53.