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Listeria placental infection

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Abstract

The gram-positive facultative intracellular bacterium Listeria monocytogenes is the causative agent of listeriosis, a severe foodborne infection. Pregnant women are at risk of contracting listeriosis, potentially leading to miscarriage, stillbirth, preterm birth and congenital neonatal infections. While other systemic bacterial infections may result in adverse pregnancy outcomes at comparable frequencies, L. monocytogenes has particular notoriety because fetal complications largely occur in the absence of overt illness in the mother, delaying medical intervention. Here, we briefly review the pathophysiology and mechanisms of materno-fetal listeriosis, discussed in light of a recent mBio report on Listeria transplacental infection in a nonhuman primate model.
Although many human systemic bacterial infections may result in miscarriage or stillbirth, unlike *Listeria monocytogenes*, the causative agents are not considered as primarily abortifacient. For example, recent data from UK show that of a cohort of 75 pregnant women with invasive *Hemophilus influenzae* infection, 63% had fetal loss (1). In comparison, with a similar incidence, only 40% of pregnancy-associated listeriosis cases resulted in spontaneous abortion, stillbirth or neonatal death in the same area (2). Other examples include *Brucella, Campylobacter, Coxiella* or *Salmonella* infections; while not recognized as part of the “TORCH” group of human congenital pathogens, all may lead to adverse fetal outcome in pregnant women (3-6) and are also well-known causes of septic abortion in farm animals (7). It has been suggested that essentially any invasive bacteria with the ability to survive within host cells can potentially colonize the placenta and fetus via the hematogenous route (8). What then makes *L. monocytogenes* stand out as a notorious miscarriage-associated microbe? One could argue that this is largely due to perception, owing to the mild or inapparent clinical course of maternal infection before onset of obstetric signs. This is in contrast to other systemic infections, where fetal complications are regarded as secondary to the mother’s illness.

The absence of obvious outward manifestations preceding listerial miscarriage has been experimentally observed in a study with intragastrically (i.g.) infected cynomolgus macaques published by Wolfe et al. in a recent issue of *mBio* (9). Similar findings were previously reported in rhesus macaques (10,11), confirming that the lack of specific prodromal signs appears to be characteristic of materno-fetal listeriosis. With a similar hemochorial, villous, discoidal placenta and reproductive cycle, non-human primates offer a suitable model to study human reproduction and related disorders (12,13). Macaques are naturally susceptible to *L. monocytogenes* infection, with clinical outcomes in pregnant monkeys mirroring those in human materno-fetal listeriosis. This includes miscarriage or
stillbirth in late pregnancy (14) (in pregnant women mostly during late second/third trimester [2,15]).

Timing of listerial miscarriage. The study by Wolfe et al. (9) is interesting in that the monkeys were inoculated in early gestation (single i.g. dose of $10^7$ CFU between gestation days [gd] 36-46), and all four exposed animals miscarried. By contrast, in an earlier study, Smith et al. (11) only observed six stillbirths among 23 late pregnancies following i.g. exposure of macaques at around gd 110. Wolfe et al. concluded that the macaque’s maternal-fetal interface may be particularly sensitive to listerial infection in early pregnancy, and that there might be a greater, unrecognized risk of listerial miscarriage in the first trimester of gestation (9).

However, careful analysis of existing data in macaques indicates that the suggested increased susceptibility in early pregnancy (9) may be more apparent than real, due to the high experimental infection dose used. In late pregnancy monkeys, Smith et al. (11) observed 16.6% stillbirths after i.g. exposure to $10^3$-$10^4$ CFU, 28.6% stillbirths when using $10^5$-$10^6$ CFU, whereas the only monkey administered $10^7$ CFU –i.e. the same dose as in Wolfe et al.– delivered a stillborn fetus. No adverse fetal outcomes were observed with a $10^2$ dose. For a given single-dose *L. monocytogenes* inoculum, a higher rate of bloodborne colonization per tissue mass is to be expected for a smaller uteroplacental unit than a significantly more developed one. An overwhelming placental infection in Wolfe et al. is supported by the acute course and short incubation period before fetal demise, only seven to 13 days postinoculation (9) compared to an average of 47 days until stillbirth in the Smith et al. study (11). The latter is more in keeping with the typical incubation period of materno-fetal listeriosis in humans (16), generally associated with lower levels of exposure to *L. monocytogenes* according to outbreak data ($5\times10^4$ CFU/g in the incriminated food [17]); $1.9\times10^6$ estimated dose for 50% listerial perinatal morbidity [18]).

A number of factors may explain the predominant presentation of materno-fetal
listeriosis in late pregnancy. For example, the human placenta only becomes truly hemochorial in the second trimester (19). This late shift in the nature of the materno-fetal interface appears to be unique to great apes. In the macaque, intervillous maternal circulation is established as early as three weeks after conception (12,13), which together with an earlier invasion of spiral arteries by the extravillous trophoblast (EVT) (12) (the primary placental cellular target of *L. monocytogenes*; see below) (Fig. 1) may contribute to explain the exquisite susceptibility noted by Wolfe et al. The importance of the placental configuration is unclear, however, because listerial abortion in ruminants is also predominantly observed in late pregnancy (7,20) despite having a different type of placentation (epitheliochorial, with the maternal and fetal circulations physically separated by six tissue layers) (13). Other possible explanations include: (i) predominance of low-level infections requiring a protracted incubation period, where the placenta itself may become a reservoir for maternal re-infection and amplification of the bacterial load (21); (ii) progressive increase of the uterine blood flow (and hence exposure of the placenta to bloodborne listeriae) (Fig. 2) relative to the overall cardiac output; (iii) possible exacerbation of critical immune tolerance mechanisms at the maternal-fetal interface in late gestation; (iv) physiological burden of advanced pregnancy, as suggested by the observation that pregnant women carrying multiple fetuses are at greater risk of listeriosis (22); (v) fetal death during early gestation is more likely to go unreported or remain etiologically undiagnosed.

**Mechanism of placental colonization and dissemination to fetus.** The high-dose infection used by Wolfe et al. caused an extensive neutrophilic inflammatory response with disruption of the macaques’ maternal-fetal barrier (9). Vasculitis, thrombosis and necrosis of the decidual spiral arteries, and presence of bacteria in the intervillous maternal circulation, villous capillaries and umbilical cord, were consistently observed (Fig. 1B). While inflammation-mediated hematogenous transmission to the fetus may prevail in an
acute or advanced placental infection, the importance of this dissemination pathway at the
initial stages of the process or in case of low-level infections is less clear. *L. monocytogenes*
is an actively invasive pathogen endowed with a stealthy actin-based cell-
to-cell spreading mechanism that bypasses the dependency on cell/tissue damage for
successful dissemination while allowing escape from immune control (23) (**Fig. 1A**). The
key importance of this cell-to-cell spread mechanism in transplacental colonization has
been demonstrated in vivo in non-primate animal models using *L. monocytogenes* mutants
lacking the virulence factor responsible for it, the actin-polymerizing protein ActA (24,25).

The primary hematogenous entry route to the placenta (**Fig. 2**) is more
controversial. Studies in guinea pigs, gerbils and mice show a contributing yet dispensable
role for the listerial invasins InlA and InlB (required for internalization into non-
phagocytic host cells but not for uptake by professional phagocytes) in placental and fetal
colonization (24,26,27). Thus, direct invasion by extracellular bacteria seems less likely to
be the main mechanism than cell-to-cell spread from infected maternal phagocytes
trafficking to the placenta (21) (**Fig. 1A**). The predominance of one mechanism over the
other may critically depend on the infectious dose and degree of infection of the primary
target organs (liver, spleen) after listerial intestinal translocation (**Fig. 2**).

**Placental tropism?** Wolfe et al. observed substantially larger bacterial loads in the
decidua and placenta, umbilical cord, amniotic fluid and fetal tissues (>10^7-10^8 CFU)
compared to the maternal nonreproductive tissue (liver, spleen and lymph node; <10^5
CFU). This was interpreted as a demonstration of the tropism of *L. monocytogenes* for the
gravid uterus (9). Alternatively, it may simply reflect the relative permissiveness of the
placenta to listerial infection compared to other (immunologically) more restrictive
maternal tissues and organs, i.e. “passive tropism”. The fetally-derived cytotrophoblast
which forms the maternal-fetal interface, particularly the invading EVT cells that penetrate
into the decidua and the maternal spiral arteries from the anchoring villi, appear to be the
primary target for *L. monocytogenes* placental colonization (24,25,28,29) (Fig. 1). Trophoblast cells lack class 1 HLA-A and HLA-B antigens and class II antigens while expressing non-classical HLA class 1 molecules, dampening allore cognition by uterine NK cells and T cells (30-32). This and other placental immune tolerance mechanisms prevent the rejection of the semi-allogenic fetus but at the same time may provide a protected sanctuary for the proliferation of intracellular pathogens like *L. monocytogenes*, ultimately depending on T cell-mediated immunity for clearance (33). Consistent with the rare co-occurrence of CNS involvement in materno-fetal listeriosis (0.01%) (15), neurological signs were never observed in experimentally infected pregnant macaques (9-11) despite listerial CNS infection having a shorter incubation period (one to 14 days) (16). In an immunocompetent pregnant mother, this may reflect competition between a permissive placenta and a less permissive blood-brain barrier in allowing the establishment of limited numbers of circulating listeriae (Fig. 2). The fact that the placenta and fetus are also preferential infection sites for *L. monocytogenes* in ruminants despite the structurally and cellularly distinct interhemal barrier argues against the implication of specific targeting mechanisms (unless involving a conserved, promiscuous host receptor); it suggests rather that placental invasion depends on general intrinsic features of the *Listeria* host-pathogen interaction (such as e.g. cell-to-cell spread in an immunologically relatively permissive environment).

The above does not exclude the existence of *L. monocytogenes* determinants facilitating establishment and proliferation in the maternal reproductive tract and placenta. According to recent data in guinea pigs, a listerial protein of the internalin family, InlP generally conserved in *L. monocytogenes*, appears to specifically aid placental colonization (34). Virulence heterogeneity among *L. monocytogenes* isolates is well documented, and specific “hypervirulent” clonal complexes have been epidemiologically and experimentally associated with invasive (placental and CNS indistinctly) listeriosis (35). Anecdotal
evidence hints at the possibility that particular strains might be more prone to cause materno-fetal infections vs other clinical presentations. For example, some listeriosis outbreaks, associated with a specific *L. monocytogenes* strain, have an unusually greater frequency of maternal-perinatal cases (36,37). In ruminants, materno-fetal and CNS forms of the disease seldom occur simultaneously in the same herd outbreak (20,38).

Interestingly, considered together, the studies by Wolfe et al. (9) and Smith et al. (10,11) show that two strains associated with natural materno-fetal listeriosis in humans or primates consistently caused miscarriage in experimentally infected macaques with an i.g. dose of $10^7$, while two others involved in human outbreaks with a low frequency of materno-fetal cases did not. Specifically, a *L. monocytogenes* strain (ScottA) from a listeriosis outbreak with very few pregnancy-related cases (seven of 42) (39) caused stillbirth at a dose of $10^{10}$ (1/1) but not $10^8$ (0/1) unless as part of a mix with isolates associated with materno-fetal infections (2/2) (10). Although based on very limited evidence and far from conclusive, these data show a trend that warrants further investigation.

As briefly outlined here, many areas still remain obscure in our understanding of materno-fetal listeriosis. Key points requiring clarification include the impact of the bacterial dose on placental infection dynamics, the pathophysiology and determinants of transplacental invasion, and the potential involvement of tropism factors. Also, the role of early immune signaling events prior to transplacental colonization as a precipitating factor in fetal demise (40). Experiments in relevant animal models, including macaques closely replicating the human system, should prove invaluable for illuminating the detailed mechanisms of placental listeriosis and other aspects of *Listeria* pathogenesis.

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References


FIGURE LEGENDS

**Fig. 1.** *Listeria* transplacental infection. Top, a pregnant human uterus and schematic of the maternal-fetal interface. Anchoring villi and (far right) a floating villus are represented. Bottom, magnified diagram of an anchoring villus and its main structural and cellular components illustrating two transplacental invasion scenarios: (A) low-level infection with stealthy, mainly cell-to-cell spread-based transplacental dissemination; (B) acute infection with strong inflammatory response, major disruption of the integrity of placental barrier with degeneration of syncitiotrophoblast, and significant hematogenous dissemination. The diagram represents the uterine decidua (DD), end of a spiral artery (SA) from which maternal blood flows into the intervillous space (IS), and a chorionic villus tree lined by the syncitiotrophoblast (ST) with underlying progenitor cytotrophoblast (CT) cells and a basement membrane (BM). CT cells penetrate into the decidua to anchor the villi in the uterus and invade the maternal arteries to allow blood extravasation into the IS. The villous stroma (STR) contains fetal capillaries (FC) that get closer to the villous surface as
pregnancy advances. Panel (A) illustrates the two main hematogenous placental invasion pathways: (a) actin-based cell-to-cell spread from infected phagocytes that traffic from primary infectious foci in maternal organs to the placenta (see Fig. 2); (b) invasion of the trophoblast by free bloodborne listeriae. Bacteria are in yellow (not at scale).

**Fig. 2.** Pathophysiology of foodborne listeriosis. *L. monocytogenes* bacteria cross the epithelial barrier of the intestine, translocate to the mesenteric lymph nodes and reach their primary target organs, i.e. liver and spleen. There they establish infectious foci that in an immunocompetent individual are efficiently cleared by cell-mediated immunity. In adult people with no predisposing conditions, the process is largely subclinical. In this population, exposure to larger infective doses may cause febrile gastroenteritis and, in rare cases, invasive disease. In immunocompromised adults and elderly people, unable to mount an efficient T-cell mediated immune response, the primary infectious foci are inadequately resolved and *Listeria* bacteria may be released to the bloodstream. This results in febrile bacteremia and, eventually, invasive infection of the brain. In pregnant women, *L. monocytogenes* colonizes the uterus in addition to liver and spleen. While the infection is controlled in the latter organs, the placental immune tolerance mechanisms provide a permissive niche for the proliferation of *L. monocytogenes*. Bacteria from the placental reservoir released to the bloodstream may reinfect the mother’s liver and spleen, contributing to infection maintenance and amplification (21). Transplacental dissemination to the fetus results in abortion, stillbirth or neonatal sepsis. A late onset congenital form is also observed in neonates, often accompanied by meningitis. Based on an original depiction in ref. (38).