AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity®

MINIREVIEW

Animal and Human Tissue Models of Vertical *Listeria monocytogenes* Transmission and Implications for Other Pregnancy-Associated Infections

David E. Lowe,[a] Jennifer R. Robbins,[b] Anna I. Bakardjiev[a]*

[a]Benioff Children's Hospital and Program in Microbial Pathogenesis and Host Defense, University of California San Francisco, San Francisco, California, USA

[b]Department of Biology, Xavier University, Cincinnati, Ohio, USA

ABSTRACT Intrauterine infections lead to serious complications for mother and fetus, including preterm birth, maternal and fetal death, and neurological sequelae in the surviving offspring. Improving maternal and child heath is a global priority. Yet, the development of strategies to prevent and treat pregnancy-related diseases has lagged behind progress made in other medical fields. One of the challenges is finding tractable model systems that replicate the human maternal-fetal interface. Animal models offer the ability to study pathogenesis and host defenses *in vivo*. However, the anatomy of the maternal-fetal interface is highly divergent across species. While many tools are available to study host responses in the pregnant mouse model, other animals have placentas that are more similar to that of humans. Here we describe new developments in animal and human tissue models to investigate the pathogenesis of listeriosis at the maternal-fetal interface. We highlight gaps in existing knowledge and make recommendations on how they can be filled.

KEYWORDS animal model, *Listeria*, maternal-fetal barrier, placental pathogens

Infections during pregnancy can lead to infections of the placenta, spread to the fetus, and cause serious pregnancy complications, including spontaneous abortion, preterm labor, stillbirth, neonatal sepsis and death, and neurological sequelae in the survivors. The placenta and surrounding uterine tissues have long been considered immunosuppressed in order to tolerate the fetal allograft (1). As a consequence it has been hypothesized that the maternal-fetal interface is immunocompromised, which would render it susceptible to infection. However, it is becoming increasingly clear that the placenta is surprisingly resistant to all but a few, largely intracellular pathogens (2, 3). Our knowledge of the physical and biochemical nature of the maternal-fetal barrier is just beginning to emerge, thanks in large part to the study of the few pathogens that can breach it. As multiple tissues are involved, the utility of *in vitro* models is limited, and development of animal models and advances in organ culture have thus played an indispensable role. However, much remains unanswered, and asking the next questions requires careful selection of appropriate model systems.

THE MATERNAL-FETAL INTERFACE

The maternal-fetal interface is composed of a maternal uterine layer called the decidua and the fetal villi, which are bathed in maternal blood (Fig. 1). It has two major functions: to nourish the fetus and to protect it from both maternal immune cells and pathogens. In multiple ways, these functions conflict with each other: the first requires a thin, highly permeable barrier, while the second demands a thicker, more impermeable one. Further, maternal immune tolerance is best served by excluding certain

Accepted manuscript posted online 26 February 2018

Citation Lowe DE, Robbins JR, Bakardjiev AI. 2018. Animal and human tissue models of vertical *Listeria monocytogenes* transmission and implications for other pregnancy-associated infections. Infect Immun 86:e00801-17. https://doi.org/10.1128/IAI.00801-17.

Editor Helene L. Andrews-Polymenis, Texas A&M University Health Science Center

Address correspondence to Anna I. Bakardjiev, abakardjiev@vir.bio.

* Present address: Anna I. Bakardjiev, VIR Biotechnology, San Francisco, California, USA.

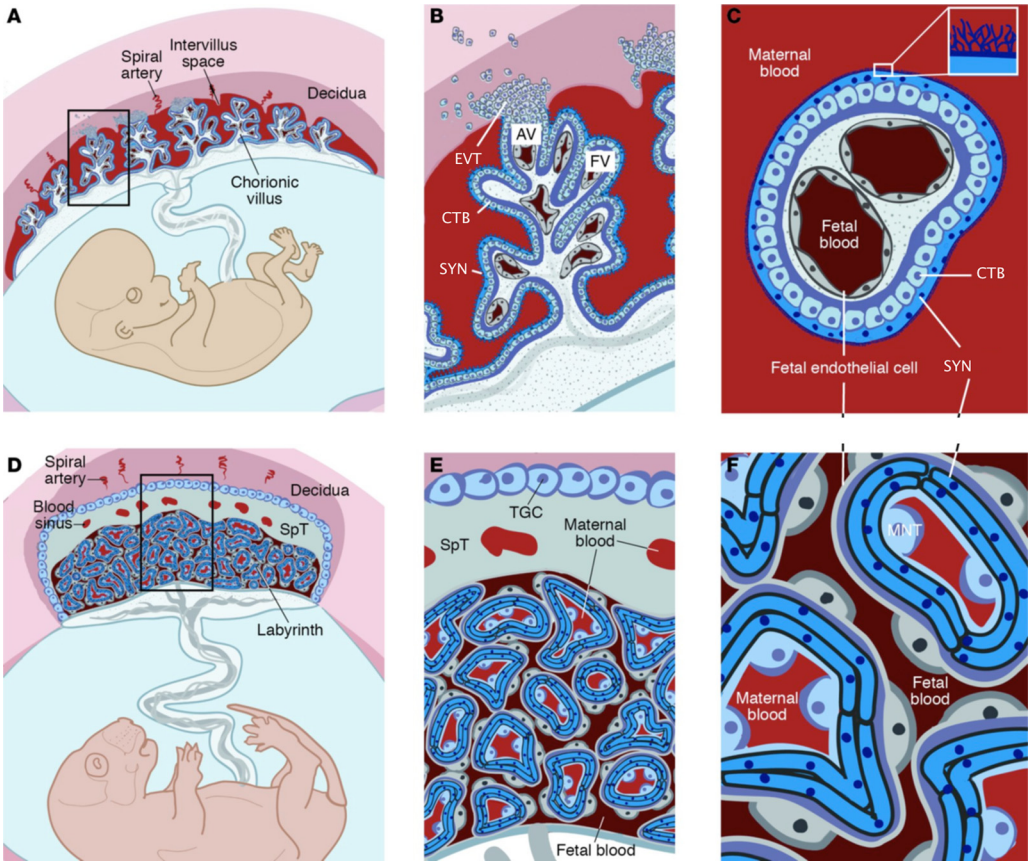

FIG 1 Human and mouse placental tissues. Humans (A to C) have a villous hemochorial placenta, while mice (D to F) have a labyrinthine hemochorial placenta. Here, maternal tissues are pink or bright red, while fetal tissues are blue, except for the fetal blood, which is dark red. (A) In humans, decidual spiral arteries perfuse the chorionic villi with maternal blood. (B) Villi can be floating freely in that blood (FV) or anchored in the maternal decidua (AV); these represent the two points of interface between maternal and fetal tissues. In AV, extravillous trophoblasts (EVTs) invade deeply into the decidua, whereas in FV, a single-cell layer of self-renewing cytotrophoblasts (CTB) underlies the continuous, single layer of syncytiotrophoblast (SYN) that is in contact with maternal blood. It is here that nutrients, gas, and wastes pass across the barrier. (C) The cross-sectional anatomy of an FV shows the fetal blood vessels within, as well as the branched microvilli on the surface of the SYN, maximizing surface area. (D) In mice, blood from spiral arteries flows through a layer of spongiotrophoblasts (SpT), progenitors of trophoblast giant cells (TGCs) that line but do not deeply invade the decidua. (E) TGCs, like EVTs, anchor the placenta more weakly than human placentas but still act as an important fetal-maternal interface. (F) Maternal blood in mice is in direct contact with a layer of mononuclear trophoblasts (MNTs), which overlays two layers of SYN. Nutrients, gasses, and wastes must cross the MNTs and SYN bilayer in order to reach fetal blood. (Republished from reference 34 with permission of the publisher.)

leukocyte populations, while host defense requires a significant immune presence. These conflicts are resolved in important histological ways.

Placental tissues develop rapidly and change over time. At different stages of gestation, the organ includes unique cell types, morphology, or functions not replicable *in vitro*. Specialized fetal cells called trophoblasts are critical for the formation of the maternal-fetal interface. Trophoblasts differentiate into subtypes depending on their function. There are two sites of direct contact between trophoblasts and maternal blood or tissues: (i) the area where extravillous trophoblasts (EVTs) invade and anchor into the decidua and (ii) the much larger surface where syncytiotrophoblast (SYN) cells are bathed in maternal blood. Underlying both EVTs and the SYN are undifferentiated trophoblasts that replenish the cells above them. Remarkably, EVTs in the decidua are juxtaposed to maternal immune cells. The decidual cytokine environment precludes a strong T cell presence (4) in favor of monocytes/macrophages, NK cells, and T regulatory cells, which are likely to promote fetal tolerance (5).

Meanwhile, the trophoblasts in contact with maternal blood fuse to become the SYN, an extensive multinucleated barrier between the maternal blood and fetal capil-

laries that nutrients and waste can cross. While this layer is thin, the lack of intercellular junctions (6) and high elastic modulus (7) likely repel both pathogens and immune cells. The SYN also lacks major histocompatibility complex (MHC) class I proteins, decreasing the likelihood of being recognized as foreign by maternal leukocytes (8). Finally, the SYN has strong antiviral defenses, including production of microRNA (miRNA)-containing exosomes and secretion of type III interferon, as well as interferon-independent host defenses that restrict colonization with *Toxoplasma gondii* (9–11).

Only a few of these differentiated trophoblasts can be derived *in vitro*, and they may not fully represent the actual cells *in vivo*. The BeWo choriocarcinoma trophoblast cell line can be induced to syncytialize with forskolin (12), but the cells are derived from a tumor, their syncytium is patchy rather than continuous, and the extent to which its gene expression mimics that of syncytia *in vivo* is not known. Few other human trophoblast-derived lines can be induced to syncytialize at all (13), and the responses of choriocarcinoma cell lines stimulated with Toll-like receptor (TLR) agonists do not mimic the responses of primary human trophoblasts (14). A recently developed three-dimensional culture model using the human choriocarcinoma JEG-3 cell line develops phenotypes more closely related to natural SYN (15). In this method, Cytodex beads are coated with human brain microvascular endothelial cells and incubated with JEG-3 cells in a bioreactor. However, these cells still cannot appreciably reconstitute the entire maternal-fetal interface. These studies underscore the difficulty of understanding the antimicrobial actions of differentiated trophoblasts *in vitro* (Table 1).

Thus, *in vivo* models are necessary, as they provide a highly differentiated placenta with all subsets of trophoblasts present and the ability to be analyzed at different gestational ages. However, a key problem with studying placental infection *in vivo* is the high divergence of the anatomy of the maternal-fetal interface across animal species (16). Broadly, placentas fall into three main types: hemochorial, epitheliochorial, and endotheliochorial. For this review, we will discuss only hemochorial placentas found in primates and rodents. In these placentas, fetal trophoblasts are in direct contact with maternal blood instead of being separated by an epithelial or endothelial layer, a crucial consideration when studying the maternal-fetal barrier.

Hemochorial placentas can be further subdivided into villous or labyrinthine. Villous placentas are found in primates, including humans, and are characterized as having branched chorionic villi surrounded by maternal blood (Fig. 1A to C). Rodents, including mice and guinea pigs, have a labyrinthine placenta (Fig. 1D to F). The areas of direct contact between trophoblasts and maternal blood/tissues vary regardless of whether the placenta is villous or labyrinthine. First, the number of SYN layers differs across species. Humans and guinea pigs have one syncytial layer, while mice have two syncytial layers and a discontinuous layer of mononuclear trophoblasts on the blood-bathed side of the syncytium. Second, the degree of trophoblast invasion into the uterus differs. Humans, primates, and guinea pigs have a very invasive placenta, with EVTs invading all the way into the inner third of the myometrium (16). In contrast, mice and gerbils show very little invasiveness (17).

The differences between animal model placentas and humans are large, but models nonetheless remain useful (Table 1). Even though the placental structure varies considerably across host clades, *Listeria monocytogenes* can infect placentas in a wide range of animals (18, 19). This suggests commonalities in infection that can be applied to human disease. As we discuss below, animal models and *ex vivo* systems have been used to study dissemination to the placenta, bacterial interaction with specialized trophoblasts, and the maternal immune responses to *L. monocytogenes*; these studies would be impossible to recapitulate *in vitro*. The advantages of using multiple models to confirm results will also be discussed. By summarizing the results, benefits, and drawbacks of these models, we hope that researchers will better be able to find the right suite of animal and tissue experiments, rather than seeking out the perfect single model.

TABLE 1 Comparison of advantages and disadvantages of various models for studying placental pathogens, particularly *L. monocytogenes*[a]

Model	High genetic variation	Robust syncytiotrophoblast	Invasive extravillous trophoblasts	Organization of tissue layers	No. of SYN layers	Genetic manipulability	GI *L. monocytogenes* inoculation (vs i.v.)	No./expt	Maternal immune cells	Human *L. monocytogenes* sequelae	Expense
Choriocarcinoma cells	+	+	+	NA	NA	+++	NA	++++	Absent	NA	$
Mice	++	++++	++	Labyrinthine	2	++++	+++	+++	Present	+++	$$
Gerbils	+++	++++	++	Labyrinthine	2	++	++	++	Present	++	$$$
Guinea pigs	+++	++++	++++	Labyrinthine	1	++	+++	++	Present	+++	$$$$
Nonhuman primates	+++	++++	++++	Villous	1	++	++++	+	Present	++++	$$$$$$$
Human placental explants	++++	+++	+++	Villous	1	+	NA	++	Mostly absent	NA	$$$$

[a]Models are evaluated on several aspects. Host genetic variation can be either an advantage or a disadvantage, depending on the desired application. Only fully *in vivo* models exhibit robust SYN; while SYN is present in explants, it may include breaks from physical damage, and syncytialized choriocarcinoma cells are patchy and possibly exhibit different gene expression. Invasive extravillous trophoblasts can be found in all *in vivo* models but are most extensive in villous placentas. +, low; ++, low intermediate; +++, high intermediate; ++++, high; NA, not applicable.

THE IDEAL PLACENTAL MODEL PATHOGEN: *LISTERIA MONOCYTOGENES*

The placenta is infected by a limited but diverse set of bacteria, viruses, and parasitic protozoa. Despite the diversity, they share key features: they are intracellular pathogens, have a tropism for phagocytes, and reach the placenta through the bloodstream (2, 3, 20). Of this set of pathogens, *Listeria monocytogenes* is a strong choice for exploring placental pathogenesis. First, *L. monocytogenes* causes a foodborne illness that is a major clinical concern during pregnancy because it can lead to serious complications, including preterm birth (50%), fetal demise (20 to 30%), infant mortality (24.5%) and long-term neurological damage in the surviving offspring (12.7%) (21, 22). It is also not rare: *L. monocytogenes* has been detected in 1.6% of second-trimester spontaneous abortions (23).

In addition to its clinical relevance, *L. monocytogenes* has several advantages for experimental research. *L. monocytogenes* is easily cultured, has a large genetic toolkit (24–26), and has been used for decades to understand intracellular pathogenesis and the host's immune responses to infection (27–29). Its intracellular life cycle is well understood. *L. monocytogenes* can enter cells in three ways: phagocytosis, receptor mediated endocytosis, and cell-to-cell spread (30). Receptor-mediated endocytosis occurs through interactions with membrane-bound listerial internalins and host cell receptors. The most significant of the internalins are internalin A (InlA), which binds E-cadherin (Ecad) (31, 32), and internalin B (InlB), which binds c-Met (33). Once internalized, the virulence factor listeriolysin O (LLO) is able form pores in the acidifying phagosome and allow *L. monocytogenes* to escape into the cytoplasm (27). Replication occurs in the cytoplasm, and the cells express ActA to induce actin-based motility. The motile bacteria can then spread to neighboring cells without exposure to the extracellular environment and restart the infection cycle. The tractability of *L. monocytogenes* is a huge advantage for the investigation of maternal-fetal infections, since genetic manipulation of the host decidua and placenta in relevant model systems is much more challenging.

MOUSE MODELS OF PLACENTAL INFECTION

Mouse models offer several advantages, such as well-understood immune responses to pathogens, a large set of genetic knockouts, and a short gestation with large litters (Table 1). However, opinions vary as to the usefulness of the pregnant mouse model to understand human disease. Like for human placentas, mouse placentas are hemochorial, trophoblasts invade the maternal decidua (though less extensively), and they a have similar decidual immune cell composition. On the other hand, the mouse placenta is labyrinthine and less invasive, and its morphology may have caused its trophoblasts to evolve characteristics distinct from those of human trophoblasts. Rather than columns of numerous, small EVTs extending deep into the decidua, the functionally homologous murine trophoblasts are organized into a near monolayer of enormous cells lining the decidua, creating a thinner maternal-fetal interface at the uterus (compare Fig. 1B and E). On the other hand, the interface with the maternal blood is thicker: fetal capillaries are surrounded by two layers of SYN, rather than the single layer found in humans (compare Fig. 1C and F). Undergirding and extending beyond these histological differences are a number of molecular differences that may play roles in preventing or resolving infection (34). However, many of the criticisms of mouse models are also concerns in other nonprimate animal models. Typically, research with mice should be corroborated with other animal models to confirm findings. Thus, the mouse model is an important, if limited, model of infection.

Because *L. monocytogenes* does not naturally infect across the mouse gut, it is typically injected intravenously (i.v.) via the tail vein. This leads to rapid colonization of the liver and spleen and initiates hematogenous dissemination. However, it may lead to experimental artifacts (35). Newer models have been developed which allow for gastrointestinal (GI) infection by humanizing mouse Ecad in order to bind wild-type InlA or mutating InlA to bind murine Ecad (36–39). However, even these newer models require a large oral or intragastric inoculum and have variable systemic dissemination. This complicates placental infection because a stringent bottleneck inhibits consistent

placental colonization without a high systemic bacterial load (40). Thus, a large inoculum or a large number of animals is required to gather sufficient data. Other new techniques for GI infection show promise for improved systemic dissemination, but these have not been tested in pregnant animals (41).

Mouse models are an economical and rapid method to test the role of virulence factors in placental infections. Because of the bottlenecks from gut to blood and from blood to placenta (40), placental infection is variable in all animals tested, leading to a large range of CFU outcomes within a single experiment that complicates statistical analysis. The large litter size and short gestational time make mice a cost-efficient model to understand which bacterial factors are critical for breaching the placental barrier. The best-studied virulence factors of *L. monocytogenes* include InlA, InlB, LLO, and ActA. Research into the role of these virulence factors has shed light on how *L. monocytogenes* is able to colonize the placenta and reach the fetus.

A central concern with murine *L. monocytogenes* infection is the lack of InlA-Ecad interactions due to a single amino acid difference from human Ecad (42). As discussed above, models have been developed to circumvent this problem (37, 39); however, these approaches have led to conflicting results as to the role of InlA in crossing the maternal-fetal barrier. Disson et al. (39) found that InlA and InlB mutants were ~5-fold less capable of placental infection without any significant differences in the colonization of maternal spleen or liver in mice expressing human Ecad. In contrast, when *L. monocytogenes* expressing murinized InlA was used to infect wild-type mice, Wollert et al. (37) did not detect any differences in bacterial burden or histology in murine placentas infected with wild-type *L. monocytogenes*, suggesting that the InlA-Ecad interaction is not required to colonize the placenta. Further, while gastrointestinal infections require either mutated Ecad or InlA, placental infection occurs in wild-type mice infected intravenously even in the absence of an InlA-Ecad interaction (43). Since Disson et al. (39) used a competitive infection and the other studies measured CFU burden, a direct comparison is difficult to make. A possible reconciliation is that InlAB interactions are sufficient and advantageous but not necessary in placental invasion of mouse models.

LLO-deficient *L. monocytogenes* cannot escape the vacuoles of infected cells and is rapidly cleared from the spleens and livers of intravenously infected mice (43). These ΔLLO bacteria are able to infect the placenta and persist over 72 h, but at very low numbers, and they are incapable of spread to the fetus. ActA mutants are able to escape the vacuole and replicate in the cytoplasm but cannot spread from cell to cell. While maternal organs are able to clear the infection over 24 to 48 h and the mutants have reduced ability to infect the placenta, the placental bacterial burden increases over 4 days. Also, despite the loss of bacterial ability to spread from cell to cell, fetal infection is possible with Δ*actA* mutants, albeit at low numbers. These mutants demonstrate that while the placenta is difficult to initially infect, the placenta cannot easily clear the bacteria, likely due to the immune restriction in the decidual environment. Simultaneously, they show that fetal infection is possible (though attenuated) as long as the bacterium reaches the host cell cytoplasm.

Mice have also been used to discover and test the roles of virulence factors that are specifically used for colonization of the placenta. The secreted internalin InlP was initially identified in a screen in pregnant guinea pigs to discover genes specifically important for placental infection (44). Deletion of InlP causes an ~1,000-fold decrease in placental CFU in the mouse compared to that of wild-type *L. monocytogenes*, with only a slight decrease of bacterial burden in maternal organs (44). Interestingly, InlP shares high homology with another secreted internalin, Lmo2027, but only InlP is consistently found in virulent *L. monocytogenes* strains (44). Mouse models also suggest the importance of a novel phosphotransferase (PTS) system found in a hypervirulent strain of *L. monocytogenes* (45). The PTS locus, termed *Listeria* pathogenicity island 4 (LIPI-4), is both necessary and sufficient for a 5-fold increase over a lab reference strain in direct competition *in vivo*. These studies not only present an opportunity to understand how *L. monocytogenes* is able to infect the maternal-fetal interface but also offer evolutionary insight into how pathogens gain this ability.

Mouse models are the most powerful animal model for dissecting the host response to placental infection due to their ability to be genetically manipulated. However, knockouts and transgenes often have multiorgan effects, which is problematic since placental colonization is dependent on the systemic burden in maternal organs, including spleen, lymph nodes, and liver (46, 47). An example of this can be seen in the role of colony-stimulating factor 1 (CSF-1) during placental infection (48). CSF-1 is a chemotactic cytokine that is important in innate immune cell development and survival. It is also expressed highly at the maternal-fetal interface (49). Pregnant CSF-1-deficient mice sustain higher burdens of *L. monocytogenes* in the placenta than heterozygous mice (48). This can be attributed to decreased chemokine production by trophoblasts, reducing neutrophil recruitment to the implantation site. However, CSF-$1^{-/-}$ mice also have immune deficiencies in maternal organs that increase the bacterial burden on the spleen and liver, creating an atypical innate immune cell response to infection (48). Given the high expression of CSF-1 by trophoblasts, the data do suggest that CSF-1 has a role in pathogen defense there. However, the systemic defects in CSF-1-deficient mice raise questions as to what role placental CSF-1 plays in infection when the maternal immune system is normal.

To circumvent these issues, some researchers use methods to specifically inactivate genes in the placenta. The short gestational period, high fecundity, and *in vitro* fertilization (IVF) technologies in mouse models allow dissection of fetal placental effects out from the maternal background. One approach uses a mixed breeding scheme, where only the maternal decidua or only the fetal trophoblasts express the mutation or transgene of interest (50, 51). For example, a cross between a wild-type male and a homozygous mutant female results in a mutant mother with wild-type placentas. Generating mutant placentas in a wild-type mother is more difficult, but mating a heterozygous female with a homozygous mutant male will result in ~50% of placentas carrying the mutation. One can also implant the IVF-produced embryos of mutant parents into a wild-type mother, although this may introduce new uncontrolled variables in placental anatomy and gene expression (52, 53). Use of a placenta-specific Cre-expressing mouse would simplify these experiments further (54, 55). In the event that the desired mutation does not exist, it is also possible to infect the blastocyst with a lentivirus construct that leads to gene expression (or repression) only in the placenta (56–58).

Finally, the pregnant mouse model can be used to understand how the host immune system balances the need for fetal tolerance with the need for defense against pathogens for both mother and fetus. There are important differences between human and mouse immune systems (59), but unlike in other animal models, mouse immunity is well understood, is serviced by a large panel of tools and reagents, and offers a wide variety of well-characterized knockouts. Humans exhibit an increase in T regulatory cells (Treg) during pregnancy that can be modeled in mice by breeding C57BL/6J females with BALB/c males; offspring have an increased number of maternal Foxp3^{+} cells that increase susceptibility to pathogens such as *L. monocytogenes* and *Salmonella enterica* serovar Typhimurium (60). Further, *L. monocytogenes* infection leads to a reduction of the Treg's suppression of CD4^{+} T cells at the maternal-fetal interface (50). Such models are useful in investigating the increased susceptibility to certain pathogens during pregnancy and in determining how colonization and dissemination occur. Mouse models can also test the roles of specific cell types and cytokines at the maternal-fetal interface. For example, in trying to understand when the maternal immune system tips from tolerating the fetus to attacking it, Perchellet et al. used a mixed mating scheme where only the fetus expresses ovalbumin in a mother whose T cells are engineered to recognize ovalbumin (51). They found that pregnancy-elevated Treg somewhat depress the helper T cell (T_H) response against the fetus but less so the cytotoxic T cell (T_C) response. It seems clear that maternal T cell activation is important for the immune rejection of infected fetuses, yet the molecular mechanisms are only beginning to emerge. Using *L. monocytogenes* and a variety of mouse genetic models and antibody depletions, Chaturvedi et al. found that neutrophils express CXCL9 and recruit fetus-specific CD8^{+} T cells into the decidua, ultimately causing fetal resorption (61).

GUINEA PIGS

Guinea pigs (*Cavea porcellus*) have been used as an animal model in infectious disease since the 19th century. For many pathogens, the guinea pig is able to exhibit human disease features not seen in mice. Guinea pig tuberculosis models have a low infectious dose and are able to form granulomas (62). Similarly, studies with *Legionella pneumophila* (63, 64) and *Staphylococcus aureus* (65–67) have demonstrated close similarities to humans in susceptibility and infection routes. This makes them a good model for preclinical drug and vaccine development for diseases that poorly infect smaller animal models. Their placentas are also more similar to those of humans than to those of mice.

Like human placentas but unlike murine ones, guinea pig placentas invade deep into the decidua, with trophoblasts almost reaching the myometrium (Table 1). Also as in humans, there is only one layer of syncytium between the maternal blood and the fetal vessels, whereas mouse placentas have two syncytial layers. Additionally, guinea pigs can vertically transmit both cytomegalovirus (68) and *Chlamydia* (69), which has not been reported in mouse models. However, like murine placentas, guinea pig placentas are labyrinthine and include specialized trophoblasts that may differ in function than those in human villous placentas. There are practical drawbacks as well: their gestation period is about three times longer than that of mice, which extends the wait time between experiments. Guinea pigs also lack genetic deletion or transgenic models, and fewer antibodies are available. Also, their relatively large size adds to the cost of housing and limits the number of animals that a lab can use in studies.

In terms of *L. monocytogenes* infections, guinea pigs have the advantage of expressing an Ecad that can interact with InlA (38); however, the InlB/c-Met interaction does not induce internalization (70). Intravenous infection of pregnant guinea pigs with either an InlA- or InlAB-deficient mutant does not show a defect by competitive infection (70). This could suggest that these internalins do not play a role in colonizing the placenta. Alternatively it may be that in animal models where either the InlA or InlB entryway is closed, these internalin pathways are not necessary for invasion of the placenta (39).

Since guinea pig gastrointestinal Ecad can mediate InlA-based internalization, an oral model of infection is possible. Initial oral infections used high doses of *L. monocytogenes* in order for infection to occur (38). This results in modest but inconsistent infection of the epithelium and mesenteric lymph nodes. Since placental infection relies on systemic maternal infection, such gavage methods have led to poor systemic spread and placental colonization (A. I. Bakardjiev, unpublished observation). As such, most pregnant guinea pig infections have used intravenous injections to ensure sufficient placental infection (70). More recently, oral infection colonized placentas more reliably in lower doses when the animals were fed *L. monocytogenes*-inoculated whipping cream (71, 72). This opens new opportunities to understand the roles of virulence factors in placental colonization and drug development.

Pregnant guinea pigs have also been used to understand how *L. monocytogenes* traffics to and from the placenta (40, 47). In trafficking studies, it is especially important to use an animal with a placenta more anatomically similar to that of humans, as differences in the thickness and structure of maternal-fetal interfaces can have a significant effect on trafficking dynamics. Analysis of a competitive index using a wild-type strain and an antibiotic resistant strain demonstrated that very few *L. monocytogenes* organisms (perhaps just one!) are sufficient to colonize the placenta. These few bacteria then rapidly amplify in the unique immune environment of the placenta and are able to spread back to the maternal organs; the maternal immune system may be primed to reject an infected fetus for good reason.

GERBILS

The Mongolian gerbil (*Meriones unguiculatus*) has become a popular model for pregnant animal infection models because it has a functional InlA-Ecad and InlB–c-Met internalization process (Table 1) (39). The litter size of gerbils is similar to that of guinea pigs (1 to 8 offspring) and smaller than that of mice (10 to 14 offspring), but the gerbil

gestation period is ~25 days, similar to that of mice and much less than that of guinea pigs (~65 days), meaning experiments can be performed more quickly. However, there are many disadvantages. Gerbils are poorly characterized compared to guinea pigs and mice. Limited antibodies and no genetic tools are available. As with mice and guinea pigs, gerbils have a labyrinthine placenta, rather than a villous placenta (73). Gerbils also require more housing costs than mice but typically less than guinea pigs.

Gerbils' capacity for InlA/InlB-mediated internalization has allowed investigators to develop oral models of *L. monocytogenes* infection for pregnant gerbils that exhibit effective placental colonization at dosages similar to those required for nonhuman primates (NHPs) (39, 74). Oral infection of pregnant gerbils with an InlAB mutant shows reduced infection of the placentas, suggesting their usefulness for colonization of the placenta, including in intravenous inoculation (39). As with guinea pigs (40), the placenta appears to serve as a reservoir for pathogen amplification, worsening outcomes for the pregnant versus nonpregnant animal (74). However, while low doses of *L. monocytogenes* in pregnant mice, guinea pigs, and nonhuman primates can all lead to stillborn or reabsorbed fetuses, this important sequela has not been observed in gerbils (74). The reason for this is unknown, but it does warrant caution in using gerbils to understand the immune response to pathogens during pregnancy.

NONHUMAN PRIMATES

Of course, nonhuman primates (NHPs) have the placental structure and immune response most similar to those of humans (Table 1). Like humans, NHPs such as rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*) have a villous hemochorial placenta that invades into the decidua, although less extensively than in humans (75). Also, it is common for these models to have two discoid placentas for a single fetus, while humans have one discoid placenta for each fetus (or, rarely, one for two fetuses). Immune regulation in NHP placentas is by far the most similar to that in humans (76). Additionally, NHPs express Ecad and c-Met isoforms that interact with InlA and InlB, allowing for *L. monocytogenes* gastrointestinal infections (77, 78), and they are permissive to other important transplacental human pathogens as well, including cytomegalovirus (79), *Toxoplasma* (80, 81), and *Trypanosoma cruzi* (82). However, there are many disadvantages to NHPs. First are ethical and legal considerations placing an increasingly high bar on the justification for use of these animals (83, 84), coupled with the high cost and veterinarian expertise required to house them adequately. Second, NHPs have long gestation periods. While this allows researchers to more easily determine if certain trimesters are more susceptible than others, it also adds to time spent on the experiments. Further, the number of fetuses per pregnancy is low, typically only one for rhesus macaques, requiring larger numbers of animals for adequate statistical analysis. Lastly, the genetic and histological toolkits for NHPs are quite small. While depleting antibodies for some species exist, the tools lag far behind the repertoire available for mice and guinea pigs.

Given the close similarities between humans and rhesus macaques, most studies have focused on histology, disease progression, and determining the dose of pathogen that would warrant clinical attention. The dosage question is not straightforward; listeriosis during pregnancy, while very serious, is a rare event that limits how much information can be gleaned from clinical reports. Like humans, pregnant rhesus and cynomolgus macaques do not show overt signs of illness after inoculation (77, 85). Further, hematological studies show few changes in leukocyte populations during infections (77, 78). However, one of the earliest and most consistent signs of *L. monocytogenes* infection in NHPs is fecal shedding of *L. monocytogenes* (78).

In terms of how it traffics through and affects the body, *L. monocytogenes* in pregnant rhesus macaques appears clinically quite similar to *L. monocytogenes* in pregnant humans. Bacterial burdens in maternal organs of cynomolgus macaques are low, but decidual and placental tissues exhibit high bacterial loads. Dose-response curves for rhesus macaques demonstrated that even low inoculums of *L. monocytogenes* are sufficient to induce fetal rejection, similar to findings in rodent studies (85, 86).

NHP studies may indicate differential susceptibility based on gestational age. Typ-

ically, listeriosis is considered a concern in the third trimester due to the high incidence of clinical reports. However, rhesus macaques infected in the third trimester had stillbirth occur in ~30% of animals, whereas cynomolgus macaques infected in the first trimester all had stillborn fetuses (78, 85). These differences could also be due to variations in the *L. monocytogenes* or host strain used in the two studies. Given the asymptomatic nature of infection in most pregnant humans and macaques, it is possible that the highest risk occurs early in gestation and that infection is diagnosed only in clinical settings at later times.

HUMAN PLACENTAL ORGAN EXPLANTS

Ex vivo placental organ cultures help researchers to understand how the human placenta resists infection (Table 1). They remove the concern of a species artifact. The placenta is highly organized, with significant cellular differentiation over space and time. A large number of differentiated states and cellular phenotypes can be found only in explant tissue (87). Tissues donated from each trimester and at term can be used, but one must consider that the placenta and decidua are changing throughout pregnancy. Thus, a first-trimester sample differs considerably from a sample obtained after delivery. For example, first-trimester villi that are 7 gestational weeks or younger can invade into Matrigel and form EVTs. Older villi can still be cultured but will be floating villi that lack EVTs. Additionally, there can be large variability in results due to genetic differences between donors. Second- and third-trimester placentas in particular may be problematic, since the reasons for pregnancy termination may involve fetal or placental abnormalities. Therefore, multiple experimental replicates must be performed in order to test for statistical significance of results. Since obtaining villi from donors and properly isolating them is challenging, this can add considerable time and expense to projects. In many localities, laws may restrict donations or ban them entirely (88). Lastly, human tissue *in situ* cannot be genetically manipulated by standard cell culture techniques such as small interfering RNA (siRNA) knockdown or lentiviral transduction.

Despite the drawbacks, human placental cultures have led to key discoveries in how the placenta resists infection. EVTs, the invasive placental cells found within the decidua and uterine blood vessel walls, were found to be the initial portal of entry for *L. monocytogenes* in explants (6). SYN was found to be an initial infection site in one study using floating explants (89); however, other studies using invasive explants have indicated that unless it is damaged, SYN is highly resistant to *L. monocytogenes* (6, 7) and *Toxoplasma gondii* (2). Syncytialized JEG-3 choriocarcinoma cells are also resistant to *T. gondii* (15). These differences may be due to different gestational ages and preparations of the villous cultures, as floating villi lack EVT; however, Díaz-Luján et al. (90) also found that SYN protected term placental explants without EVTs from *Trypanosoma cruzi*. Further investigation of the villi demonstrated that differentiated EVTs, but not the underlying undifferentiated cytotrophoblasts, sequester *L. monocytogenes* to the vacuole and reduce the amount of bacteria that could replicate in the cytosol (91). Therefore, the ability to image and infect human placental tissue with differentiated and spatially organized cell types can be a useful tool in understanding placental defenses.

Decidual organ cultures may also be used in order to explore how the maternal uterine layer defends against pathogens (92). During pregnancy, the endometrium undergoes dramatic transformation during decidualization (93). Colonization of the decidua is critical for infection of the placenta (94, 95). However, only ~0.02% of the inoculum is capable of colonizing decidual organ cultures (96). Once they have successfully colonized, however, the bacteria are able to proliferate in foci. It is unknown how the decidua resists colonization. A possibility raised by Rizzuto et al. (96) is a cell-autonomous resistance similar to what is seen in EVTs. Interestingly, $CD68^+$ macrophages do not seem to associate with the listerial foci in the organ cultures. This may suggest that decidual macrophages are not capable of responding to infections after they are established.

FUTURE DIRECTIONS AND CONCLUSIONS

There remain many questions that can be addressed in these models. *L. monocytogenes* continues to be useful in probing maternal and fetal defenses and in drawing cross-species comparisons. However, many other pathogens are worthy of further investigation. With the emergence of Zika virus on new continents, the mechanisms by which viruses cross the placenta are of increasingly urgent concern (95). Transplacental cytomegalovirus infections are the leading cause of congenital deafness, and other neurological sequelae remain an important problem worldwide (97). Also, while most cases of vertical HIV transmission occur perinatally, about 2% are due to placental crossing, and this is hypothetically related to breaks in the trophoblast layer (98). Learning more about how the significantly larger, neglected kinetoplastid parasites *Leishmania* and *Trypanosoma* cross the placenta could be beneficial to tropical medicine (99). Further, while congenital syphilis has been all but eliminated from high-income countries, it is still a significant problem in low-income ones (100, 101), yet almost nothing is known about how it crosses the maternal-fetal barrier.

While several studies have investigated the roles of *L. monocytogenes* virulence factors, fewer have looked at the host responses to pathogens at the maternal-fetal interface. Does the maternal inflammatory response during early gestation (8) coincide with more aggressive protection of the mother? If so, this may explain why early-trimester infections in NHPs lead to more stillbirths. Does the EVT-decidua boundary possess unique immune responses that limit infections without inducing a maternal immune response? Answering these questions could help elucidate the role of the immune response in supporting a healthy pregnancy. It may also lead to important discoveries about life-threatening inflammatory conditions not usually associated with infection, such as preeclampsia (102).

Finally, further experiments with these models could begin to answer questions of evolution. The diversity of placental forms suggests that mammals have solved the central conflicts of the placenta (being permeable but protective and immunotolerant but inflammatory) in different ways likely linked to gestational time, offspring number, microbial communities, and other variables (103, 104). Some bacterial pathogens appear to have explicitly evolved to transmit by causing spontaneous abortion, and yet the mechanisms by which they do so are quite unknown (105, 106). Additionally, the question of whether the placenta has a nonpathogenic, commensal microbiome during an uncomplicated pregnancy prior to the start of labor is an active area of investigation (107).

Placental infection is a rare but serious complication to pregnancy that deserves greater scholarly attention. We have presented several animal models and described their advantages, disadvantages, and uses in understanding placental infection. Unfortunately, placental anatomy varies greatly between species, leading some to conclude that only humans have human placentas (76). Nevertheless, we believe that the use of animal and organ culture models will improve our mechanistic understanding of host-pathogen interactions in these unique tissues. The use of multiple complementary models and comparison to phenotypic characterization of human tissues will increase the likelihood that the resulting discoveries are relevant for human health.

REFERENCES

1. Medawar PB. 1953. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol 7:320–338.
2. Robbins JR, Bakardjiev AI. 2012. Pathogens and the placental fortress. Curr Opin Microbiol 15:36–43. https://doi.org/10.1016/j.mib.2011.11.006.
3. Brasil P, Pereira JP, Jr, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, Rabello RS, Valderramos SG, Halai UA, Salles TS, Zin AA, Horovitz D, Daltro P, Boechat M, Raja Gabaglia C, Carvalho de Sequeira P, Pilotto JH, Medialdea-Carrera R, Cotrim da Cunha D, Abreu de Carvalho LM, Pone M, Machado Siqueira A, Calvet GA, Rodrigues Baiao AE, Neves ES, Nassar de Carvalho PR, Hasue RH, Marschik PB, Einspieler C, Janzen C, Cherry JD, Bispo de Filippis AM, Nielsen-Saines K. 2016. Zika virus infection in pregnant women in Rio de Janeiro. N Engl J Med 375:2321–2334. https://doi.org/10.1056/NEJMoa1602412.
4. Nancy P, Tagliani E, Tay CS, Asp P, Levy DE, Erlebacher A. 2012. Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. Science 336:1317–1321. https://doi.org/10.1126/science.1220030.
5. van Mourik MS, Macklon NS, Heijnen CJ. 2009. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. J Leukoc Biol 85:4–19. https://doi.org/10.1189/jlb.0708395.
6. Robbins JR, Skrzypczynska KM, Zeldovich VB, Kapidzic M, Bakardjiev AI.

2010. Placental syncytiotrophoblast constitutes a major barrier to vertical transmission of Listeria monocytogenes. PLoS Pathog 6:e1000732. https://doi.org/10.1371/journal.ppat.1000732.
7. Zeldovich VB, Clausen CH, Bradford E, Fletcher DA, Maltepe E, Robbins JR, Bakardjiev AI. 2013. Placental syncytium forms a biophysical barrier against pathogen invasion. PLoS Pathog 9:e1003821. https://doi.org/10.1371/journal.ppat.1003821.
8. Moffett A, Loke C. 2006. Immunology of placentation in eutherian mammals. Nat Rev Immunol 6:584–594. https://doi.org/10.1038/nri1897.
9. Delorme-Axford E, Donker RB, Mouillet JF, Chu T, Bayer A, Ouyang Y, Wang T, Stolz DB, Sarkar SN, Morelli AE, Sadovsky Y, Coyne CB. 2013. Human placental trophoblasts confer viral resistance to recipient cells. Proc Natl Acad Sci U S A 110:12048–12053. https://doi.org/10.1073/pnas.1304718110.
10. Bayer A, Lennemann NJ, Ouyang Y, Bramley JC, Morosky S, Marques ET, Jr, Cherry S, Sadovsky Y, Coyne CB. 2016. Type III interferons produced by human placental trophoblasts confer protection against Zika virus infection. Cell Host Microbe 19:705–712. https://doi.org/10.1016/j.chom.2016.03.008.
11. Ander S, Rudzki E, Arora N, Sadovsky Y, Coyne CB, Boyle JP. 2018. Human placental syncytiotrophoblasts restrict Toxoplasma gondii vertical transmission at two distinct stages and induce CCL22 in response to infection. mBio 9:e01678-17. https://doi.org/10.1128/mBio.01678-17.
12. Orendi K, Gauster M, Moser G, Meiri H, Huppertz B. 2010. The choriocarcinoma cell line BeWo: syncytial fusion and expression of syncytium-specific proteins. Reproduction 140:759–766. https://doi.org/10.1530/REP-10-0221.
13. Ji L, Brkic J, Liu M, Fu G, Peng C, Wang YL. 2013. Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. Mol Aspects Med 34:981–1023. https://doi.org/10.1016/j.mam.2012.12.008.
14. Gierman LM, Stodle GS, Tangeras LH, Austdal M, Olsen GD, Follestad T, Skei B, Rian K, Gundersen AS, Austgulen R, Iversen AC. 2015. Toll-like receptor profiling of seven trophoblast cell lines warrants caution for translation to primary trophoblasts. Placenta 36:1246–1253. https://doi.org/10.1016/j.placenta.2015.09.004.
15. McConkey CA, Delorme-Axford E, Nickerson CA, Kim KS, Sadovsky Y, Boyle JP, Coyne CB. 2016. A three-dimensional culture system recapitulates placental syncytiotrophoblast development and microbial resistance. Sci Adv 2:e1501462. https://doi.org/10.1126/sciadv.1501462.
16. Benirschke K, Burton GJ, Baergen RN. 2012. Pathology of the human placenta, 6th ed. Springer, Berlin, Germany.
17. Fischer TV, Floyd AD. 1972. Placental development in the Mongolian gerbil (Meriones unguiculatus). I. Early development to the time of chorio-allantoic contact. Am J Anat 134:309–320.
18. Low JC, Donachie W. 1997. A review of Listeria monocytogenes and listeriosis. Vet J 153:9–29. https://doi.org/10.1016/S1090-0233(97)80005-6.
19. Hoelzer K, Pouillot R, Dennis S. 2012. Animal models of listeriosis: a comparative review of the current state of the art and lessons learned. Vet Res 43:18. https://doi.org/10.1186/1297-9716-43-18.
20. Vigliani MB, Bakardjiev AI. 2014. Intracellular organisms as placental invaders. Fetal Matern Med Rev 25:332–338. https://doi.org/10.1017/S0965539515000066.
21. Mylonakis E, Paliou M, Hohmann EL, Calderwood SB, Wing EJ. 2002. Listeriosis during pregnancy: a case series and review of 222 cases. Medicine (Baltimore) 81:260–269. https://doi.org/10.1097/00005792-200207000-00002.
22. Lamont RF, Sobel J, Mazaki-Tovi S, Kusanovic JP, Vaisbuch E, Kim SK, Uldbjerg N, Romero R. 2011. Listeriosis in human pregnancy: a systematic review. J Perinat Med 39:227–236. https://doi.org/10.1515/jpm.2011.035.
23. Giraud JR, Denis F, Gargot F, Fizazi T, Babin P, Rautlin de la Roy YD, Hoppeler A, Brisou J, de Tourris H. 1973. Listeriosis. Occurrence in spontaneous interruptions of pregnancy. Nouv Presse Med 2:215–218.
24. Smith K, Youngman P. 1992. Use of a new integrational vector to investigate compartment-specific expression of the Bacillus subtilis spoIIM gene. Biochimie 74:705–711. https://doi.org/10.1016/0300-9084(92)90143-3.
25. Lauer P, Chow MY, Loessner MJ, Portnoy DA, Calendar R. 2002. Construction, characterization, and use of two Listeria monocytogenes site-specific phage integration vectors. J Bacteriol 184:4177–4186. https://doi.org/10.1128/JB.184.15.4177-4186.2002.
26. Bahjat KS, Liu W, Lemmens EE, Schoenberger SP, Portnoy DA, Dubensky TW, Jr, Brockstedt DG. 2006. Cytosolic entry controls CD8+-T-cell potency during bacterial infection. Infect Immun 74:6387–6397. https://doi.org/10.1128/IAI.01088-06.
27. Portnoy DA, Auerbuch V, Glomski IJ. 2002. The cell biology of Listeria monocytogenes infection: the intersection of bacterial pathogenesis and cell-mediated immunity. J Cell Biol 158:409–414. https://doi.org/10.1083/jcb.200205009.
28. Witte CE, Archer KA, Rae CS, Sauer JD, Woodward JJ, Portnoy DA. 2012. Innate immune pathways triggered by Listeria monocytogenes and their role in the induction of cell-mediated immunity. Adv Immunol 113:135–156. https://doi.org/10.1016/B978-0-12-394590-7.00002-6.
29. Rolhion N, Cossart P. 2017. How the study of Listeria monocytogenes has led to new concepts in biology. Future Microbiol 12:621–638. https://doi.org/10.2217/fmb-2016-0221.
30. Ireton K. 2007. Entry of the bacterial pathogen Listeria monocytogenes into mammalian cells. Cell Microbiol 9:1365–1375. https://doi.org/10.1111/j.1462-5822.2007.00933.x.
31. Mengaud J, Ohayon H, Gounon P, Mege RM, Cossart P. 1996. E-cadherin is the receptor for internalin, a surface protein required for entry of L. monocytogenes into epithelial cells. Cell 84:923–932.
32. Schubert WD, Urbanke C, Ziehm T, Beier V, Machner MP, Domann E, Wehland J, Chakraborty T, Heinz DW. 2002. Structure of internalin, a major invasion protein of Listeria monocytogenes, in complex with its human receptor E-cadherin. Cell 111:825–836. https://doi.org/10.1016/S0092-8674(02)01136-4.
33. Niemann HH, Jager V, Butler PJ, van den Heuvel J, Schmidt S, Ferraris D, Gherardi E, Heinz DW. 2007. Structure of the human receptor tyrosine kinase met in complex with the Listeria invasion protein InlB. Cell 130:235–246. https://doi.org/10.1016/j.cell.2007.05.037.
34. Maltepe E, Bakardjiev AI, Fisher SJ. 2010. The placenta: transcriptional, epigenetic, and physiological integration during development. J Clin Invest 120:1016–1025. https://doi.org/10.1172/JCI41211.
35. D'Orazio SE. 2014. Animal models for oral transmission of Listeria monocytogenes. Front Cell Infect Microbiol 4:15. https://doi.org/10.3389/fcimb.2014.00015.
36. Tsai YH, Disson O, Bierne H, Lecuit M. 2013. Murinization of internalin extends its receptor repertoire, altering Listeria monocytogenes cell tropism and host responses. PLoS Pathog 9:e1003381. https://doi.org/10.1371/journal.ppat.1003381.
37. Wollert T, Pasche B, Rochon M, Deppenmeier S, van den Heuvel J, Gruber AD, Heinz DW, Lengeling A, Schubert WD. 2007. Extending the host range of Listeria monocytogenes by rational protein design. Cell 129:891–902. https://doi.org/10.1016/j.cell.2007.03.049.
38. Lecuit M, Vandormael-Pournin S, Lefort J, Huerre M, Gounon P, Dupuy C, Babinet C, Cossart P. 2001. A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. Science 292:1722–1725. https://doi.org/10.1126/science.1059852.
39. Disson O, Grayo S, Huillet E, Nikitas G, Langa-Vives F, Dussurget O, Ragon M, Le Monnier A, Babinet C, Cossart P, Lecuit M. 2008. Conjugated action of two species-specific invasion proteins for fetoplacental listeriosis. Nature 455:1114–1118. https://doi.org/10.1038/nature07303.
40. Bakardjiev AI, Theriot JA, Portnoy DA. 2006. Listeria monocytogenes traffics from maternal organs to the placenta and back. PLoS Pathog 2:e66. https://doi.org/10.1371/journal.ppat.0020066.
41. Bou Ghanem EN, Jones GS, Myers-Morales T, Patil PD, Hidayatullah AN, D'Orazio SE. 2012. InlA promotes dissemination of Listeria monocytogenes to the mesenteric lymph nodes during food borne infection of mice. PLoS Pathog 8:e1003015. https://doi.org/10.1371/journal.ppat.1003015.
42. Lecuit M, Dramsi S, Gottardi C, Fedor-Chaiken M, Gumbiner B, Cossart P. 1999. A single amino acid in E-cadherin responsible for host specificity towards the human pathogen Listeria monocytogenes. EMBO J 18:3956–3963. https://doi.org/10.1093/emboj/18.14.3956.
43. Le Monnier A, Autret N, Join-Lambert OF, Jaubert F, Charbit A, Berche P, Kayal S. 2007. ActA is required for crossing of the fetoplacental barrier by Listeria monocytogenes. Infect Immun 75:950–957. https://doi.org/10.1128/IAI.01570-06.
44. 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. https://doi.org/10.1128/IAI.00625-16.
45. Maury MM, Tsai YH, Charlier C, Touchon M, Chenal-Francisque V, Leclercq A, Criscuolo A, Gaultier C, Roussel S, Brisabois A, Disson O, Rocha EPC, Brisse S, Lecuit M. 2016. Uncovering Listeria monocytogenes hypervirulence by harnessing its biodiversity. Nat Genet 48:308–313. https://doi.org/10.1038/ng.3501.

46. Le Monnier A, Join-Lambert OF, Jaubert F, Berche P, Kayal S. 2006. Invasion of the placenta during murine listeriosis. Infect Immun 74:663–672. https://doi.org/10.1128/IAI.74.1.663-672.2006.
47. Melton-Witt JA, Rafelski SM, Portnoy DA, Bakardjiev AI. 2012. Oral infection with signature-tagged Listeria monocytogenes reveals organ-specific growth and dissemination routes in guinea pigs. Infect Immun 80:720–732. https://doi.org/10.1128/IAI.05958-11.
48. Guleria I, Pollard JW. 2000. The trophoblast is a component of the innate immune system during pregnancy. Nat Med 6:589–593. https://doi.org/10.1038/75074.
49. Pollard JW, Bartocci A, Arceci R, Orlofsky A, Ladner MB, Stanley ER. 1987. Apparent role of the macrophage growth factor, CSF-1, in placental development. Nature 330:484–486. https://doi.org/10.1038/330484a0.
50. Rowe JH, Ertelt JM, Xin L, Way SS. 2012. Listeria monocytogenes cytoplasmic entry induces fetal wastage by disrupting maternal Foxp3+ regulatory T cell-sustained fetal tolerance. PLoS Pathog 8:e1002873. https://doi.org/10.1371/journal.ppat.1002873.
51. Perchellet AL, Jasti S, Petroff MG. 2013. Maternal CD4(+) and CD8(+) T cell tolerance towards a fetal minor histocompatibility antigen in T cell receptor transgenic mice. Biol Reprod 89:102. https://doi.org/10.1095/biolreprod.113.110445.
52. Delle Piane L, Lin W, Liu X, Donjacour A, Minasi P, Revelli A, Maltepe E, Rinaudo PF. 2010. Effect of the method of conception and embryo transfer procedure on mid-gestation placenta and fetal development in an IVF mouse model. Hum Reprod 25:2039–2046. https://doi.org/10.1093/humrep/deq165.
53. de Waal E, Vrooman LA, Fischer E, Ord T, Mainigi MA, Coutifaris C, Schultz RM, Bartolomei MS. 2015. The cumulative effect of assisted reproduction procedures on placental development and epigenetic perturbations in a mouse model. Hum Mol Genet 24:6975–6985. https://doi.org/10.1093/hmg/ddv400.
54. Zhou CC, Chang J, Mi T, Abbasi S, Gu D, Huang L, Zhang W, Kellems RE, Schwartz RJ, Xia Y. 2012. Targeted expression of Cre recombinase provokes placental-specific DNA recombination in transgenic mice. PLoS One 7:e29236. https://doi.org/10.1371/journal.pone.0029236.
55. Wenzel PL, Leone G. 2007. Expression of Cre recombinase in early diploid trophoblast cells of the mouse placenta. Genesis 45:129–134. https://doi.org/10.1002/dvg.20276.
56. Okada Y, Ueshin Y, Isotani A, Saito-Fujita T, Nakashima H, Kimura K, Mizoguchi A, Oh-Hora M, Mori Y, Ogata M, Oshima RG, Okabe M, Ikawa M. 2007. Complementation of placental defects and embryonic lethality by trophoblast-specific lentiviral gene transfer. Nat Biotechnol 25:233–237. https://doi.org/10.1038/nbt1280.
57. Georgiades P, Cox B, Gertsenstein M, Chawengsaksophak K, Rossant J. 2007. Trophoblast-specific gene manipulation using lentivirus-based vectors. Biotechniques 42:317–318, 320, 322–325. https://doi.org/10.2144/000112341.
58. Fan Y, Li R, Huang J, Yu Y, Qiao J. 2013. Diploid, but not haploid, human embryonic stem cells can be derived from microsurgically repaired tripronuclear human zygotes. Cell Cycle 12:302–311. https://doi.org/10.4161/cc.23103.
59. Mestas J, Hughes CC. 2004. Of mice and not men: differences between mouse and human immunology. J Immunol 172:2731–2738. https://doi.org/10.4049/jimmunol.172.5.2731.
60. Rowe JH, Ertelt JM, Aguilera MN, Farrar MA, Way SS. 2011. Foxp3(+) regulatory T cell expansion required for sustaining pregnancy compromises host defense against prenatal bacterial pathogens. Cell Host Microbe 10:54–64. https://doi.org/10.1016/j.chom.2011.06.005.
61. Chaturvedi V, Ertelt JM, Jiang TT, Kinder JM, Xin L, Owens KJ, Jones HN, Way SS. 2015. CXCR3 blockade protects against Listeria monocytogenes infection-induced fetal wastage. J Clin Invest 125:1713–1725. https://doi.org/10.1172/JCI78578.
62. Clark S, Hall Y, Williams A. 2014. Animal models of tuberculosis: guinea pigs. Cold Spring Harb Perspect Med 5:a018572. https://doi.org/10.1101/cshperspect.a018572.
63. Berendt RF, Young HW, Allen RG, Knutsen GL. 1980. Dose-response of guinea pigs experimentally infected with aerosols of Legionella pneumophila. J Infect Dis 141:186–192. https://doi.org/10.1093/infdis/141.2.186.
64. Williams A, Featherstone AS. 1988. The early diagnosis of Legionnaires' disease in a Legionella pneumophila aerosol-infected guinea pig model; comparison of a method developed for detecting Legionella pneumophila antigens in urine and the demonstration of circulating antibody by enzyme-linked immunosorbent assay. J Infect 16:47–54. https://doi.org/10.1016/S0163-4453(88)96095-1.
65. Maurin M, Lepidi H, La Scola B, Feuerstein M, Andre M, Pellissier JF, Raoult D. 1997. Guinea pig model for Staphylococcus aureus native valve endocarditis. Antimicrob Agents Chemother 41:1815–1817.
66. Stratford AF, Zoutman DE, Davidson JS. 2002. Effect of lidocaine and epinephrine on Staphylococcus aureus in a guinea pig model of surgical wound infection. Plast Reconstr Surg 110:1275–1279.
67. Kim HK, Falugi F, Thomer L, Missiakas DM, Schneewind O. 2015. Protein A suppresses immune responses during Staphylococcus aureus bloodstream infection in guinea pigs. mBio 6:e02369-14. https://doi.org/10.1128/mBio.02369-14.
68. Kumar ML, Nankervis GA. 1978. Experimental congenital infection with cytomegalovirus: a guinea pig model. J Infect Dis 138:650–654. https://doi.org/10.1093/infdis/138.5.650.
69. De Clercq E, Kalmar I, Vanrompay D. 2013. Animal models for studying female genital tract infection with Chlamydia trachomatis. Infect Immun 81:3060–3067. https://doi.org/10.1128/IAI.00357-13.
70. Bakardjiev AI, Stacy BA, Fisher SJ, Portnoy DA. 2004. Listeriosis in the pregnant guinea pig: a model of vertical transmission. Infect Immun 72:489–497. https://doi.org/10.1128/IAI.72.1.489-497.2004.
71. Williams D, Dunn S, Richardson A, Frank JF, Smith MA. 2011. Time course of fetal tissue invasion by Listeria monocytogenes following an oral inoculation in pregnant guinea pigs. J Food Prot 74:248–253. https://doi.org/10.4315/0362-028X.JFP-10-163.
72. Williams D, Irvin EA, Chmielewski RA, Frank JF, Smith MA. 2007. Dose-response of Listeria monocytogenes after oral exposure in pregnant guinea pigs. J Food Prot 70:1122–1128. https://doi.org/10.4315/0362-028X-70.5.1122.
73. Fischer TV, Floyd AD. 1972. Placental development in the Mongolian gerbil (Meriones unguiculatus). II. From the establishment of the labyrinth to term. Am J Anat 134:321–326.
74. Roulo RM, Fishburn JD, Amosu M, Etchison AR, Smith MA. 2014. Dose response of Listeria monocytogenes invasion, fetal morbidity, and fetal mortality after oral challenge in pregnant and nonpregnant Mongolian gerbils. Infect Immun 82:4834–4841. https://doi.org/10.1128/IAI.01514-14.
75. Ramsey EM, Houston ML, Harris JW. 1976. Interactions of the trophoblast and maternal tissues in three closely related primate species. Am J Obstet Gynecol 124:647–652. https://doi.org/10.1016/0002-9378(76)90068-5.
76. Schmidt A, Morales-Prieto DM, Pastuschek J, Frohlich K, Markert UR. 2015. Only humans have human placentas: molecular differences between mice and humans. J Reprod Immunol 108:65–71. https://doi.org/10.1016/j.jri.2015.03.001.
77. Smith MA, Takeuchi K, Brackett RE, McClure HM, Raybourne RB, Williams KM, Babu US, Ware GO, Broderson JR, Doyle MP. 2003. Nonhuman primate model for Listeria monocytogenes-induced stillbirths. Infect Immun 71:1574–1579. https://doi.org/10.1128/IAI.71.3.1574-1579.2003.
78. Wolfe B, Wiepz GJ, Schotzko M, Bondarenko GI, Durning M, Simmons HA, Mejia A, Faith NG, Sampene E, Suresh M, Kathariou S, Czuprynski CJ, Golos TG. 2017. Acute fetal demise with first trimester maternal infection resulting from Listeria monocytogenes in a nonhuman primate model. mBio 8:e01938-16. https://doi.org/10.1128/mBio.01938-16.
79. Bialas KM, Tanaka T, Tran D, Varner V, Cisneros De La Rosa E, Chiuppesi F, Wussow F, Kattenhorn L, Macri S, Kunz EL, Estroff JA, Kirchherr J, Yue Y, Fan Q, Lauck M, O'Connor DH, Hall AH, Xavier A, Diamond DJ, Barry PA, Kaur A, Permar SR. 2015. Maternal CD4+ T cells protect against severe congenital cytomegalovirus disease in a novel nonhuman primate model of placental cytomegalovirus transmission. Proc Natl Acad Sci U S A 112:13645–13650. https://doi.org/10.1073/pnas.1511526112.
80. Schoondermark-Van de Ven E, Melchers W, Galama J, Camps W, Eskes T, Meuwissen J. 1993. Congenital toxoplasmosis: an experimental study in rhesus monkeys for transmission and prenatal diagnosis. Exp Parasitol 77:200–211. https://doi.org/10.1006/expr.1993.1077.
81. Araujo FG, Wong MM, Theis J, Remington JS. 1973. Experimental Toxoplasma gondii infection in a nonhuman primate. Am J Trop Med Hyg 22:465–472. https://doi.org/10.4269/ajtmh.1973.22.465.
82. Grieves JL, Hubbard GB, Williams JT, Vandeberg JL, Dick EJ, Jr, Lopez-Alvarenga JC, Schlabritz-Loutsevitch NE. 2008. Trypanosoma cruzi in nonhuman primates with a history of stillbirths: a retrospective study (Papio hamadryas spp.) and case report (Macaca fascicularis). J Med Primatol 37:318–328. https://doi.org/10.1111/j.1600-0684.2007.00271.x.
83. Phillips KA, Bales KL, Capitanio JP, Conley A, Czoty PW, t Hart BA,

Hopkins WD, Hu SL, Miller LA, Nader MA, Nathanielsz PW, Rogers J, Shively CA, Voytko ML. 2014. Why primate models matter. Am J Primatol 76:801–827. https://doi.org/10.1002/ajp.22281.
84. Abbott A. 2014. Biomedicine: the changing face of primate research. Nature 506:24–26. https://doi.org/10.1038/506024a.
85. Smith MA, Takeuchi K, Anderson G, Ware GO, McClure HM, Raybourne RB, Mytle N, Doyle MP. 2008. Dose-response model for Listeria monocytogenes-induced stillbirths in nonhuman primates. Infect Immun 76:726–731. https://doi.org/10.1128/IAI.01366-06.
86. Williams D, Castleman J, Lee CC, Mote B, Smith MA. 2009. Risk of fetal mortality after exposure to Listeria monocytogenes based on dose-response data from pregnant guinea pigs and primates. Risk Anal 29:1495–1505. https://doi.org/10.1111/j.1539-6924.2009.01308.x.
87. Genbacev O, Schubach SA, Miller RK. 1992. Villous culture of first trimester human placenta—model to study extravillous trophoblast (EVT) differentiation. Placenta 13:439–461. https://doi.org/10.1016/0143-4004(92)90051-T.
88. Eckroad K. 2016. An interpretive approach: a call for state statutory reform governing fetal tissue donation. Univ Md Law J Race Relig Gender Class 16:92–114.
89. Lecuit M, Nelson DM, Smith SD, Khun H, Huerre M, Vacher-Lavenu MC, Gordon JI, Cossart P. 2004. Targeting and crossing of the human maternofetal barrier by Listeria monocytogenes: role of internalin interaction with trophoblast E-cadherin. Proc Natl Acad Sci U S A 101:6152–6157. https://doi.org/10.1073/pnas.0401434101.
90. Diaz-Lujan C, Triquell MF, Castillo C, Hardisson D, Kemmerling U, Fretes RE. 2016. Role of placental barrier integrity in infection by Trypanosoma cruzi. Acta Trop 164:360–368. https://doi.org/10.1016/j.actatropica.2016.09.021.
91. Zeldovich VB, Robbins JR, Kapidzic M, Lauer P, Bakardjiev AI. 2011. Invasive extravillous trophoblasts restrict intracellular growth and spread of Listeria monocytogenes. PLoS Pathog 7:e1002005. https://doi.org/10.1371/journal.ppat.1002005.
92. Rizzuto GA, Kapidzic M, Gormley M, Bakardjiev AI. 2016. Human placental and decidual organ cultures to study infections at the maternal-fetal interface. J Vis Exp https://doi.org/10.3791/54237.
93. Gellersen B, Brosens IA, Brosens JJ. 2007. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. Semin Reprod Med 25:445–453. https://doi.org/10.1055/s-2007-991042.
94. Redline RW, Shea CM, Papaioannou VE, Lu CY. 1988. Defective antilisterial responses in deciduoma of pseudopregnant mice. Am J Pathol 133:485–497.
95. Redline RW, Lu CY. 1987. Role of local immunosuppression in murine fetoplacental listeriosis. J Clin Invest 79:1234–1241. https://doi.org/10.1172/JCI112942.
96. Rizzuto G, Tagliani E, Manandhar P, Erlebacher A, Bakardjiev AI. 2017. Limited colonization undermined by inadequate early immune responses defines the dynamics of decidual listeriosis. Infect Immun 85:e00153-17. https://doi.org/10.1128/IAI.00153-17.
97. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. 2013. The "silent" global burden of congenital cytomegalovirus. Clin Microbiol Rev 26:86–102. https://doi.org/10.1128/CMR.00062-12.
98. Soilleux EJ, Coleman N. 2003. Transplacental transmission of HIV: a potential role for HIV binding lectins. Int J Biochem Cell Biol 35:283–287. https://doi.org/10.1016/S1357-2725(02)00132-2.
99. Grinnage-Pulley T, Scott B, Petersen CA. 2016. A mother's gift: congenital transmission of Trypanosoma and Leishmania species. PLoS Pathog 12:e1005302. https://doi.org/10.1371/journal.ppat.1005302.
100. Wijesooriya NS, Rochat RW, Kamb ML, Turlapati P, Temmerman M, Broutet N, Newman LM. 2016. Global burden of maternal and congenital syphilis in 2008 and 2012: a health systems modelling study. Lancet Global Health 4:e525–e533. https://doi.org/10.1016/S2214-109X(16)30135-8.
101. Arnesen L, Serruya S, Duran P. 2015. Gestational syphilis and stillbirth in the Americas: a systematic review and meta-analysis. Rev Panam Salud Publica 37:422–429.
102. James JL, Whitley GS, Cartwright JE. 2010. Pre-eclampsia: fitting together the placental, immune and cardiovascular pieces. J Pathol 221:363–378. https://doi.org/10.1002/path.2719.
103. Bainbridge DR. 2014. The evolution of pregnancy. Early Hum Dev 90:741–745. https://doi.org/10.1016/j.earlhumdev.2014.08.013.
104. Capellini I, Nunn CL, Barton RA. 2015. Microparasites and placental invasiveness in eutherian mammals. PLoS One 10:e0132563. https://doi.org/10.1371/journal.pone.0132563.
105. Goodswen SJ, Kennedy PJ, Ellis JT. 2013. A review of the infection, genetics, and evolution of Neospora caninum: from the past to the present. Infect Genet Evol 13:133–150. https://doi.org/10.1016/j.meegid.2012.08.012.
106. Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, Mege JL, Maurin M, Raoult D. 2017. From Q fever to Coxiella burnetii infection: a paradigm change. Clin Microbiol Rev 30:115–190. https://doi.org/10.1128/CMR.00045-16.
107. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. 2014. The placenta harbors a unique microbiome. Sci Transl Med 6:237ra65. https://doi.org/10.1126/scitranslmed.3008599.

David E. Lowe received his B.S. in biology from the University of the South and earned his Ph.D. in Dr. Ian Glomski's lab at the University of Virginia, studying bacterial dissemination. During his graduate work, he became interested in how the host limits pathogen dissemination and how the pathogen can overcome host responses. This led him to a postdoctoral fellowship in Dr. Anna Bakardjiev's lab at UCSF to better understand the placental defenses to listeria infections. He is currently a Laboratory Leadership Fellow at the Centers for Disease Control, where he is combining his scientific background with leadership skills and risk and quality management in order to manage a public health laboratory. He is currently investigating immune responses and potential medical countermeasures for rabies virus and divergent lyssaviruses.

Jennifer R. Robbins completed her Ph.D. at Stanford in Dr. Julie Theriot's lab, studying *Listeria* pathogenesis. She currently serves as a Professor and Associate Chair of Biology at Xavier University in Cincinnati, OH, where she primarily teaches undergraduates. However, she has remained active in infectious disease-related research both with Xavier students and through many years of visiting faculty/scientist appointments at University of California—San Francisco, University of California—Berkeley, and University of Cincinnati.

Anna I. Bakardjiev has spent her career understanding the mechanisms of pathogenesis, as well as treatment and prevention. She received her M.D. from Medizinische Hochschule Hannover in Hannover, Germany. After a postdoctoral fellowship in Dr. Dan Portnoy's lab at UC Berkeley, she began her lab at UCSF and became an Associate Professor in the Division of Pediatrics. Dr. Bakardjiev's lab used *L. monocytogenes* to study the host-pathogen interactions that occur at the maternal-fetal interface. Her lab used multiple animal models, human organ culture, and molecular bacteriology to define the bacterial factors that cause disease and the host responses that prevent infections. Recently, she accepted a position at VIR Biotechnology, where she is advancing treatments for infectious diseases as the Director for Clinical Research.