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Letter to the Editor
Murine Fetoplacental Infection Models

We read with interest the recent article (1) addressing unique aspects of murine placental infection by *Salmonella enterica* serovar Typhimurium. We would like to call the authors' attention to two other murine fetoplacental infection models that we have studied.

Listeria monocytogenes is a facultative intracellular pathogen causing severe fetoplacental infections in many species. We found that placental infection was a stochastic process determined by the size of the initial inoculum on day 14.5 of pregnancy (4). Infection always began in the uterine decidua, with subsequent spread into the placental labyrinth in some mice. Once organisms localized to the uterine decidua, they proliferated to extremely high titers ($>10^8$/g) even in preimmunized animals that were protected from infection in other organs. Bacterial titers in the liver and spleen and mortality did not differ between nonpregnant females and mothers without placental organisms. However, mothers with placental infection developed overwhelming sepsis and eventually succumbed. Ineffective immune responses to listeria differed in each placental region (5). In the uterine decidua, there was a paucity of the monocytes-macrophages required in other organs to control infection. Only neutrophils were able to enter the infected tissue. In the labyrinth, virtually no inflammatory cells were present until late in the course of infection. In fetal regions of the placenta, monocytes-macrophages were present but could not be activated.

Fusobacterium nucleatum is an anaerobic pathogen implicated in periodontal infections and placental chorioamnionitis. Unlike listeria, intravenous inoculation of organisms on day 14.5 of pregnancy resulted in only transient maternal systemic colonization (2). Similar to the case with listeria, localization of small numbers of organisms to the uterine decidua resulted in overwhelming placental infection, with premature delivery and/or stillbirth. Organisms entered the labyrinth only after ischemic necrosis. The latter point is an important caveat for murine fetoplacental infection models. The possibility that spread into the labyrinth occurs after tissue infarction caused by decidual vascular thrombosis secondary to severe infection cannot be excluded and complicates the interpretation of placental immune responsiveness. Interestingly, infection of Toll-like receptor 4 (TLR4) but not TLR2 knockout mice prevented necrosis and inflammation and reduced stillbirth but did not affect bacterial titers in the placenta (3).

Our interpretation of this data is that the uterine decidua is an immunoprivileged environment that normally protects placental tissue from the maternal immune response. The price of protection is enhanced susceptibility to small numbers of bacteria that enter the maternal bloodstream in women with foodborne illnesses (listeria and salmonella) and periodontal infections (fusobacterium) or that ascend from the cervix in acute chorioamnionitis. Once in the placental environment, proliferation of organisms is unchecked, leading to a neutrophil-dominated innate immune response that triggers premature labor and delivery, saving the mother from the risks of systemic infection.

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Author's Reply

I agree with Redline et al. that the maternal decidua may act as an immunoprivileged site which inadvertently supports the unchecked proliferation of select pathogens. Indeed, many pathogens, such as *Salmonella*, *Listeria*, *Fusobacterium*, *Clostridium*, *Plasmodium*, *Toxoplasma*, etc., reach the feto-maternal interface via the hematogenous route. Often, only small numbers of pathogens reach various organs via this route. Nevertheless, in the immunoprivileged sites, such as decidua, they may proliferate profoundly. Interestingly, most pathogens capable of crossing the placental barrier have a facultative or obligate intracellular lifestyle, suggesting that pathogen-dependent immune evasive strategies may also be at play. Moreover, the paradigm of infection-induced fetal loss and/or premature labor correlates with overt local inflammation.

As suggested by Redline et al., the entry of pathogens into the labyrinth trophoblast may be preceded by tissue infarction caused by inflammation and neutrophil infiltration following severe infection. However, in the context of *Salmonella* infection, it also cannot be ruled out that inflammation is triggered only upon interaction of a virulent pathogen with pathogen recognition receptors of labyrinth trophoblast. As reported in our study (5), both virulent and avirulent *Salmonella* bacteria

invade and profoundly proliferate in the placental tissue. Distinctly, only virulent *Salmonella* triggered an inflammatory signature that damaged placental integrity, leading to fetal loss. We also observed that larger numbers of the virulent *Salmonella* bacteria were dispersed throughout the placenta whereas the avirulent strain was mainly localized to the deciduas at 72 h of infection (5). Importantly, even the avirulent strain breached the placental barrier, and small numbers of bacteria were retrieved from fetal livers by 72 h (data not shown). Nevertheless, the mothers went on to have full-term pregnancies, although the pups were born infected and often exhibited growth retardation (data not shown). The latter observation supports the hypothesis that *Salmonella* has evolved invasive mechanisms to cross the placental barrier. Indeed, *Salmonella* utilizes a specialized type III secretion system to invade nonphagocytic cells (13). Furthermore, *Salmonella* bacteria are known to be highly evasive, devoting >4% of their genome to virulence, secreting ~30 different effector proteins into infected cells via their type III secretion apparatus (3). Often, *Salmonella*-infected cells, such as macrophages, undergo rapid apoptosis. Therefore, once *Salmonella* bacteria gain access to maternal decidua either directly or via infection of decidual macrophages, they can rapidly and easily spread across placental layers. Whether such specific pathogen interaction facilitates infection of the labyrinth trophoblast needs further investigation.

I speculate that each pathogen may distinctly and differentially interact with decidual and/or placental cells. For example, in a murine model of *Campylobacter rectus* and *Porphyromonas gingivalis* infection, focal necrosis and increased inflammatory cell infiltrate and more specifically increased TLR-4 immunofluorescence were evident in labyrinth trophoblasts (2). This suggests that for some infections, innate immune signaling may be augmented after the pathogen reaches the labyrinth trophoblast. In another recent study, in infection of mice with muridherpes virus, initially only the decidua was infected, but by 9 days of infection, the bacterial burden had decreased in the deciduas but increased in the placenta. Interestingly, this did not result in any adverse pregnancy outcome, which correlated to inhibition of inflammatory placental cytokines and chemokines, such as interleukin 6 (IL-6), MIP-1β, and RANTES (4). Thus, for some other infections, breaching of the placental barrier may occur independently of inflammation and consequently in the absence of tissue infarction. As indicated by Redline et al. above, they previously reported that in a murine model of *Fusobacterium*, blocking of TLR-4 responses prevents fetal loss (9), implicating inflammation rather than bacterial burden as a cause of adverse pregnancy outcome. Our study also supports this notion that the inflammatory signature evoked by *Salmonella* is damaging to the fetus. Occasionally, the price of overt inflammation during pregnancy may be maternal fatality due to systemic sepsis, as in the case of *Salmonella* infection. Consequently, blocking of IL-6, a key inflammatory cytokine, reduces systemic infection in maternal hosts (11).

The complexity of pathogen-specific interactions at the feto-maternal interface is also revealed from studies on human pregnancy. For example, in human first- and second-trimester placentas, the expression of Toll-like receptors (TLRs) is limited to inner cytotrophoblast layers, and only pathogens that breach the outer TLR-negative syncytiotrophoblast are able to evoke inflammatory damage (1). Additionally, the syncytiotrophoblast may act as a physical barrier to infection, as demonstrated with such pathogens as *Listeria monocytogenes*, *Trypanosoma gondii*, herpes simplex virus, and cytomegalovirus (6, 7, 12). At the same time, the human uterine decidua also expresses TLRs (8) and can be infected with various pathogens (10). Therefore, both the pregnant host and pathogen-specific adaptations may govern pregnancy success and/or failure. In this scenario, inflammation and tissue infarction may go hand-in-hand, and additional experiments are needed to decisively delineate the temporal relationship between the two events in the context of *Salmonella* infection.

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