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OPINION

INTRACELLULAR ORGANISMS AS PLACENTAL INVADERS

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A SURPRISE PATHOGEN

In an era of evidence-based medicine, physicians sometimes forget the value of anecdotes in stimulating thought about clinical problems. Our recent report on typhoid fever in a pregnant woman at 12 weeks of gestation is a good example[1]. In spite of culture-proven diagnosis and appropriate treatment of the mother with antibiotics, fetal loss occurred at 16 weeks of gestation. *Salmonella typhi* was found in the fetal lung on autopsy, consistent with vertical transmission of the organism. None of the clinicians caring for the patient had imagined that gram-negative bacteria could cross the placenta and kill the fetus in spite of early diagnosis and treatment with appropriate antibiotics.

In marked contrast, large animal veterinarians are trained to quarantine aborting animals until contaminated areas are disinfected, prophylactic antibiotics are given and the specimens of placenta, fetus, blood, vaginal swabs, feces, and fetal stomach contents are systematically collected and sent to veterinary diagnostic labs for necropsy, histopathology, culture and sensitivity and polymerase chain reaction (PCR) analysis for various organisms. This practice is normal for veterinarians in order to prevent huge economic damages that might occur should one animal ingest an infected placenta and pass on the infection to the whole herd. In humans, *Mycoplasma hominis* or *Brucella* spp. might be in the differential diagnosis of recurrent miscarriage, but culture techniques are not generally used for the evaluation of miscarriage. We do not think about infection very often, and we do not culture routinely. Indeed culturing every spontaneous human miscarriage might add significantly to the cost of medical care without yielding much useful information. Thus, in our case, even the admitting physician who had knowledge of this patient's typhoid fever in the first trimester did

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Table 1 Placental invaders (adapted with permission from Robbins and Bakardjiev 2010[2])

Pathogen (illness)	Type	Lifestyle	Cells infected
Brucella spp. (Brucellosis)[a, b, c, d, e]	Bacteria	Facultative	Leukocytes, epithelial
Coxiella burnetii (Q fever)[c, e, h]	Bacteria	Obligate	Leukocytes
Listeria monocytogenes (Listeriosis)[a, b, c, d, g, h]	Bacteria	Facultative	Epithelial, phagocytes
Mycobacterium tuberculosis (TB)[d, g]	Bacteria	Facultative	Leukocytes
Treponema pallidum (Syphilis)[b, c, f, g]	Bacteria	Facultative	Unknown
Leishmania spp. (Leishmaniasis)[h]	Parasite	Obligate	Leukocytes
Plasmodium falciparum (Malaria)[b, c, e, g, h]	Parasite	Obligate	Erythrocytes, hepatocytes
Toxoplasma gondii (Toxoplasmosis)[a, b, d, g]	Parasite	Obligate	All nucleated cells
Trypanosoma spp. (Chagas disease, African sleeping sickness)[e, g]	Parasite	Facultative	Epi/endothelial
Cytomegalovirus (CMV)[a, b, d, g]	Virus	Obligate	Leukocytes, trophoblasts
Lymphocytic choriomeningitis virus (LCMV)[a, b, f, g]	Virus	Obligate	Leukocytes
Parvovirus B19[a, b, c, d, f]	Virus	Obligate	Hematopoietic, endothelial
Rubella virus (German measles)[g]	Virus	Obligate	Many
Varicella zoster virus (Chicken pox)[g, h]	Virus	Obligate	Leukocytes, neurons, epithelial

Notes: [a]First trimester fetal death.; [b]second trimester fetal death; [c]stillbirth; [d]preterm labor; [e]intrauterine growth restriction; [f]fetal hydrops; [g]severe neonatal infection; [h]increased severity of maternal disease.
Many other intracellular organisms, including *Babesia* spp., Coxsackie B virus, Japanese encephalomyelitis virus, *Leptospira* spp., Cryptococcus neoformans, *Pasteurella, Shigella*, *Salmonella typhi*, *Wucheria bancrofti*, Candida spp., *Campylobacter*, and many gingival bacteria, including *Fusobacterium nucleatum*, merit further study because of human case reports and/or animal studies.
Epstein-Barr virus, Hepatitis B virus, HIV, and HSV are known to cause perinatal infections via exposure to maternal secretions and blood but rarely via vertical transmission across the placenta.

not order Gram stain or bacterial cultures of the placenta at 16 weeks of gestation because infection is not in the differential diagnosis for second trimester fetal loss.

BEYOND TORCH

Perhaps we clinicians depend too much on the TORCH mnemonic that we learned in medical school. Although it is well known that the TORCH list addresses only a few of the blood-borne pathogens known to enter the fetal compartment, it is important to remember that the "O" in TORCH suggests that "other" microbes can infect the placenta as well. Table 1[2] lists the diverse placental pathogens that have been associated with pregnancy complications and fetal damage in humans. This list contains viruses, bacteria and protozoan microbes. Many of these are the same pathogens that cause embryonic and fetal demise in cattle, sheep, goats and camelids[3]. All these zoonotic infections are known in the veterinary literature to be caused by intracellular organisms.

Similarly, every organism listed in Table 1 is able to survive in host cells. Some, such as the viruses, have obligate intracellular lifecycles; but others are facultative

intracellular. Whether obligate or facultative, intracellular microbes have evolved many ways to elude host recognition. Many have learned to take up residence inside circulating blood cells that facilitate systemic dissemination. For example, the bacterial pathogens *L. monocytogenes* and *Salmonella* can hitch a ride in monocytes to reach the central nervous system[4] or the liver[5]. Thus, it is plausible that infected monocytes can provide transportation for these organisms on their way to the maternal-fetal interface, giving new meaning to the concept of hematogenous spread.

THE DECIDUA IS THE INITIAL SITE OF INFECTION

In vivo studies with murine and guinea pig models have all pointed to the decidua as the preferred site of initial placental colonization for the hematogenously spread organisms: *Toxoplasma gondii*, *Chlamydia psittaci*, *Coxiella burnetii*, *Fusobacterium nucleatum*, *Salmonella typhimurium*, *Brucella abortus*, *L. monocytogenes* and Cytomegalovirus (CMV)[2,6]. The decidua actively recruits leukocytes to the endometrium during the secretory phase of the menstrual cycle and during the implantation phase of early pregnancy[7]. Once blood monocytes are localized in the decidua, they differentiate into decidual macrophages. Microbes hiding inside these infected macrophages have the opportunity to infect trophoblasts. We know that trophoblast infection does happen based on histological examination of human placentas infected by *L. monocytogenes*[8–10] and by the recent finding of intracellular bacteria of diverse morphologies inside trophoblasts at the basal plates of 27% of all placentas[11], including those without clinical or pathological evidence of chorioamnionitis.

ENTERING THE TROPHOBLAST

Trophoblast infection occurs most likely via multiple mechanisms depending on the armamentarium of the specific pathogen such as virulence factors that facilitate adherence or invasion of host cells (e.g. adhesins, invasins and Type III secretion systems). Another possibility is bacterial cell-to-cell spread from infected maternal cells to trophoblasts without exposure to the extracellular environment. Inoculation of pregnant guinea pigs with wild-type and mutant *L. monocytogenes* has shown that the predominant mechanism for placental invasion by this organism is cell-to-cell spread[12].

Figure 1 shows the maternal-fetal interface at the placenta. The largest surface area of fetal contact with maternal blood is the syncytiotrophoblast (SYN). The SYN is a continuous layer of fused multinucleated trophoblasts undergirded by cytotrophoblasts and a basement membrane. SYN has a distinct polarity and there are no intercellular junctions. Microbes have difficulty adhering to SYN, and in human placental organ cultures, SYN is highly resistant to infection by several organisms.

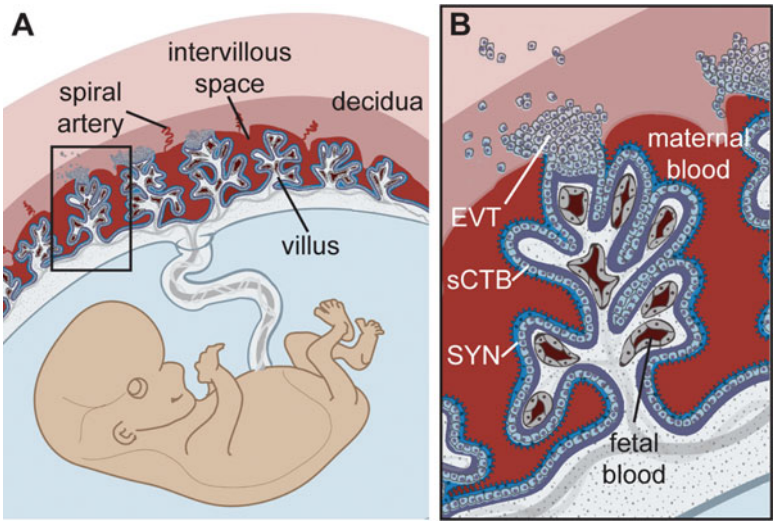

Figure 1 Structure of the human placenta. (*a*) Maternal blood from spiral arteries in the decidua flows into the intervillous space, where it surrounds thousands of fetally derived chorionic villi. (*b*) Villi are freely floating in maternal blood, or invade the decidua to form anchoring villi. The entire villous surface is covered with a continuous layer of multinucleated SYN, which is the major fetal surface in contact with maternal blood. The apical side of the SYN comprises profuse, branched microvilli, which provide abundant surface area for gas and nutrient exchange between the mother and the fetus. The SYN is undergirded by cytotrophoblasts (sCTB), which are separated from fetal capillaries in the villous stroma by a basement membrane. Some sCTB leave the basement membrane and differentiate along the invasive pathway to form anchoring villi. Columns of unpolarized sCTB attach to and then penetrate the uterine wall where they give rise to extravillous cytotrophoblasts (EVT). (Figure adapted with permission from Robbins and Bakardjiev 2010[2].)

Those that have been studied include *L. monocytogenes*[13], *T. gondii*[14], Herpes simplex virus[15] and CMV[16].

Also shown is the extravillous trophoblast (EVT) that forms the anchoring villi to attach the placenta to the decidua and the uterine wall. EVT is intimately juxtaposed to a variety of leukocytes at the decidua. In vitro placental culture techniques have directly shown the ability of *L. monocytogenes*[13], *T. gondii*[14], Herpes simplex virus[15] and CMV[16] to infect EVT.

IMMUNE TOLERANCE

Maternal leukocytes are plentiful in the decidua, but their functions are modulated to help with angiogenesis or fetal immune tolerance[7]. Most of the leukocytes in the decidua are specialized natural killer cells (dNK) and macrophages. T-cells and B-cells are scarce due to epigenetic silencing of chemoattractants in the decidua[17]. Cytolytic functions of dNKs, macrophages and T-cells in the decidua are all suppressed perhaps due to cross talk with EVT, which expresses the immunosuppressant human leukocyte

antigen G (HLA-G)[7], but not the Major Class I Histocompatibility (MHC) molecules HLA-A or HLA-B[7]. The intimate relationship of EVT to maternal macrophages in the decidua might be permissive to infection transmitted from these cells directly to trophoblasts. EVT could be a tolerant ecological niche for intracellular organisms because of the many specialized immune adaptations of the decidua to allow for tolerance of fetal tissues[7]. For example, due to the lack of classical MHC Class I molecules infected EVT cannot present microbial antigens residing in their cytoplasm.

HOST DEFENSES

That being said, the maternal-fetal interface has defense mechanisms that limit infection by intracellular organisms. This has probably been most closely characterized in experimental models with *L. monocytogenes*. In pregnant guinea pigs, only one in a million bacteria injected intravenously is able to colonize the maternal-fetal interface[18]. Furthermore, EVT have the ability to eliminate approximately 80% of intracellular *L. monocytogenes* within 24 hr[19]. However, the bacterial load can at times overcome host defenses such that the organism can no longer be eliminated and the placenta becomes a nidus for infection, leading to continuous seeding and reseeding of the maternal organs and the fetus until the placenta and the products of conception are expelled, or the mother dies[18]. Further research is needed to better understand the molecular and cellular mechanisms of host defenses against pathogens at the maternal-fetal interface, and how these are breached.

BREACHING THE BARRIER

Pathogens vary in their ability to elicit host responses, and hence disease expression. *Mycobacterium tuberculosis*, for example, may not leave a significant inflammatory signature, and uterine infection can co-exist with pregnancy rarely causing fetal damage or vertical transmission because of the organism's slow replication rate and ability to live for long periods inside macrophages. Other organisms, such as the obligate intracellular CMV, tend to evoke host cell apoptosis and a great deal of tissue necrosis, inflammation and edema in placental tissues, leading to abruption and fetal loss[16]. Damage to SYN by CMV opens the door to secondary invaders such as Herpes simplex virus, which might not cause damage on its own[20]. In general, co-infection by more than one organism is probably worth remembering as a possible mechanism by which damage to SYN might be permissive to infection by a second organism. Another example is placental infection with *Plasmodium falciparum*, the parasite that causes malaria. Infected erythrocytes sequester in the intervillous spaces adhere to SYN and cause SYN damage[21]. This damage might explain increased rates of vertical transmission of HIV-1[22] in pregnant women who are co-infected with both malaria and HIV.

LOOKING AHEAD

We hope that this discussion will serve to increase awareness among obstetrics/gynecology clinicians that all maternal illnesses during pregnancy should be regarded with suspicion, because host immune cells can carry intracellular organisms to the placenta, and breach the placental barrier. Antibiotic therapy of infections, such as typhoid fever and listeriosis, might have to be extended beyond the two weeks recommended for non-pregnant individuals, especially if infection occurs during the first trimester[23,24].

We do not know if histological examination of the placenta or bacterial cultures are the most important investigations in cases of fetal loss, preterm labor, or abruption. We do hope, however, that this discussion might stimulate some clinical research into culture-independent methods of diagnosing infection by intracellular organisms at the maternal-fetal interface, whether it be by quantitative PCR for pathogen DNA, immunohistochemistry, electron microscopy, or some other method.

CONFLICT OF INTEREST

None of the authors have a conflict of interest.

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