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Review

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Risks associated with viral infections during pregnancy

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Introduction

Viral infections during pregnancy have been associated with adverse pregnancy outcomes and birth defects in the offspring; unfortunately, we have limited therapeutic or preventative tools to protect the mother and the fetus during pandemics. Viruses rarely cross the placental barrier, but when the virus does reach the fetus, it can result in severe birth defects such as microcephaly or even fetal death. It has been well established that viral infection of the cells at the maternal-fetal interface can affect placental function, which may result in pregnancy complications such as miscarriage, intrauterine growth restriction (IUGR), or preterm birth (PTB). Furthermore, a growing body of evidence suggests that viral infection of the decidua and/or placenta may result in the production of soluble immune factors that could reach the fetus and might affect fetal development.

The maternal-fetal interface includes multiple cell types that contribute to the development of the fetus, regulation of the maternal immune system, and protection against microorganisms. The maternal side is made from the stroma of the uterus, or decidual cells, and a wide range of immune cells including NK cells, macrophages, DCs, and Tregs. The fetal side consists of the placental villus, which contains fetal blood vessels surrounded by fibroblasts and fetal macrophages (known as Hofbauer cells), cytotrophoblasts, and, finally, the multinucleated syncytiotrophoblast, an epithelial covering that is in direct contact with maternal blood (Figure 1). In addition, the extravillous trophoblast is in direct contact with cells from the decidua, including maternal immune cells, endothelial cells, and microorganisms present in the uterus.

Many factors can influence the incidence, longevity, and severity of viral infection at the maternal-fetal interface. Viruses gain access to the cells within the decidua and placenta by ascending from the lower reproductive tract or via hematogenous transmission (1, 2). Following access to the upper reproductive tract, viral tropism for the decidua and/or placenta is then dependent on both viral entry receptor expression by the cellular component of these tissues and the specific maternal immune response to the virus. These factors vary by cell type and gestational age and can be affected by changes to the in utero environment and maternal immunity. Therefore, the virus-host interaction during pregnancy is complex and highly variable (Figure 1).

Innate immune cells, including NK cells, DCs, and macrophages, and the maternal humoral response play a critical role in regulating and controlling the infection and, consequently, determining its severity. Innate cells phagocytize virus complexes and can kill infected cells, while antibodies facilitate viral clearance. Contrary to nonpregnant women, during pregnancy, the function of the innate immune system is influenced and regulated by the fetal/placenta unit. In summary, the route of viral transmission, abundance of permissive cell types (which changes with gestational age), and maternal immune function all influence viral infections at the maternal-fetal interface.

Viral infection at the maternal-fetal interface

The most common virus identified to date at the maternal-fetal interface is CMV, a member of the *Herpesviridae* family. CMV interacts with ubiquitously expressed heparin sulfate on the cell surface and then penetrates cells via interactions with integrin subunits (3, 4). Roles for other receptors, such as EGFR and PDGFR-α, have also been reported, but those findings have been contradicted, and the role of these receptors in CMV infection remains disputed (5, 6). The known receptors for CMV entry are expressed by multiple cell types, including epithelial cells, endothelial cells, muscle cells, fibroblasts, trophoblasts, and monocytes/macrophages; therefore, these cells can all be permissive to CMV infection. At the maternal-fetal interface, specifically, CMV is more likely to be detected in the maternal decidua, where it infects and replicates

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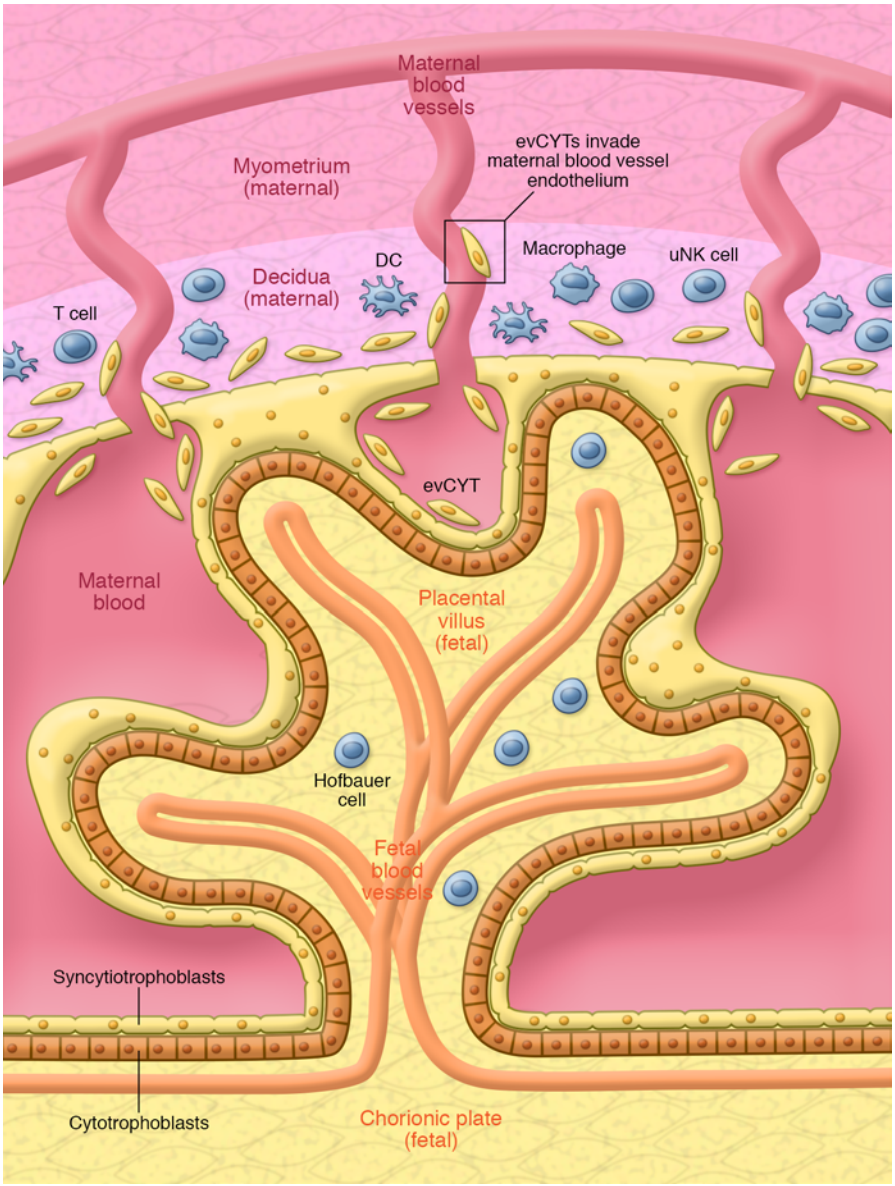

Figure 1. Cell types at the maternal-fetal interface. Maternal and fetal cells make up the maternal-fetal interface. The maternal decidua consists of pregnancy-specific differentiated stromal cells that house the maternal blood vessels and maternal immune cells including T cells, uterine NK (uNK) cells, macrophages, and DCs. Extravillous cytotrophoblasts (evCYTs) invade the decidua and reach the maternal spiral arteries, establishing nutrient circulation between the embryo and the mother. The placental villus is in direct contact with the maternal blood and thus facilitates gas, nutrient, and communication exchange between the mother and developing fetus. It is formed by a double cell layer consisting of syncytiotrophoblasts and cytotrophoblasts. The villus contains the fetal blood vessels that are surrounded by fibroblasts and fetal macrophages (termed Hofbauer cells).

in endothelial cells, invasive cytotrophoblast, fibroblasts, and the glandular epithelium (7, 8), than in the placenta. Decidual infection can occur in the first, second, or third trimester and is affected by variations in local maternal innate immune cells. For example, in pregnant women, higher levels of local DCs and macrophages containing phagocytosed viral proteins in the decidua are associated with milder infection, potentially because of the robust innate response to infection in these individuals (7, 8).

Interestingly, placental CMV infection is less common than decidual infection. This is because the syncytiotrophoblast does not express the receptors for CMV entry, and the virus must traverse this layer to infect the susceptible cytotrophoblast localized underneath the syncytiotrophoblast layer. Because of this tissue organization, the severity of infection is dependent on maternal factors such as humoral immunity and microbial coinfections of the placenta (7–9) that would promote the breach of the syncytiotrophoblast layer. Low-avidity neutralizing IgG can bind the virus and interact with syncytiotrophoblast Fc receptors, thus catalyzing viral transcytosis to the underlying cytotrophoblast (2, 9, 10). As discussed below, coinfections, or polymicrobial infections, are an important factor in allowing pathogens to damage the syncytial layer, thereby permitting the virus to cross and reach the fetal side. Alternatively, the presence of other pathogens could activate latent CMV in the decidual-immune reservoir (7).

Another member of the *Herpesviridae* family, herpes simplex virus (HSV), is estimated to infect the decidua and/or placenta in 6% to 14% of pregnancies (7, 11) and, like CMV, is more likely to be identified in the decidua than in the placenta (7, 12). Deciduitis and villitis have been described in relation to HSV infections (13), which may explain the association between primary maternal infection with HSV and increased risk of miscarriage and fetal death (14). Heparan sulfate, herpesvirus entry mediator A (HveA), HveB, and HveC are the entry receptors for HSV-1 and -2. They are not expressed on the surface of the syncytiotrophoblast but are expressed on the extravillous trophoblast (15, 16). Finger-Jardim et al. reported a 9% prevalence of HSV-2 in placental sam-

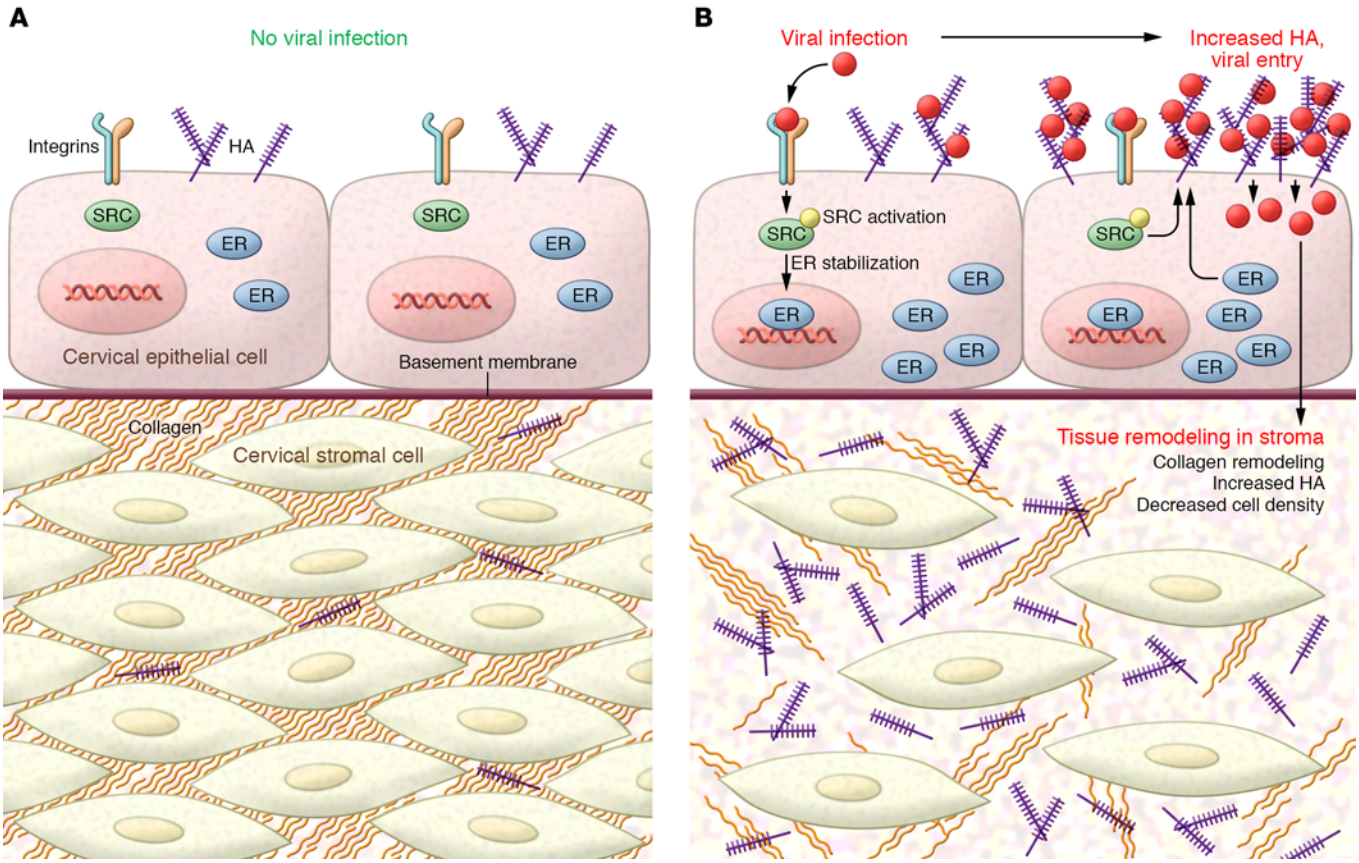

Figure 2. Mouse models of viral infection during pregnancy. Herpesviruses target the pregnant cervix and induce dramatic molecular and functional changes. (**A**) High sex hormone levels associated with pregnancy enhance cervical susceptibility to infection by causing upregulation in the cervix of integrins and HA, receptors for herpes infection. (**B**) Infection of cervical epithelial cells results in activated SRC kinase, which stabilizes the ER. These changes are also associated with reduced expression of innate immune factors such as TLRs and defensins, as well as tissue remodeling in the stroma that is reminiscent of cervical ripening.

ples in asymptomatic patients at the time of delivery, and none reported genital herpes (11). These results suggest that HSV-2 can infect the placenta even in asymptomatic patients. The clinical and biological relevance of HSV-2 infection on those placentas is poorly understood, but as discussed below, the antiviral response originated at the placenta might reach the fetal side and influence fetal development.

The herpesviruses varicella zoster virus and EBV have been isolated in placental tissue and can infect the developing fetus, but cases are extremely rare, and little is known about the conditions for infection (12, 17). While not herpesviruses, human papillomaviruses HPV6, -11, -16, -18, and -31 can also infect the extravillous trophoblast (18, 19); HPV16 and HPV62 have been identified in gestational week-12 chorionic villus (20); and HPV16, -6, -83, and -39 have been characterized full-term placenta (19, 21–23). Zika virus (ZIKV) can also infect multiple cell types of the decidua and placenta and will be discussed in further detail in a later section.

Viral infections and fetal development

Viral infections that are capable of crossing the placental barrier and reaching the fetus can have devastating effects on fetal development (24). Gregg first described the association between fetal viral infection and abnormal development when he discovered the association between prenatal rubella infection and cataract in 1941 (25). There is now a large body of work demonstrating that direct infection of the fetus with CMV, HSV-2, or rubella can cause major neurosensory deficits (26–28), learning disabilities, and psychiatric disorders (29–31). Microcephaly has also been associated with fetal CMV and ZIKV infection (ZIKV is further discussed below) (32, 33). While these examples of direct infection of the fetus have the most severe individual consequences, in utero fetal viral infections are rare. Neonatal HSV infection has been identified in approximately 0.06% of neonates in the US, and only 5% of those infections are associated with intrauterine infection (34). Fetal infection with CMV has an estimated prevalence of 0.5% (28), and congenital rubella has been virtually eliminated in the US (35–37). Conversely, the prevalence of maternal infection or reactivation with CMV, HSV-2, or influenza is high (60%–90%, 18%–22%, and 40%, respectively) (8, 11, 27, 38–43). All these data support the notion that the placenta is actively preventing viral transmission to the fetus; however, viral infections of the placenta, such as that by HSV-2, can occur in asymptomatic patients (11) and can induce systemic and local (placental/decidual) changes, which could still affect fetal development.

While viral infections that do not cross the placenta can affect fetal development, specifically neuronal development, the mech-

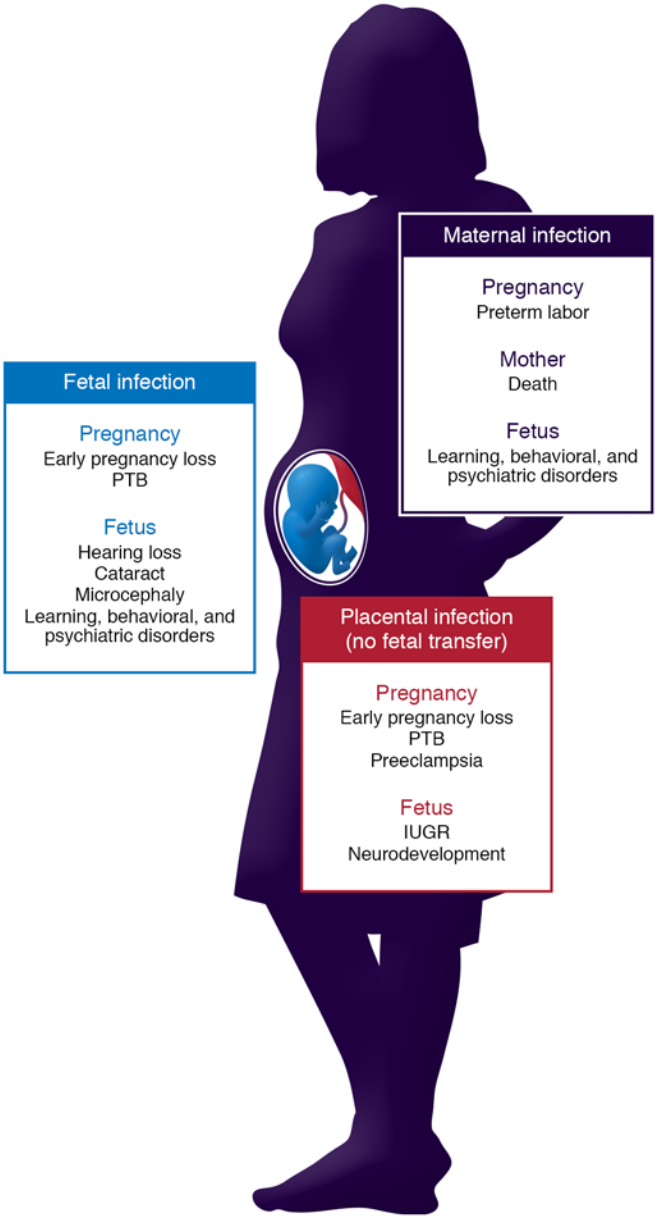

Figure 3. Maternal viral infections and associated outcomes. Viral infection at the maternal-fetal interface can affect the mother as well as fetal development. The placenta functions as a physiologic and immunologic barrier to prevent viral transfer from the mother to the fetus. However, the immunologic response to infection might reach the fetal circulation or predispose the mother to abnormal responses to other microorganisms, with potential pregnancy complications such as IUGR, PTB, or even early pregnancy loss. Fetal infection can cause pregnancy loss and is associated with hearing loss, cataract, microcephaly, and psychiatric disorders in the fetus.

anism is still poorly understood. Original observations identified individuals from a Finnish birth cohort whose mothers were in their second trimester of pregnancy during the 1957 influenza pandemic as having an increased chance of being admitted to a psychiatric hospital and diagnosed with schizophrenia (44). Although several studies following this initial observation had inconclusive results, when researchers identified mothers with influenza only using serological tests, they confirmed that influenza during early-to-mid gestation was associated with an increased risk of schizophrenia in the offspring (45, 46).

The epidemiologic studies referenced above indicate that maternal viral infections do not need to bypass the placental barrier to affect fetal development, and animal models have been invaluable tools for improving our understanding of this complex mechanism. With these models, potential maternal immune pathways and cytokines that could be responsible for behavioral changes in offspring have been identified (47, 48). Recently, in a polyinosinic-polycytidylic acid (poly(I:C)) mouse model of maternal immune activation (MIA), maternal Th17 cells and their effector IL-17a were identified as critical drivers of maternal inflammation–associated defects in fetal brain development and offspring behavior (49). In another study, using the same mouse model of MIA, it was determined that maternal IL-6 is also a critical effector (50). In this study, injecting the pregnant mother with IL-6 alone was able to affect offspring behavior (50). Furthermore, neutralizing maternal IL-6 with antibodies following poly(I:C) treatment prevented the exploratory and social deficits caused by the MIA (50).

To evaluate the role of viral infection in pregnancy and fetal development, we developed a murine model that consists of exposing pregnant mice to murine herpes virus 68 (MHV68). We used the gammaherpes virus MHV68, since it is a latent DNA virus from the *Herpesviridae* family, the same family for nearly all of the viruses that infect the woman's reproductive tract (HSV, CMV, EBV, and human herpesviruses HHV6 and HHV7). Our data suggested that, even in the absence of placental passage of the virus, the fetus could be adversely affected by viral invasion of the placenta (51). Morphological analysis of fetuses from mothers with viral infections revealed major developmental changes. Despite the absence of fetal infection, the fetuses of infected mothers had delayed differentiation of the eyes, tails, and limbs (51). They also had hydrocephalus, defined by an increase in the subarachnoid space in the brain, but no changes in the lateral ventricles, abnormal immune infiltration, or white matter damage (51). In the thoracic cavity, the pathological changes were characterized by the presence of hemorrhage inside the lungs and pericardium in all treated animals compared with the controls (51). Interestingly, we also observed a marked increase in the levels of fetal proinflammatory cytokines, including high levels of IFN-γ and TNF-α (51). Collectively, these findings provide strong evidence that, even when the virus does not reach the fetus, the fetus is still affected by the maternal immune response to the infection. This could be a result of the proinflammatory response of the placenta, or it could be due to other physiological changes in the mother or the placenta that are associated with the infection.

Viral infection and PTB: cervical infection

Cervical viral infection can increase the risk for PTB, defined as birth before 37 weeks' gestation in women. Many viruses have been reported to infect the cervix, including HHV6, HHV7 (52), HSV (53), CMV (2, 54), EBV (55), and HPV (56), the most common viral infection of the cervix. Despite the prevalence, there were historical challenges associated with measuring viral infections in biological fluids and tissues from the lower reproductive track. With improved molecular techniques (sensitive PCR assays), we

are beginning to identify viral infections as potential risk factors for PTB (53, 56–58). For example, Zuo et al. showed that cervical HPV infection was associated with PTB (56), while others determined that genital HSV was also associated with PTB (53, 59). One of these studies included nearly 700,000 women and reported that untreated genital herpes infection nearly doubled the risk of PTB (53). In a mouse model, we have shown that the murine herpesvirus MHV68 targets the pregnant cervix and induces dramatic molecular and functional changes in that tissue (58). We observed that high levels of sex hormones associated with pregnancy enhanced the susceptibility to cervical infection by upregulating cervical integrins (58). In addition, MHV68 infection of the cervix in pregnant mice reduced the expression of innate immune factors such as TLRs and defensins (58). These changes in the pregnant cervix were associated with increased ascending infection compared with that detected in uninfected pregnant mice (Figure 2A) (58). In a separate study, pregnant mice received HSV-2 intravaginally, which induced tissue remodeling in the stroma that was reminiscent of cervical ripening (60). Infection of human cervical epithelial cells with HSV-2 caused activation of SRC kinase, which stabilized the estrogen receptor (ER) (60). Interestingly, HSV-2 also upregulated hyaluronic acid (HA), which was dependent on the increased ER levels (60). Since HA is associated with changes in tissue structure and function, we postulate that viral infection of cervical epithelial cells results in increased HA synthesis, thus affecting stromal architecture and premature cervical ripening (Figure 2A).

Viral infection and PTB: in utero or systemic infection

Several studies have demonstrated an association between maternal, placental, or amniotic fluid viral detection and PTB. One case-control study identified a higher frequency of HPV in the extravillous trophoblast of women suffering from spontaneous PTB compared with women delivering at term, with no association with low- or high-risk HPV strains (18). Another retrospective study examined over 2,400 cases of pregnancy over an 11-year period and discovered that cervical infection with high-risk HPV was highly associated with abnormal placental pathology and PTB (56). The presence of adeno-associated virus (AAV) in amniotic cells was also associated with an increased risk of PTB, although the authors did not determine whether AAV DNA was present in the placenta (61).

Maternal infections with influenza and hepatitis have also been associated with preterm labor. Data collected from the 2009 influenza H1N1 pandemic revealed that women with H1N1 were more likely to have adverse pregnancy outcomes, such as spontaneous miscarriage and preterm birth (62–64). The rate of PTB correlated with maternal disease severity (65), and, moreover, vaccination against H1N1 reduced the rates of PTB and low birth weight (66–69). Maternal hepatitis B virus (HBV) infection was also associated with PTB, although the underlying mechanism was not determined (70).

The only mechanistic insights we have with regard to virus-associated PTB are those gleaned from animal models. Initial studies consisted of administration of TLR ligands, such as Poly(I:C), to pregnant WT or *Tlr3*-KO mice to mimic viral signaling during pregnancy (71, 72). This induced preterm labor in WT but not *Tlr3*-KO mice, indicating that TLR3 signaling could cause PTB (71). Although these animal studies strongly support a role for viral infections in PTB, the majority of the clinical findings in patients with PTB are associated with an inflammatory process caused by bacterial infections (73). Our animal studies using a live virus have suggested that the actual mechanism associated with infection-induced PTB is more complex. We showed that pregnant mice injected with a DNA virus of the *Herpesviridae* family did not suffer PTB, but the virus infected the placenta (51). The viral infection of the placenta was associated with hyperresponsiveness to low concentrations of bacterial endotoxin, which led to PTB (51). At the molecular level, viral infections modified the function of pattern recognition receptors (PRRs), such as TLRs, and affected the quantity as well as the quality of their responses. We demonstrated in both in vivo and in vitro studies that the virus downregulated the placental type I IFN, IFN-β, thus releasing the intrinsic regulation of TLR4-mediated proinflammatory cytokines in the trophoblast and allowing its cells to respond to the endotoxin challenge (ref. 74 and Figure 2B). Given these findings, we proposed that many of these infection-related pregnancy complications are polymicrobial in nature and involve an initial infection, viral (first hit), that modifies the response of the PRRs to the second infection, bacterial (second hit). Together, these pathogens result in a dysregulated inflammatory response that triggers preterm delivery. This mechanism needs further elucidation if we are to develop new methods for prevention and therapy.

Viral infections and early placental development

The invasive extravillous trophoblast is responsible for anchoring the placenta and invading the maternal spiral arteries early in pregnancy, thus ensuring adequate blood flow and communication between mother and fetus (75–77). If these cells are dysregulated and this process disrupted, there is increased risk for pregnancy complications (78). For example, first-trimester trophoblasts infected with CMV demonstrate reduced cell invasion, increased apoptosis (2, 79), higher expression of proinflammatory cytokines (80), and reduced HLA-G expression (81, 82). CMV infection is also associated with fetal growth restriction, spontaneous pregnancy loss (82–84), and preeclampsia (85, 86), all of which are outcomes that can result from insufficient placental development. HSV infection also results in loss of HLA-G (87), cell death, and reduced human chorionic gonadotropin secretion (88). These changes in trophoblast function could explain why both HSV-1 and HSV-2 have been associated with spontaneous pregnancy loss (89) and IUGR pregnancies (82, 84). AAV2 also induces trophoblast apoptosis and reduces cell invasion (18, 90, 91) and is associated with spontaneous miscarriage, stillbirth (92), and preeclampsia (82, 91, 93). Collectively, these results demonstrate that viral infection of the trophoblast can alter placental function and could result in suboptimal conditions for fetal growth and development.

Viral infection and maternal health

Pregnant women have higher mortality rates and complications associated with viral infections compared with the general population, but the reason for the increased susceptibility is not well defined (94). The maternal immune response can be beneficially or detrimentally affected by pregnancy, depending on the envi-

ronmental conditions and the stage of the pregnancy. It is well established that the immunological changes associated with pregnancy can result in the amelioration of some autoimmune disorders, such as multiple sclerosis or rheumatoid arthritis (95), but can also increase the severity of several types of viral infections. For example, pregnant women have higher mortality rates associated with varicella virus infection, which is 10 times more likely to be complicated by pneumonia during pregnancy (94, 96, 97). They are more susceptible to rubeola (also known as measles), and the infection is more likely to cause death (98, 99). Furthermore, during the 2009 H1N1 influenza pandemic, pregnant women developed more severe flu-related complications, in some cases leadings to hospitalization and death, when compared with the general population (41, 62, 64, 100–105), and this was confirmed to have also occurred during the 1918 H1N1 (106, 107) and 1957 H5N1 pandemics (108, 109).

Despite these clear associations between pregnancy and virus-induced morbidity, there is still little known about how pregnancy affects the mother's response to viral pathogens. There is growing evidence suggesting that the placental response to virus is directly responsible for disease severity. For example, pregnant women infected with Lassa fever had higher mortality rates than did nonpregnant women with the infection (110). The Lassa virus replicates at very high levels in the placenta (111), and the risk of maternal death increases with the length of gestation (110) and the size of the placenta. Furthermore, evacuation of the uterus significantly improves the mother's chances of survival (110). Since the placenta regulates the maternal immune system and can itself respond to pathogens, it is probably an important mediator of the maternal response to viral infection, regardless of whether the placenta is directly infected (112).

ZIKV and pregnancy

The recent ZIKV outbreak in Brazil has refocused our attention on the risks associated with viral infections during pregnancy. This virus, a member of the family *Flaviviridae,* is transmitted by mosquito bite and is also potentially sexually transmitted (113–117). While historically an infection has been described as causing symptoms ranging from fever and rash to Guillain-Barre syndrome (118–124), during the most recent outbreak there was also a startling increase in the incidence of fetal brain and CNS abnormalities when mothers acquired the virus during pregnancy (32, 125–134). A causal link between ZIKV and these defects was established when the ZIKV genome was identified in the amniotic fluid of women whose fetuses had microcephaly detected during fetal ultrasound (125–127, 132, 133, 135). Researchers also identified ZIKV in placentas from miscarriages and IUGR pregnancies, suggesting that ZIKV also affects placental function and could increase the risk of several pregnancy complications (126, 132, 136). There is now a global push to understand how ZIKV affects pregnancy and fetal brain development.

Like all viruses, ZIKV requires cellular expression of receptors that permit viral binding and entry into that cell. Several of these receptors have been identified, including the TAM kinase receptors AXL and TYRO3, in addition to the C-type lectin DC-SIGN and the glycoprotein TIM1 (137). An important study by the Harris and Pereira laboratories recently determined that AXL and TYRO3 receptors had variable expression in placental cells that could be affected by the culture of primary cells. These proteins were expressed by amniotic epithelia, and AXL was also expressed by the cytotrophoblast, Hofbauer cells, and placental fibroblasts, but this expression was variable depending on the gestational stage, the donor, and whether the cells were cultured (138). In contrast, TIM1 was invariably expressed at mid- and late gestation in amniotic epithelia, syncytiotrophoblast, Hofbauer cells, and invasive cytotrophoblast (138). They also examined ZIKV in primary placental cells and explants. Amniotic epithelia from mid-gestation had higher viral titers than did late-gestation epithelia, and mid-gestation proliferating cytotrophoblast and invasive cytotrophoblast were also viral targets (138). Of note, infection of the cytotrophoblast in early gestation was associated with loss of proliferation (138), which could contribute to ZIKV-associated miscarriage and growth restriction. Finally, this study found that placental fibroblasts and Hofbauer cells were infected by ZIKV (138), with similar findings reported by others (139). These cells are closely associated with the fetal vasculature and could be responsible for harboring virus that can be more easily transmitted to the fetus.

We found that, unlike *Flavivirus* yellow fever, ZIKV infection induces apoptosis in first-trimester trophoblasts, prevents differentiation of these cells into spheroid cultures, and induces the collapse of preformed trophoblast-derived spheroids (140). Furthermore, there is a growing interest in additional factors responsible for the teratogenic effects observed in some patients infected with ZIKV. Since the seroprevalence of HSV-1 and HSV-2 was found to be higher in Brazil's North region (141), which reported the majority of microcephaly cases (132), we tested the hypothesis that the immune response to HSV-2 could have an effect on ZIKV infection. Indeed, we observed that HSV-2 infection of trophoblasts could enhance the expression of TAM receptors, which facilitate entry of ZIKV into the cell. Using a mouse model resistant to ZIKV infection, we demonstrate that HSV-2 infection enhances placental sensitivity to ZIKV infection (140).

Microcephaly is the most severe birth defect associated with ZIKV and occurs via direct infection of the fetus during the first or second trimester of pregnancy (126, 132, 133, 135). The mechanism of virus-associated microcephaly is still unknown, but recent studies suggest that direct infection of the brain dysregulates development. Viral antigens infecting glial cells and neurons have been identified in the brains of fetuses with microcephaly, and these infections are associated with microcalcifications (126, 132, 136). Human cortical progenitors and human brain organoids can also be infected by ZIKV, which results in increased cell death and fewer proliferative zones, respectively (142). While animal models will require further validation, preliminary work with mice has demonstrated that similar placental types are susceptible to infection (143), the virus infects the fetal brain (113, 142), and fetal infection results in signs of microcephaly (113, 142). ZIKV infection of the lower reproductive tract of pregnant mice has also been shown to result in viral transmission to the fetus (113).

Conclusions and perspectives

Viral infections during pregnancy can affect fetal development and maternal mortality and are therefore a major clinical problem worldwide (Figure 3). Unfortunately, we do not yet have the appropriate tools to prevent infection and treat pregnant women during

pandemics such as influenza, Ebola, and ZIKV. The complexity of pregnancy and the immunologic changes associated with the acceptance of the fetus makes it challenging to improve the way we treat pregnant women. To progress, we must gain a better understanding of how viruses infect and affect the placenta at different stages of gestation and how direct and indirect fetal infections affect development. We also need to determine why pregnant women respond differently to infections than do their nonpregnant counterparts. These studies will provide the first step toward improving the clinical care provided to pregnant women and their unborn children.

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