frontiers in Virology

REVIEW
published: 24 March 2022
doi: 10.3389/fviro.2022.801778

Vertical Zika Virus Transmission at the Maternal-Fetal Interface

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Zika virus (ZIKV) is spread by mosquito bites or *via* sexual or vertical transmission. ZIKV-infected adults are generally asymptomatic, but can display mild symptoms including fever, joint pain, rash and conjunctivitis. However, during pregnancy, vertical ZIKV transmission can cause placental dysfunction and elicit severe fetal defects, including microcephaly, retinopathy, fetal growth restriction and/or stillbirth. Since no FDA-approved vaccine or anti-viral agents are currently available, ZIKV infection poses a global maternal-fetal health challenge. The maternal-fetal interface consists of maternal decidual and immune cells as well as fetal-derived trophoblasts. Compared to other cell types at the maternal-fetal interface, syncytiotrophoblasts, which form the outer layer of floating villi, are less-permissive to ZIKV, thereby preventing ZIKV transmission to the underlying cytotrophoblasts and/or other cells such as Hofbauer cells or fetal endothelium in the villi. However, anchoring villi are tightly attached to the decidua and their cytotrophoblastic cell columns are ZIKV-permissive, suggesting this location as the most likely site of ZIKV vertical transmission. Thus, at the maternal-fetal interface, maternal decidual cells likely serve as a reservoir of ZIKV persistence since they: 1) overexpress viral entry molecules compared to trophoblasts; 2) are highly permissive to ZIKV infection in a gestational age-dependent manner (more easily infected earlier in gestation); 3) augment ZIKV infection of weakly permissive primary cytotrophoblast cultures; and 4) display local maternal-immune tolerance, which prolongs ZIKV survival to facilitate fetal transmission. This review focuses on molecular mechanisms underlying ZIKV infection of cells at the human maternal-fetal interface, thus highlighting how decidual cells enhance propagation of ZIKV in extravillous cytotrophoblasts and why development of agents that eliminate ZIKV persistence in reproductive tissues before pregnancy is crucial to prevent perinatal ZIKV transmission.

Keywords: Zika virus, decidual cells, trophoblasts, maternal-fetal interface, vertical transmission

OPEN ACCESS

Edited by:
Kristina M. Adams Waldorf, University of Washington, United States

Reviewed by:
Julie Eggenberger, University of Washington, United States
Dawn Dudley, University of Wisconsin-Madison, United States

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Specialty section:
This article was submitted to Translational Virology, a section of the journal Frontiers in Virology

Received: *25 October 2021*
Accepted: *04 March 2022*
Published: *24 March 2022*

Citation:
Guzeloglu-Kayisli O, Kayisli UA, Schatz F and Lockwood CJ (2022) Vertical Zika Virus Transmission at the Maternal-Fetal Interface. Front. Virol. 2:801778. doi: 10.3389/fviro.2022.801778

INTRODUCTION

Throughout pregnancy, the placenta is a specialized tissue that acts as a physical and immunological barrier against invading pathogens to protect the developing fetus from infectious agents. Unlike most viruses, which cannot cross the placental barrier, Zika virus (ZIKV), varicella zoster virus, rubella, and cytomegalovirus are transmitted from the mother to the fetus by infecting various

placental cell types (1–3). Serving as major antecedents of infection-related global morbidity and mortality during pregnancy, these pathogens cause congenital anomalies and placental dysfunction resulting in adverse pregnancy outcomes such as preterm birth or fetal growth restriction and/or miscarriage (1–8). Therefore, pregnant women represent a vulnerable population for viral infections since pregnancy confers a unique immune status that facilitates maternal tolerance of the semi-allogenic fetus and enables viral infections (9, 10). Better understanding of the role and mechanism(s) responsible for viral infections during pregnancy has become increasingly relevant because of the risk of current pandemic.

ZIKV utilizes vertical route to cross the placenta and reach fetal neuronal tissues causing severe fetal defects (11, 12). Vertical transmission was confirmed by detection of ZIKV RNA in the cerebral tissues of the aborted fetuses and placental tissues as well as the increased numbers of neonatal neurodevelopmental defects during the ZIKV outbreak (13, 14). Identification of ZIKV-infected cell types as well as better understanding of the cellular and molecular mechanisms utilized by the ZIKV to cross the placenta are particularly important in preventing viral transmission to the fetus as well as ameliorating fetal prognosis and adverse pregnancy outcomes. Therefore, improved understanding ZIKV pathogenesis during pregnancy may guide the design and/or development of therapeutic agents against ZIKV.

This review focuses comprehensively on underlying molecular mechanisms of vertical ZIKV transmission at the maternal-fetal interface and highlights the trimester-dependent role of decidual stromal cells in promoting ZIKV replication as well as describes the potential use of Food and Drug Administration (FDA) approved drugs against ZIKV infection, thereby preventing perinatal ZIKV transmission.

ARCHITECTURE OF THE MATERNAL-FETAL INTERFACE

The placenta is a unique, multifunctional organ that supplies oxygen and nutrients to the fetus to promote its development and functions as a physical barrier that protects the fetus against infections (9, 10). In humans, following implantation, collaboration between trophoblasts and extraembryonic mesodermal cells permits formation of the placenta's floating and anchoring villi (15–17). Subsequently, the anchoring villi attach to the decidua basalis (17–19). **Figure 1A** represents schematized cells in the human maternal-fetal interface, which is composed of the maternally derived decidua and fetal-derived placenta. Both cytotrophoblasts (CTBs) and syncytiotrophoblasts (STBs) originate from the trophectoderm layer of the blastocyst (19, 20). CTBs are highly proliferative mononuclear cells that are attached to a basal membrane within the placental villi (**Figure 1A**). Villous

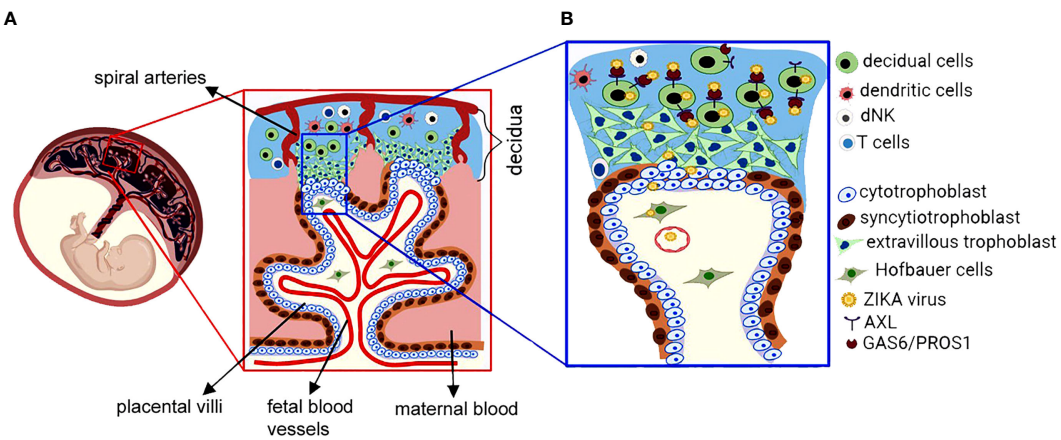

FIGURE 1 | Schematic presentation of the maternal-fetal interface and ZIKA virus infection. **(A)** The fetus-derived placental villi, which are bathed in maternal blood within intervillous space are composed of floating villi and anchoring villi that invade the maternal decidua. The maternal decidua consists of decidual cells that differentiate from uterine endometrial stromal cells, maternal blood vessels and maternal immune cells consisting of decidual natural killer (NK), dendritic cells (DC) and T cells. The placental villi are composed of fetal blood vessels, villous fibroblasts and Hofbauer cells in the villous core as well as proliferative cytotrophoblasts and multinucleated syncytiotrophoblasts, which cover the surface of the placental villous and direct contact to maternal blood. Extravillous trophoblasts are differentiated from cytotrophoblasts and invade the maternal decidua and maternal spiral arteries. Substantial crosstalk exists between maternal decidual cells and fetal trophoblasts that are essential for a successful pregnancy. **(B)** Representative presentation of decidual cell-mediated vertical transmission of the ZIKA virus. The enlarged inset represents the potential route of ZIKA virus (ZIKV) transmission from decidual cells to fetal cells. At the maternal-fetal interface, decidual cells exhibit higher viral entry/attachment factors AXL, GAS6 and PROS1 and they are more permissive to direct ZIKV infection in a gestational age-dependent manner compared to extravillous trophoblast or cytotrophoblast cells. Thus, ZIKV infects and spreads *via* decidual cells to fetal trophoblasts or Hofbauer cells or fetal endothelial cells. Therefore, decidual cells act as reservoirs for trimester-dependent placental transmission of ZIKV, thereby accounting for the higher ZIKV-infection susceptibility and more severe fetal sequelae observed in early *versus* late pregnancy.

CTBs are progenitor cells for STBs, which are multinucleated, terminally differentiated cells formed by fusion of CTBs. STBs cover the outer cell layer of placental floating villi and are in direct contact with the maternal circulation (**Figure 1A**) (3). Thus, STBs serve as the initial site of defense against pathogen(s) attempting to cross the placental barrier. In addition to their defensive role, STBs facilitate maternal-fetal oxygen exchange and nutrient transport and produce several growth factors and hormones that are critical for fetal development (3, 21). In the anchoring villi (**Figure 1A**), proliferation of CTBs forms extravillous trophoblasts (EVTs) of the cytotrophoblastic cell column, which differentiate into interstitial and endovascular CTBs that invade the maternal decidua and spiral arteries, respectively, thereby facilitating the spiral artery remodeling to markedly increase utero-placental blood flow required for fetal survival and growth (21–23).

In humans, the maternally derived decidua originates from the endometrium and promotes immunological tolerance of the semi-allogenic fetus as well as host defense against pathogens (24–28). The decidua is comprised of decidual stromal cells, glandular epithelial cells, maternal blood vessels and an immune cell population dominated by decidual natural killer (NK) cells and macrophages, with smaller percentages of dendritic cells and T lymphocytes (**Figure 1A**) (29, 30). Decidual cells are large, round, polyploid, epithelial-like cells derived from progesterone-induced decidualization of endometrial fibroblast-like stromal cells and are required for the establishment and maintenance of a normal pregnancy. Impaired decidualization is strongly associated with recurrent pregnancy loss or placental accreta or maternal hemorrhage confirming the importance of decidual cells in maintaining pregnancy (31–33). In the decidua, maternal immune cells play a crucial role in defending against infection with their numbers and subtypes changing dynamically during pregnancy. As the dominant lymphocyte population in the decidua, decidual NK cells participate in trophoblast invasion and spiral artery remodeling attaining maximum numbers during the first trimester then declining near term (34). Moreover, decidual NK cells play critical roles in promoting anti-viral innate immunity as well as placental development by producing several soluble factors (34–38). Conversely, lower T cell numbers are present in the first *versus* third trimester (39). In addition to decidual NK cells, decidual $CD8^+$ T cells play a key role in balancing the paradoxical requirement for induction of maternal–fetal tolerance and anti-viral immunity (40).

EPIDEMIC OF ZIKV INFECTION

ZIKV is a member of *Flaviviridae* that includes Dengue, West Nile, Japanese encephalitis, and yellow fever viruses and is primarily spread by the bite of the infected female *Aedes* mosquito (41, 42). The ZIKV was first isolated from a rhesus monkey in the Zika forest of Uganda in 1947 (43, 44). The first ZIKV infection outside of Africa occurred in Indonesia in 1981 (45), and then spread across the South Pacific islands in 2007, reaching Brazil in 2015 (43, 46). In 2016, a major ZIKV outbreak occurred in Central and South America and became a worldwide public health problem (46–48). ZIKV infections in adults are generally asymptomatic, but they can display mild symptoms including fever, joint pain, rash, and conjunctivitis (49, 50). However, during the 2015-16 epidemic in South America, ZIKV became a global health threat because of the dramatic increase in accompanying developmental defects *e.g.*, microcephaly, ocular changes and retinopathy in up to 20% of children of affected mothers as well as a remarkable increase in adults with Guillain-Barré syndrome (46–48). The Centers for Disease Control and Prevention reported 42,750 symptomatic ZIKV cases in the U.S. and territories including 7,407 ZIKV-infected pregnant women delivering 283 live infants with ZIKV-associated birth defects and 17 pregnancy losses; among 1450 babies born to ZIKV infected mothers, 6% had ZIKV-associated birth defects such as small head size, eye damage *etc.*, whereas 9% developed postpartum nervous system problems *e.g.*, seizures, swallowing, movement problems, or developmental delays (51). In one large Brazilian series, Brasil et al. (11) reported that the rate of fetal death in ZIKV-infected pregnancies was 7% and overall adverse outcomes were 46% *vs.* 11.5% among newborns from ZIKV-noninfected women. Additionally, the recent findings from population-based birth defects surveillance data reported a four-fold increase in the prevalence of birth defects related to ZIKV infection during pregnancy (12). These observations suggest delayed postnatal sequalae and the need for long-term monitoring of children that were exposed to ZIKV *in utero* and born with a normal head circumference (52). Although no additional ZIKV infections have recently been reported in the Americas, ZIKV infections and outbreaks still occur in India (53) and China (54), indicating that ZIKV continues to pose a maternal-fetal health challenge.

ZIKV GENOME ORGANIZATION

The ZIKV genome consists of a single-strand positive-sense (ss) RNA with approximately 10.7 kilobases in length that contains a single open reading frame flanked with ~100 nucleotides at 5' and ~400 nucleotides at 3' untranslated region (UTR) (41, 55). As displayed in **Figure 2A**, the open reading frame of the ZIKV genome encodes three structural proteins (the capsid, precursor membrane, and envelope) and seven non-structural (NS) proteins (NS1, 2A, 2B, 3, 4A, 4B and 5). The encoded single polyprotein is subsequently cleaved by both viral and host proteases to yield structural and NS proteins. The structural proteins play a role in viral attachment, entry, assembly, and pathogenicity, whereas the NS proteins are involved in viral replication, polyprotein processing and attenuating host antiviral responses (55–58). The viral promoter is located at 5' UTR with a cap site, and the terminal end of the 3' UTR contains a conserved dinucleotide (CU) instead of polyadenylation (59–61). The mature capsid (C) protein protects the viral genome and is produced by cleavage of the immature C-protein by the viral protease. The membrane (M) protein, which is located on the viral surface, is generated by cleavage of precursor (pr) M protein by a host furin protease (62). The envelope (E) protein is a major

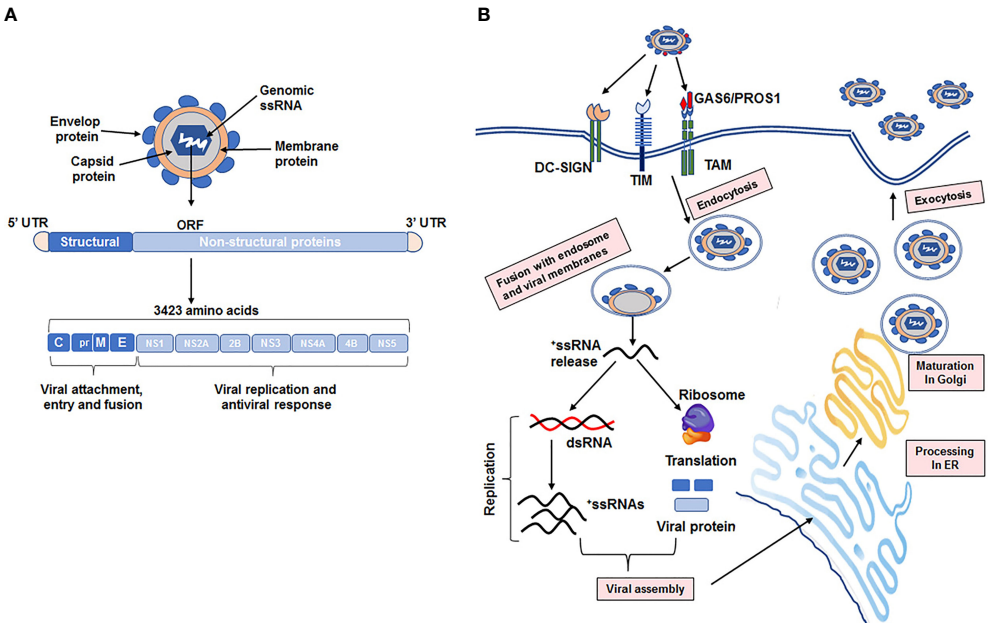

FIGURE 2 | Detailed structure of the ZIKA virus genome, replication, and maturation into host cells. **(A)** The ZIKA virus (ZIKV) genome is single stranded positive sense RNA, which is approximately 10.7 kilobase in length and contains a single open reading frame (ORF) with two flanking untranslated regions on the capped 5′-end and the 3′-end lacking a polyA-tail. ORF encodes a single polyprotein comprised of 3423 amino acids, which is post-transcriptionally cleaved by viral and host cell proteases into 3 structural proteins (the capsid (C), precursor membrane (prM), and envelope E) and 7 non-structural proteins (NS; NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5). Structural proteins mediate viral attachment, entry, and fusion into host cells, whereas non-structural proteins are involved in viral replication and the host antiviral response. **(B)** Schematic presentation of ZIKV entry, replication, and maturation in host cells. The initial step in ZIKV infection is viral attachment *via* the viral envelop (E protein and phosphatidylserine; PS) to receptors (DC-SIGN, TIM, and TAM family members) on the host cell membrane. Subsequently, this binding mediates internalization into host cells *via* clathrin-mediated endocytosis. Then, the viral RNA is released into the host cytoplasm following fusion of viral and host endosomal membranes. The released RNA is immediately bound onto host ribosomes and translated into a polyprotein that is cleaved by viral and host cell proteases to form mature structural and nonstructural proteins. Viral replication is mediated by viral RNA-dependent RNA polymerase and methyltransferase on the surface of the endoplasmic reticulum (ER), resulting in a double stranded (ds) RNA. Transcription and replication of the dsRNA result in the formation of new viral mRNAs and ssRNA genomes, respectively. Following viral assembly of capsid proteins at the ER, the virion is transported to the Golgi apparatus where virion prM protein is cleaved to form the mature M protein. Finally, the host cell releases the mature virus by exocytosis.

viral surface protein that mediates viral entry by binding to host cell surface receptors (63, 64). Among NS proteins, central enzymatic activities are primarily encoded by NS3 and NS5 proteins. NS3 protein displays serine protease, RNA triphosphatase, and helicase activities, whereas NS5 possesses methyltransferase and RNA-dependent RNA polymerase (RdRP) activities (65, 66). The methyltransferase activity is required for methylation of the 5'-end caping of viral genomic RNA. Thus, viral RNA cannot be distinguished from host RNAs and consequently is translated into viral proteins by host ribosomes. RNA-dependent RNA polymerase generates a negative sense RNA using the viral positive sense ssRNA as a template, and thus is essential for viral genome replication and transcription (41, 67).

As depicted in **Figure 2B**, the initial step in ZIKV infection is viral attachment to host cell surface receptors followed by endocytosis, enabling the virus to release ssRNA into the host cell cytoplasm (63, 65, 66). Subsequently, the ssRNA acts as a genome template that is translated into a viral polyprotein, which functions as a viral genome replicator by recruiting ssRNA to viral replication complexes. ZIKV replication occurs on the surface of the endoplasmic reticulum, resulting in a double stranded RNA genome synthesized from the genomic ssRNA by viral RdRP (68–70). The resultant double stranded RNA genome is then either transcribed to viral mRNAs for viral translation or replicated to produce new ssRNAs. Assembly of immature viral proteins and genome occurs in the endoplasmic reticulum, then transported to the Golgi apparatus for viral prM protein cleavage, which then fuse to form competent mature virions. These mature virions are then released by exocytosis (69, 70).

ZIKV ENTRY RECEPTORS

Like other viruses, ZIKV can infect and replicate in various cell types by attaching to several surface proteins that facilitate viral binding and entry into host cells (71). Since ZIKV displays a wide range of cellular tropisms, identification of ZIKV entry factor(s) is/are crucial to delineate details of ZIKV tropisms and pathogenesis. Several cellular receptors that contribute to ZIKV infection have been identified. These include C-type lectins *aka* dendritic cell-specific intercellular adhesion molecule-3-

grabbing nonintegrin (DC-SIGN) and phosphatidylserine (PS) receptors (71), which serve as entry co-factors for ZIKV, include members of the T-cell immunoglobulin (TIM) (72) and the TYRO3, AXL, MERK (TAM) family (73, 74). TIM-family members (TIM1-4) bind viral PS in the viral membrane, whereas TAM members bind PS indirectly, through the soluble intermediates growth arrest-specific 6 (GAS6) and protein S1 (PROS1) that act as a bridge for ZIKV-TAM receptor binding, which induces viral endocytosis (**Figure 2B**) (73, 74). Among these putative ZIKV receptors, AXL is the most studied receptor for ZIKV infection. AXL is a transmembrane receptor that contains an extracellular and intracellular tyrosine kinase domain. The AXL extracellular domain acts a ZIKV entry factor by binding to its ligands GAS6 and PROS1, whereas the tyrosine kinase domain of AXL is involved in mediating an innate immune response as well as other biological processes including cell proliferation, differentiation, and survival (75, 76). Previous studies demonstrated that AXL-overexpressing human fetal neuronal stem and glial cells, astrocytes, endothelial and microglial cells are highly susceptible to ZIKV infection (74, 77), implicating the role of AXL in ZIKV-induced neuropathology. Later studies showed that the reduced AXL levels elicited by small interfering RNA or neutralizing antibodies dramatically diminished ZIKV infection in dermal fibroblast or glial cell cultures, thereby supporting the crucial role of AXL in ZIKV infection (74, 78). In contrast to *in vitro* studies, *in situ* results using *Axl*-deficient mice (79, 80) indicated that Axl may not be required for ZIKV infection in mice, suggesting that Axl expression and/or Axl-mediated signaling pathway(s) in mice are different than in human primary cells.

ZIKV TRANSMISSION

ZIKV is primarily spread to humans by the bite of infected female *Aedes aegypti* and *albopictus* mosquitoes (43, 81, 82), thereby, accepting the skin as the initial host target. Infected mosquitoes transmit ZIKV into the epidermis or directly into the circulation (78, 83, 84). Thus, epidermal cells serve as the initial site of ZIKV infection since epidermal keratinocytes, dermal fibroblasts, and immature dendritic cells are reported to be permissive to ZIKV infection (78).

Sexual transmission from male-to-female or *vice-versa* is also common and is responsible for about 32–54% of ZIKV infections (85, 86). Although the highest transmission occurs from male-to-female, female-to-male and male-to-male transmission are also reported (87–91). Detection of ZIKV RNA in semen (92), vaginal and cervical secretions (93, 94) supports the contribution of sexual ZIKV transmission in the increased numbers of infected people in high-risk areas as well as travelers to areas with risk of ZIKV. In the male reproductive tract, ZIKV infects testicular somatic and germ cells such as Sertoli cells, spermatozoa *etc.* (92, 95). In a mouse model, ZIKV persistence was reported in testicular tissues up to four weeks post-infection resulting in testicular inflammation, atrophy, and infertility (96, 97). Moreover, in humans, ZIKV RNA was detected in semen for 6 months (92) and in semen used for assisted reproductive technology up to 112 days (95), indicating that infected men serve as a potential long-term reservoir for sexual transmission.

Similarly, prolonged ZIKV shedding was initially reported in vaginal secretions up to 14 days after ZIKV infection (94). However, a later investigation by Reyes et al. reported prolonged viral shedding in vaginal secretions up to 6 months (98). This difference in ZIKV persistence in vaginal tissues is likely associated with the menstrual cycle phase during ZIKV infection. In support of this suggestion, vaginal ZIKV inoculation during diestrus in AG129 mice causes longer ZIKV survival (~10 days post-infection) and greater lethality compared with inoculation during estrus (~3 days post-infection) (99), indicating that ZIKV shedding is affected by estrous cycle stages. Additionally, vaginal inoculation of pregnant wild-type mice resulted in fetal brain infection and FGR (100), suggesting that ZIKV infection *via* sexual transmission route can amplify the severity of vertical transmission. ZIKV RNA was also detected in ovaries and uterus in animal models (101, 102), as well as in human oocytes (103). *In vitro* studies indicate that human endometrial stromal cells (HESCs) obtained from cycling endometrium are highly permissive to ZIKV infection (104, 105), and that viral replication is increased during decidualization (105). Taken together, these studies indicate that ZIKV persistence in both male and female reproductive tissues plays an important role in the management of ZIKV infection, since both male and female reproductive tissues serve as sanctuary and reservoir for prolonged ZIKV survival (72, 89). Thus, eradication of ZIKV persistence in the reproductive tissues is expected to reduce the risk of perinatal transmission yielding invaluable public health benefits.

Vertical transmission of ZIKV from the infected mother to the fetus causes severe fetal outcomes *aka* congenital ZIKA syndrome, which includes microcephaly, seizures, hypertonia, and other neurological problems as well as ocular and skeletal anomalies *etc.* (106–108),. During pregnancy, maternal ZIKV infection elicits placental dysfunction resulting in adverse pregnancy outcomes such as FGR, miscarriage and/or stillbirths. Araujo et al. (109) reported that 83% of neonates exposed to prenatal ZIKV infection were small for gestational age in Brazil during the 2015 epidemic, whereas in the United States, the rate of small for gestational age was reported to be 11.2% in women with antenatal ZIKV infection (110). Another study from Brazil reported that the rate of overall adverse outcomes was 46%, which included 7% of fetal death in ZIKV-infected pregnancies (11). Recently, Mercado-Reyes et al. (7) reported the pregnancy outcomes among pregnant women with ZIKV symptoms in Columbia between 2016-2018. Among the 1180 pregnancies, adverse pregnancy outcomes were found to be 22.4%, which included 1.4% pregnancy losses, 9.7% PTBs, 6.9% low birth weights and 4.6% small for gestational age. All these studies indicated that following placental transmission, ZIKV reaches the fetal brain where it causes severe detrimental defects by interrupting proliferation, migration, and differentiation as well as inducing apoptosis of neuronal progenitor cells (111–115). Recent studies demonstrated that ZIKV can infect a broad

range of cells in the human placenta including cytotrophoblasts, endothelial cells, fibroblasts, amniotic epithelial, and/or Hofbauer cells (71, 115–118) as well as maternal decidual cells (104) and cause severe histopathological changes (119, 120). Initially, STBs were assumed to be the site of ZIKV entry. However, STBs are reported to resist ZIKV attachment and replication (121), therefore they are less-permissive to ZIKV infection, suggesting that maternal-fetal ZIKV transmission occurs at other placental site(s). The ZIKV resistance in STBs may be related to low expression of ZIKV attachment molecules such as TAM receptors (104) and/or high expression of interferon (IFN)-induced antiviral genes *RIG-1*, *IFIH1*, *ISG15*, *etc.* (121), as well as high production of type III IFNs, specifically IFNλ1, which protects host cells against ZIKV infection (122). Moreover, recently, Miranda et al. reported (123) the reduced expression of tight junction proteins, particularly claudin-4, and increased paracellular permeability of STBs obtained from ZIKV infected women, displayed an alternative mechanism of ZIKV transmission to other placental cells. On the other hand, compared to STBs, CTBs are ZIKV-permissive, suggesting that ZIKV replication in CTBs mediates its transmission to the fetus. However, ZIKV infection of CTBs in the floating villi is highly unlikely since the outer layer of the villi formed by STBs is in direct contact with maternal blood, thereby preventing ZIKV infection of CTBs in the floating villi. Thus, the anchoring villi are the most likely site of ZIKV infection of CTBs. Guzeloglu-Kayisli et al. (104) provided strong evidence that decidual cell-derived factors facilitate placental transmission by amplifying ZIKV replication in CTBs, the primary cell type in anchoring villi, which attach directly to the decidua. Collectively, these findings indicate that ZIKV persistence in immune privileged sites such as testis, brain and placenta potentiate sexual and vertical ZIKV transmission (77, 87, 89, 124). Of note, transmission of ZIKV *via* blood transfusion has also been documented, especially in Brazil (125, 126).

ZIKV INFECTION OF DECIDUAL CELLS IS THE PRIMARY SITE OF VERTICAL TRANSMISSION

Fetal dissemination of any infectious agent requires transmission through the placenta attached to the immunologically active uterine decidua or *via* hematogenous transmission (1–3). As mentioned above, STBs are less permissive to ZIKV infection. Thus, maternal-fetal ZIKV transmission must occur at other potential placental site(s) at the maternal-fetal interface, suggesting that decidual cells or EVTs or both are the most likely site(s) of ZIKV transmission to the fetus. *Ex vivo* and *in vitro* decidual cell cultures demonstrated that decidual cells act as both a reservoir and source of ZIKV transmission to adjacent anchoring villi at the maternal-fetal interface, thereby representing a primary vertical transmission site (**Figure 1B**) (104, 118, 127). Guzeloglu-Kayisli et al. (104) reported that human decidual cell cultures are more permissive to ZIKV infection, replication, and viral release than human primary cultured CTBs. Similarly, cultured human decidual cells express significantly higher levels of viral entry and bridging molecules *AXL, GAS6* and *PROS1* (**Figure 1B**) than primary cultures of either CTBs or STBs. In addition, compared to cultured CTBs and STBs, decidual cells differentially express a set of genes that are involved in viral replication and/or infection (104). Similarly, Tabata et al. (116) found high AXL expression in decidual cells and EVTs obtained from second trimester placentas, but low levels of AXL expression in villous CTBs obtained from second and term trimester placentas, suggesting that AXL expression varies depending on the gestational age and placental cell types. This study also reported ZIKV infection in decidual cells, CTBs, EVTs, and Hofbauer cells, supporting ZIKV transmission from decidual cells to chronic villi and fetal circulation as the primary vertical transmission route (116). Moreover, Richard et al. (128) demonstrated that fetal endothelial cells are permissive to ZIKV infection and display higher AXL and GAS6 levels, indicating that the ZIKV infects fetal endothelial cells by using these entry molecules to cross the placental barrier. Similarly, Zheng et al. (129) reported higher AXL levels in decidual stromal cells and perivascular cells and lower levels in decidual dendritic cells and macrophages at the maternal-fetal interface using Single-Cell RNA sequencing. Additionally, Guzeloglu-Kayisli et al. (104) found that ZIKV replication and release is amplified in primary CTB cultures treated with ZIKV-infected decidual cell supernatants compared to direct ZIKV infection of these cells, indicating that decidual cells not only serve as a ZIKV reservoir, but also facilitate ZIKV infection of CTBs (**Figure 1B**). Furthermore, Wesblum et al. found that ZIKV induces expression of distinct innate immune response genes, particularly those related to anti-viral interferon signaling in decidua *vs*. chronic villi explant cultures, suggesting that this antiviral response paradoxically promotes a rapid and robust replication of ZIKV in decidual cells (127).

GESTATIONAL AGE DEPENDENCE IN ZIKV INFECTION OF DECIDUAL CELLS

The risk of vertical transmission exists throughout pregnancy, whereas the greatest risk of severe fetal abnormalities is strongly associated with ZIKV infection in the 1st and 2nd trimester (46–48). Consistent with this clinical information, higher ZIKV replication is detected in decidual cells obtained from first trimester placentas compared to decidual cells from term placentas (104). However, the biological mechanism(s) utilized by ZIKV to cross to the placenta and the cause of the inverse relationship between the gestational age of ZIKV infection and its severity remain unclear. As potential mechanism(s) responsible for gestational age dependent ZIKV infection, we reported that: 1) decidual cells isolated from first trimester placentas exhibit higher viral entry/attachment molecules *AXL*, *GAS6*, and *PROS1* than term decidual cells, indicating a higher risk of ZIKV infection in early pregnancy and greater subsequent detrimental ZIKV effects on the fetus (104); 2) decidual cells obtained from term placentas display a strong anti-viral response

to ZIKV infection that correlates with lower ZIKV replication in term *vs.* first trimester decidual cell cultures (104); and 3) mid-gestation decidua expresses higher IFNα and IFNλ levels compared with early decidua, indicating an inverse correlation between IFN levels and ZIKV susceptibility during gestation (127). In addition to these mechanisms, dynamic changes in the immune cell populations at the maternal-fetal interface during pregnancy could alter ZIKV susceptivity (130, 131). For example, decidual NK cell numbers reach a maximum during the first trimester but decline near term. Conversely, lower T cell number are present in the first trimester than at term (39).

POTENTIAL THERAPEUTIC OPTIONS AGAINST ZIKV INFECTION

Unfortunately, no approved effective treatment or vaccine currently exist against ZIKV infection. Since recent ZIKV outbreaks resulted in significant adverse health effects, several investigations focused on development of drugs and vaccines to treat or prevent *in utero* ZIKV transmission. As examples, a plasmid-based DNA vaccine (132, 133) or modified recombinant measles virus vaccine backbone (NCT02996890) or mRNA-based vaccine (134) or inactivated whole ZIKV vaccine (135) candidates have been developed and tested to prevent ZIKV infection. In several vaccine studies, viral prM and E proteins have been intensively targeted since both prM and E in the ZIKV surface are accepted as the primary antigenic target (136). Moreover, many monoclonal antibodies targeting ZIKV proteins have been described in detail (137, 138). However, the main concern related to therapeutic potential for clinical applications is viral escape due to the high mutation rates of ZIKV genome.

To provide a potential treatment against the detrimental effects of ZIKV infection, several different strategies have been employed aimed at blocking viral replication or inhibiting viral protein synthesis. Several FDA-approved drugs have been investigated for their potential rapid response to the ZIKV outbreak since their mechanisms and safety as well as their pharmacokinetic and pharmacodynamic profiles of these drugs are well documented. Predictably, screening these drugs for their effectiveness against ZIKV infection should rapidly advance their approval for clinical use than newly identified drugs. Some FDA-approved candidate drugs against ZIKV infection are given in **Table 1**. As examples, Sofosbuvir, an FDA-approved drug used for the treatment of chronic hepatitis C virus infection, was investigated as a potential inhibitor of ZIKV RNA-dependent RNA polymerase (145). Although Sofosbuvir inhibited ZIKV infection in human hepatocellular carcinoma (Huh-7) and placental choriocarcinoma cells, no inhibitory effect was observed in Vero or A549 cells (139, 140), indicating cell type dependent anti-ZIKV activity. Recently, Mesci et al. (141) reported *in vitro* and *in vivo* protective effects of Sofosbuvir against ZIKV by demonstrating inhibition of ZIKV replication and ZIKV-induced apoptosis in human neuronal progenitor cell cultures as well as reduction of ZIKV titers in the serum of Sofosbuvir administered pregnant SCID immunodeficient mice.

Nitazoxanide was investigated as another potential treatment against ZIKV by inhibiting the viral protease complexes NS2B-NS3 that play essential roles during viral polyprotein processing (146). Nitazoxanide is a potent antiparasitic drug used to treat anaerobic bacterial and protozoal infections and possesses broad spectrum activity against many viruses (147). The FDA approved Nitazoxanide for treatment of diarrhea and enteritis in adults and in children ≥12-months (148–151). Nitazoxanide ingested with food is absorbed from the gastrointestinal tract and hydrolyzed in plasma to form its active metabolite, tizoxanide with serum levels attaining up to 10 μg/mL (152). Clinical trials revealed its efficacy against rotavirus and norovirus gastroenteritis in children and adults (153, 154). Moreover, Nitazoxanide therapy against influenza is currently a phase III clinical trial (NCT02612922). Its broad range of anti-viral activity (147, 154–157) suggests that Nitazoxanide induces a cell-specific effect rather than a viral-specific effect. However, the mechanism(s) mediating antiviral activity of Nitazoxanide and/or tizoxanide is/are not completely elucidated. The anti-viral activities of Nitazoxanide and its bioactive metabolite tizoxanide against ZIKV were first tested in Vero and A549 cells. Both agents significantly inhibited ZIKV infection in these cell types (158). Subsequently, Li et al. (146) demonstrated that Nitazoxanide inhibited ZIKV infection by decreasing viral replication and viral protein expression in human placental epithelial cells, human neuronal progenitor cells and human pluripotent stem cell line. Thereafter, De Souza et al. (142) found that Nitazoxanide reduced ZIKV viral loads up to 2 logs in primary cultured chorionic cells obtained from human term placentas and in a human cervical epithelial cell line. Recently, Guzeloglu-Kayisli et al. (104) evaluated the anti-ZIKV activity of tizoxanide in primary cultures of HESCs obtained from cycling

TABLE 1 | Summary of candidate anti-ZIKV drugs and their mechanisms.

Drugs	Known mechanism	Anti-Zika activity tested cell types	Reference
Sofosbuvir	RNA polymerase inhibitor	. human hepatocellular carcinoma (Huh-7)	(139, 140)
		. human neuronal progenitor cell cultures	(141)
Nitazoxanide	protease complexes inhibitor	. chorionic cells and cervical epithelial cell line	(142)
		. human endometrial cells, decidual cells and cytotrophoblasts	(104)
Atovaquone	RNA synthesis inhibitor	. JEG3, chronic villous	(143)
Efavirenz	Nucleoside inhibitor	. human neuroblastoma cells, astrocytes, Vero	(144)
Tipranavir	Protease inhibitor	. human neuroblastoma cells, astrocytes, Vero	(144)
Dasabuvir	RNA polymerase inhibitor	. human neuroblastoma cells, astrocytes, Vero	(144)

endometrium, decidual cells obtained from first trimester and term placentas, as well as in primary cultures of CTBs from term placentas and found that tizoxanide significantly reduces ZIKV replication in all these cell types. Cao et al. (158) demonstrated that pre-treatment with either Nitazoxanide or tizoxanide failed to inhibit ZIKV replication in Vero cell line, but adding either drug post-infection exerted an anti-ZIKV effect, suggesting that both drugs inhibit infection after viral attachment. Taken together, these results provide solid evidence supporting the potential use of Nitazoxanide or tizoxanide to prevent ZIKV infection and associated fetal abnormalities.

Additionally, Yamamoto et al. (159) screened a library of 1017 FDA-approved drugs targeting ZIKV E protein and identified Atovaquone as an effective drug against ZIKV infection in both mammalian Vero and mosquito-derived C6/36 cells *in vitro*. Atovaquone is a well-known anti-malaria and anti-parasitic drug (143) and is a coenzyme Q analogue that inhibits the mitochondrial cytochrome complex III and pyrimidine synthesis (160). The anti-viral effect of Atovaquone against ZIKV infection was tested in JEG3 trophoblast cells as well as *ex vivo* chorionic villous explants, suggesting that Atovaquone may protect placental transmission of ZIKV and could be a potential candidate against ZIKV during pregnancy (160). Similarly, Stefanik et al. also (144) identified potential anti-ZIKV candidates by screening FDA-approved drugs that interact with ZIKV NS3 and NS5 proteins and found that only three drugs: Efavirenz, an antiretroviral drug against HIV, Tipranavir, a HIV protease inhibitor, and Dasabuvir, a RNA polymerase inhibitor against Hepatitis C virus, inhibited ZIKV titers in Vero cells as well as in primary human brain cortical astrocytes and a neuroblastoma cell line (144).

CONCLUSION REMARKS

This review discusses in detail the putative mechanism(s) responsible for ZIKV infection at the maternal-fetal interface. Specifically, it reveals the role of immunologically active decidual cells, which are highly permissive to ZIKV infection and likely act as both a reservoir and source of ZIKV transmission to adjacent anchoring villi at the maternal-fetal interface. Moreover, the trimester-dependent responses of decidual cells to ZIKV infection could elucidate the clinically important questions such as why pregnant women are highly susceptible to ZIKV infection and why the subsequent effects are more detrimental in the first trimester than in late pregnancy. Finally, this review discusses the anti-ZIKV effects of FDA-approved candidate drugs that were demonstrated to inhibit ZIKV replication and dissemination. Accordingly, these drugs represent potential therapeutic candidate(s) that block perinatal ZIKV transmission, thereby averting its harmful effects on the fetus.

In conclusion, both current and previous pandemics demonstrated that viral infections pose a major risk during pregnancy because of their detrimental effects on the fetus and adverse pregnancy outcomes. Therefore, determination of viral tropisms and host factors at the maternal-fetal interface are crucial to improve understanding the mechanism(s) and/or route (s) employed by emerging viruses. Predictably, prevention of viral infections during pregnancy will be more rapidly accomplished by screening of anti-viral effects of FDA-approved drugs that were previously verified as to their modes of action, safety, and pharmacokinetic and pharmacodynamic profiles.

AUTHOR CONTRIBUTIONS

The authors OG-K, UK, FS, and CL made substantial contributions to this work and approved the final version of the manuscript.

FUNDING

This study was supported by the Florida Department of Health with ZIKA Research Grant Initiative (7ZK23) to OG-K and CJL.

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