

MINIREVIEW

Cellular and molecular mechanisms of viral infection in the human placenta

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One sentence summary: One current overview about viral entry and dissemination mechanism at the maternal–fetal interface.

Editor: Alfredo Garzino-Demo

ABSTRACT

The placenta is a highly specialized organ that is formed during human gestation for conferring protection and generating an optimal microenvironment to maintain the equilibrium between immunological and biochemical factors for fetal development. Diverse pathogens, including viruses, can infect several cellular components of the placenta, such as trophoblasts, syncytiotrophoblasts and other hematopoietic cells. Viral infections during pregnancy have been associated with fetal malformation and pregnancy complications such as preterm labor. In this minireview, we describe the most recent findings regarding virus–host interactions at the placental interface and investigate the mechanisms through which viruses may access trophoblasts and the pathogenic processes involved in viral dissemination at the maternal–fetal interface.

Keywords: viruses; maternal–fetal interface; viral entry; trophoblasts; viral pathogenesis; vertical infection

INTRODUCTION

During pregnancy, congenital viral infections can affect the health status of the fetus through different pathogenic mechanisms that affect its health and result in complications such as fetal abnormalities, post-natal infections and fetal death. It has been suggested that these outcomes are related to alteration in fetal programming, the study of which is an emerging field in perinatal medicine and involves analysis of the developmental origin of adult disease (Elfving et al. 2008; Pham et al. 2013; Piedimonte and Perez 2014; Bebell and Riley 2015; Slatter et al. 2015; Melo et al. 2016).

Several studies have shown that different viral agents can be transmitted from the mother to her offspring (Table 1). This list has been growing over the years because some viruses have not been studied sufficiently in relation to the maternal–fetal interface. This is the case for arboviruses like dengue (DENV), chikungunya and Zika virus (ZIKV) and respiratory viruses like coronavirus and human respiratory syncytial virus, which are related to a range of ailments, for example, the common cold, and even to death (Ng et al. 2006; Piedimonte, Walton and Samsell 2013; Brasil et al. 2016; Torres et al. 2016; Ribeiro et al. 2017). Knowledge regarding the mechanism of action of these pathogens in the different cell types of the placenta could aid in developing antiviral therapies.

Transmission of these pathogens to fetal–placenta tissues may occur through four different routes: (i) through the maternal vascular endothelium to the endovascular extravillous trophoblasts (EVTs); (ii) through infected maternal blood macrophages, which transmit the infection to placental trophoblasts; (iii) through ascendant infection of the urogenital tract, resulting in vertical infection; (iv) through paracellular routes from the maternal blood to fetal capillaries (Delorme-Axford, Sadovsky and Coyne 2014; Coyne 2016). The molecular mechanisms that these pathogens employ to cross the maternal circulation to the fetal compartment is a subject of current research, which provides very interesting perspectives. In this minireview, we discuss different findings that have contributed to our understanding of how viruses can access cellular components of the placenta and the pathogenic mechanisms that facilitate the process of infection at the maternal–fetal interface, particularly in trophoblasts.

STRUCTURE OF THE MATERNAL–FETAL INTERFACE

The maternal–fetal interface is a complex structure that is essential for maintaining equilibrium between control of the maternal immune response and biochemical factors involved in successful development during human gestation. The placenta and fetal membranes represent a selective barrier with two main functions: nurturing and protection of the developing fetus (Brett et al. 2014; Wong and Cox 2016). The human placenta is a chimeric tissue that contains maternal and fetal components, which are organized so as to promote communication between the mother and fetus (Aplin 2000; Coyne 2016). Placental development starts in the endometrium 7 days after fertilization; the placenta is formed by different cell types that possess specific functions, such as the synthesis of molecules essential for fetal development, nutrient transport and other functions (Aplin 2000). Chorionic villi are the main functional component of the placenta and can be classified into floating villi and anchoring villi. The former are in intimate contact with maternal fluids, participating in the transport of nutrients, gases and waste between the fetus and the mother, whereas the latter are associ-

ated with the uterine wall, forming a support structure between the mother and the fetus (Huppertz 2008).

Among the multiple cellular components that constitute the placenta, trophoblasts play a fundamental role in the architecture and functionality of this organ. The floating villi and anchoring villi are composed of trophoblasts (Benirschke and Kaufmann 2012). An individual chorionic villus has a polarized epithelium of cytotrophoblast progenitors (CTBs) anchored to a basal membrane. These cells possess high proliferative and migratory capacity, which allows them to leave the basal membrane and permits their differentiation into floating villi and anchoring villi. In the case of floating villi, the CTBs fuse to form a group of multinucleated cells known as syncytiotrophoblasts (STBs); these cells attach to fetal villous trees in the placenta, and the rest of the villi are in contact with the maternal circulation. The route leading to the assembly of the anchoring villi is formed by aggregates of differentiated CTBs, which organize into non-polarized mononuclear columns known as extravillous trophoblasts. EVT's have the capacity to attach and penetrate the uterine wall for forming a bridge between the mother and fetus. In the region between EVT columns and the endometrium, CTBs acquire an invasive phenotype for invading the maternal decidua and replacing the endothelial cells that constitute the uterine veins and arteries. The invasion of EVT's into the maternal decidua culminates with the formation of hybrid cells that allow fusion of maternal and fetal circulation (Fig. 1). This process defines placenta formation, which plays a very important role during gestation by promoting nurturing, protection and support in the uterine cavity (Cross, Werb and Fisher 1994; Norwitz, Schust and Fisher 2001; Maltepe, Bakardjiev and Fisher 2010).

ENTRY MECHANISMS OF VIRUSES INTO MATERNAL–FETAL INTERFACE TROPHOBLASTS

Viruses are obligate intracellular parasites that recruit and employ different mechanisms to achieve the key events in the infection cycle and generate new infectious particles. During viral infection, one of the main challenges that viruses face is penetrating the target cell (Blaas 2016). For this, the virus employs host cell surface molecules as receptors. This interaction between the virus and host cell induces conformational changes on viral proteins that promote viral entry into the host cell by two main mechanisms: (i) through direct fusion with the cell plasma membrane or (ii) through an internalization process within endosomes and further release into the cytoplasm (Schneider-Schaulies 2000; Yamauchi and Greber 2016).

Several groups have demonstrated the presence of specific receptors for different viruses on the plasma membrane of placental trophoblasts and choriocarcinoma cell lines such as BeWo, JAR and JEGIII (Halwachs-Baumann et al. 2006; Delorme-Axford et al. 2013; Delorme-Axford, Sadovsky and Coyne 2013). These cell line models maintain many of the structural and functional characteristics of trophoblasts and can therefore be widely used for studying viral infections occurring at the maternal–fetal interface (Vargas et al. 2012). Pioneering studies with adenoviruses showed that trophoblasts express anchoring receptors that are necessary for the infection process (Koi et al. 2001). The entry of adenoviruses into the target cell first requires interaction between the viral fiber protein and the coxsackievirus B (CBV) and adenovirus receptor (CAR); this is followed by interaction of the base of the viral protein penton

Table 1. Viral infections *in vivo* and *in vitro* from different placental and embryo tissues.

Family	Type of virus	Tissue or cell culture	Reference
Herpesviridae	Epstein-Barr virus	Extravillous trophoblast, syncytiotrophoblast and corium	Devergne et al. (2001)
	Varicella virus	Fetal membranes and derived mesenchymal stromal-stem cells	Avanzi et al. (2013)
	Human cytomegalovirus	Decidua, villi, amniotic membrane and syncytiotrophoblast and derived mesenchymal stromal-stem cells	Avanzi et al. (2013); Weisblum et al. (2014); Tabata et al. (2016)
	Herpes simplex 1 y 2	Extravillous trophoblast, cytotrophoblast (<i>in vitro</i>) and fetal membranes	Avanzi et al. (2013)
	Herpesvirus 6	Syncytiotrophoblasts, embryo	Csoma et al. (2002); Avanzi et al. (2013)
Filoviridae	Ebola virus	Syncytiotrophoblast and mononuclear cells of placenta	Muehlenbachs et al. (2016)
Retroviridae	Human immunodeficiency virus	Hofbauer cells and amniotic fluid	Johnson and Chakraborty (2016)
	T-cell leukemia-lymphoma virus	Syncytiotrophoblasts (<i>in vitro</i>)	Toth et al. (1995)
Picornaviridae	Coxsackievirus	Trophoblast and amnion	Delorme-Axford et al. (2013)
	Hepatitis A virus	Fetus from infected mother	Renge et al. (2002)
Papillomaviridae	Human papilloma virus	Amnion, placental cells, uterine epithelium, syncytiotrophoblast	Freitas et al. (2013)
Hepadnaviridae	Hepatitis B virus	Umbilical cord and fetus, polymorphonuclear cells, Hofbauer cells and cytotrophoblast (<i>in vitro</i>)	Wang et al. (2008); Bai et al. (2014); Zhou and Wang (2015)
Hepeviridae	Hepatitis E	Placental villous and placental connective tissue and syncytiotrophoblast tissue	Bose et al. (2014)
Parvoviridae	Parvovirus B19	Amniotic fluid, extravillous trophoblast (<i>in vitro</i>), fetal progenitor cells	Parry et al. (1997)
Flaviviridae	Adeno-associated virus	Amnion and embryo	Burguete et al. (1999)
	Hepatitis C virus	Primary trophoblast (<i>in vitro</i>), fetus, placental tissue	Ranger-Rogez et al. (2002)
	Dengue virus	Adventitious tunic, umbilical and placental cord, macrophages, placental villi and endothelium	Dos Santos et al. (2016)
Togaviridae	Zika virus	Hofbauer cells, trophoblasts, cytotrophoblast, decidua, chorio-amniotic membrane, umbilical cord, chorionic villi	El Costa et al. (2016); Tabata et al. (2016)
	Rubella virus	Chorionic villi, placental basal plate and fetus	Terry et al. (1986); Lazar et al. (2015)

with the integrins $\alpha V\beta 3$ and $\alpha V\beta 5$ that are present in both differentiated and undifferentiated trophoblasts (Koi et al. 2001). CAR expression varies with gestational age and the differentiation state of the trophoblast. This receptor is expressed only in villous trophoblasts and at very low levels in STBs in first-trimester placentas. However, CARs are not expressed in TBs or STBs in third-trimester placentas; this receptor's expression remains constant in EVT's (Fig. 2A). These data suggest that the susceptibility to adenovirus infection depends on the stage of trophoblast differentiation (MacCalman et al. 1996).

The entry of human cytomegalovirus (HCMV) into fibroblasts is mediated by epidermal growth factor receptor (EGFR); co-receptors-like integrins $\alpha 5\beta 3$, $\alpha 2\beta 1$ and $\alpha 6\beta 1$ are necessary for internalization of viral particles (Wang et al. 2003; Feire, Koss and Compton 2004). Some of these receptors have been identified in CTBs; viral entry can be inhibited by blocking them with specific antibodies, which may help avoid the entry of HCMV into the placenta (Feire, Koss and Compton 2004). Integrin receptors are differentially expressed in several types of trophoblasts, which suggests that susceptibility to HCMV infection in the placenta is related to change from an epithelial to endothelial phenotype (Fig. 2A). This transition is distinguished by a repertoire of specific integrins that are expressed during the natural development of the human placenta (Maidji et al. 2007).

Several anchoring and high-affinity receptors have been reported for other viral agents that infect the maternal-fetal

interface, such as ZIKV, human papilloma virus (HPV), influenza A virus (H1N1) and herpes simplex virus-1. However, it has not been determined if these receptors are differentially regulated during the process of infection or if this process depends on the differentiation state of trophoblasts (Koi et al. 2002; You et al. 2003; Komine-Aizawa et al. 2012; Tabata et al. 2016).

Viruses take advantage of cellular factors and processes to establish their cycle of infection in trophoblasts, for example, via transcytosis, which is a mechanism for access to trophoblasts that is employed by certain viruses (Lagaye et al. 2001). Transcytosis is a type of transport that several cell types employ to mobilize molecules between two cellular compartments or environments. The movement of molecules may occur from the apical zone to the basolateral zone or vice versa; this will depend on the nature of the transported molecule and its impact on the cellular physiology (Tuma and Hubbard 2003). Although transcytosis has been mainly studied in epithelial cells, this process is not restricted to this cell type; it has also been observed in osteoclasts, neurons and cells that constitute the human placenta (Coxon and Taylor 2008; Herve, Ghinea and Scherrmann 2008; Kzhyshkowska et al. 2008). Both CTBs and STBs employ this mechanism for selective transport of a large amount of nutrients, hormones, growth factors and cytokines, as well as for the transfer of passive immunity to the foetus, since transcytosis is also utilized for the transport of maternal antibodies to the fetal circulation (Ellinger et al. 1999; Fuchs and Ellinger 2004).

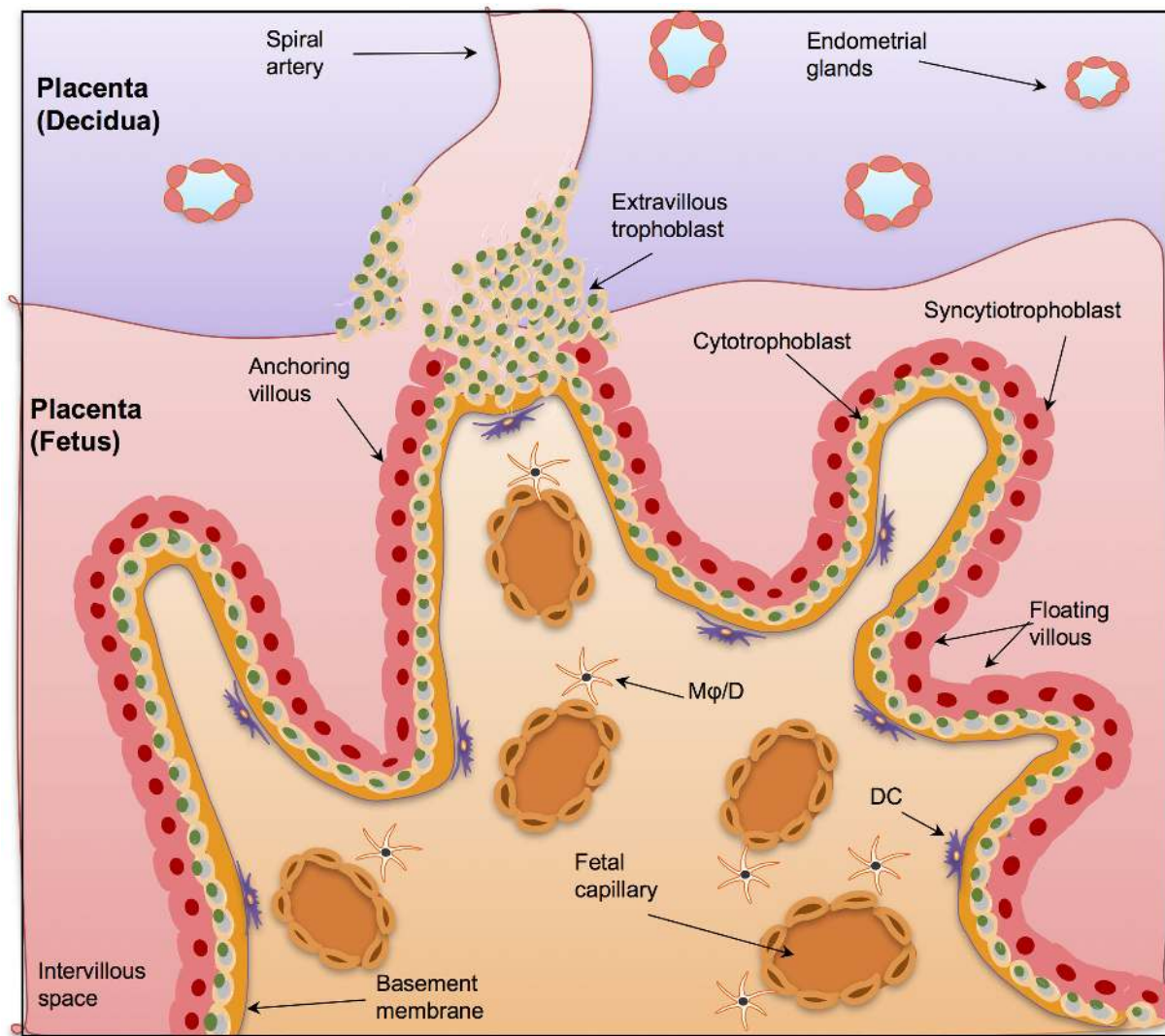


Figure 1. Graphical representation of the maternal–fetal interface. The human placenta is formed by chorionic villi, which can be classified into floating villi and anchoring villi. The first participates in the transport of nutrients, gases and waste between the fetus and the mother, and the anchoring villi is associated to the uterine wall forming a support structure between the mother and the fetus. The chorionic villus has a polarized epithelium of CTB anchored to a basal membrane. The floating villi is formed by CTB that fuse to form a group of multinucleated cells known as STB; whereas, the anchoring villi will be formed by aggregates of CTBs which organize into EVT. The EVT invades the maternal decidua and begins the replacement of the endothelial lineage that coats the uterine veins and arteries, inducing the formation of a hybrid of maternal and fetal circulation. Mφ/D = macrophage/dendritic cells; DC = dendritic cells.

Studies performed with human immunodeficiency virus (HIV-1) were the first to describe the involvement of transcytosis as a mechanism for viral infection of trophoblasts (Parry et al. 2006). It has been proposed that CTBs obtained from first-trimester placentas are susceptible to HIV-1 infection and that STBs from third-trimester placentas develop latent infection (Moussa et al. 1999; Sheikh, Polliotti and Miller 2000). This strongly suggests that the cellular components of the placenta with specific differentiation states work as a barrier against HIV-1 infection. Parry and co-workers demonstrated that CD4, CCR5 and CXCR4 receptors, which are important for the entry mechanisms of HIV-1, are not expressed in villous trophoblasts isolated from second- and third-trimester placentas, as well as from the BeWo choriocarcinoma cell line. This finding has been correlated with the fact that free HIV-1 viral particles cannot infect these cells. Therefore, it has been suggested that the infection process in trophoblasts occurs through primary infection of cells of hematopoietic origin (lymphocytes, macrophages and

dendritic cells), since it was observed that BeWo cells were infected when they were co-cultured with HIV-1-infected PBMCs and that this effect was not observed when the cells were incubated with free viral particles. Furthermore, infection of BeWo cells co-cultured with HIV-1-infected PBMCs was not observed when the cells were treated with transcytosis inhibitors, such as colchicine, supporting the importance of this transport mechanism during infection of trophoblasts by this virus (Parry et al. 2006).

Additional studies have supported the role of transcytosis as an entry mechanism for HIV-1, since the virus employs a series of organized steps to access the trophoblasts by using this endocytic pathway. In particular, HIV-1 internalization in trophoblast cells may follow different routes: viruses can be degraded by lysosomes through the endosomal system; alternatively, viral particles may access the basolateral surface of trophoblast cells through transcytosis or may be transported by the recycling machinery to the apical surface of these cells.

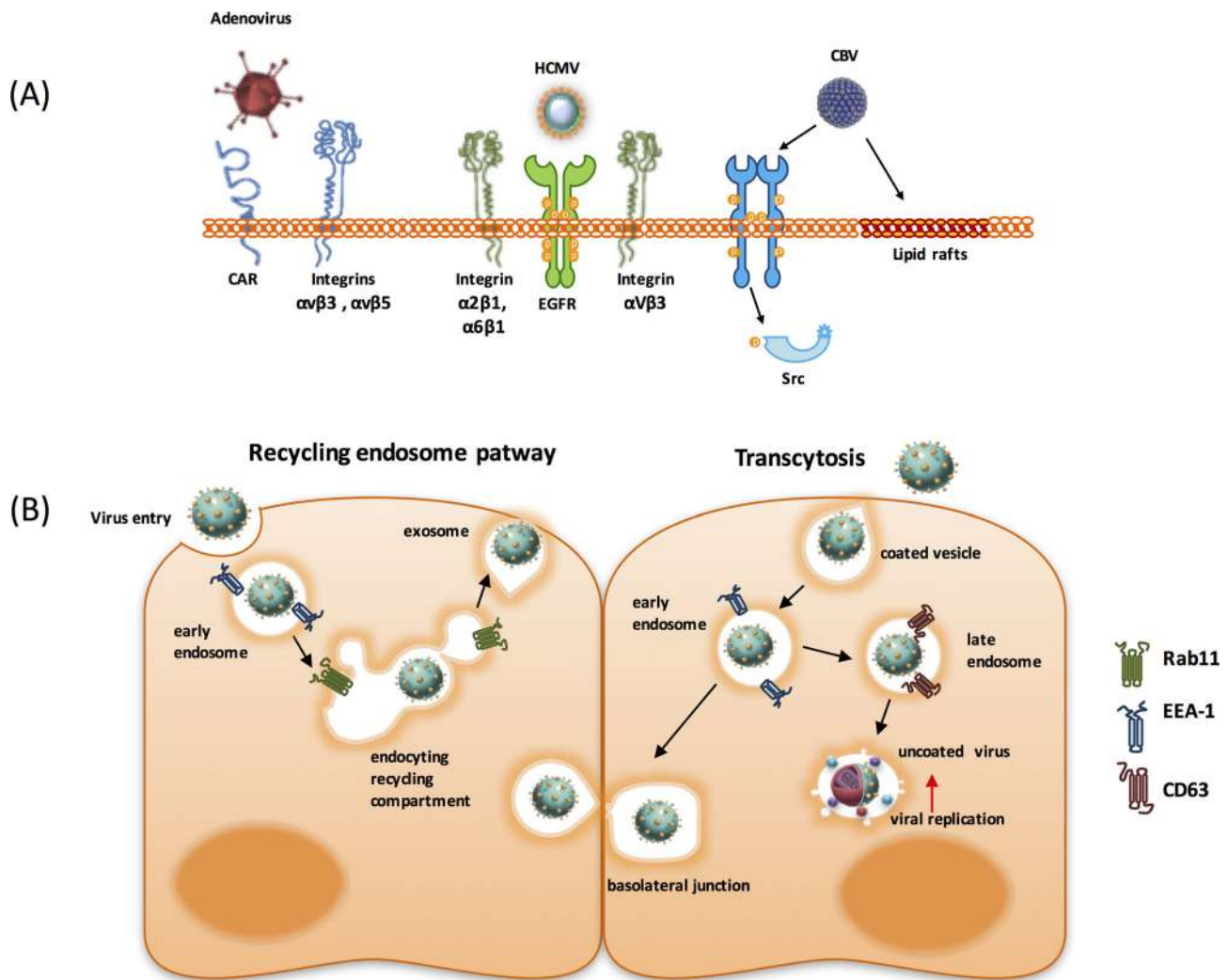


Figure 2. Viral entry mechanisms in the maternal–fetal interface trophoblasts. (A) For viral entry, different cellular receptors are used. Viral fiber and penton protein of adenovirus interacts with CBV and CAR and integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, respectively. HCMV entry is mediated by the epidermal growth factor receptor (EGFR) and co-receptors, like integrins $\alpha v\beta 3$, $\alpha 2\beta 1$ and $\alpha 6\beta 1$. CBV uses lipid rafts during the entry to the trophoblast and Src tyrosin kinase family plays a key role in this process. (B) Transcytosis as a viral access route. Internalization process of HIV particles employs early endosomes (EEA-1 positive) and accumulate in late endosomes (CD63 positive). The destination could be the degradative pathway or the fusion within the late endosome compartment and beginning of viral replication.

Viral particles employ early endosomes that are positive for the protein early endosome antigen-1 (EEA-1) in order to obtain access into trophoblasts, suggesting that this process occurs rapidly or that few viral particles obtain access through this route. Additionally, HIV-1 particles accumulate in late endosomes, which are positive for CD63, and could be degraded through fusion to lysosomes or released into the cytoplasm to initiate the cycle of infection of trophoblasts. When these cells were treated with inhibitors of endosomal acidification, reduction in the expression of viral genes was observed, which suggests that viral particles utilizing this route may access the cytoplasm, promoting viral replication in these cells (Vidricaire, Imbeault and Tremblay 2004).

Viral particles are present in cellular compartments that specialize in cell recycling. In particular, HIV-1 particles have been identified in organelles positive for the Rab11 marker (marker for recycling endosomes), which in turn allows the tracking of HIV-1 from different Rab11-positive organelles to the plasma membrane of trophoblasts. This finding suggests that some viral particles that enter trophoblasts rapidly return to the apical

membrane and possibly return to the maternal circulation in a recycling process (Fig. 2B). The observations obtained in this research determined key components of the access mechanism used by HIV-1 to enter trophoblasts, which is specific to these cells as this process occurs in a manner different from that in other cell lineages permissive to this virus (Vidricaire, Imbeault and Tremblay 2004; Vidricaire and Tremblay 2005).

The use of transcytosis as an entry mechanism for access to trophoblasts has also been identified in other viruses, like the hepatitis B virus (HBV). HBV accesses trophoblasts through the apical surface by a process that requires the cytoskeletal machinery and culminates in the release of viral particles to the basolateral surface of these cells. The transport of HBV particles depends on the differentiation states of the trophoblast, which suggests that HBV-specific receptors are present on the membrane of the trophoblast only during the early stages of the pregnancy, similar to that observed in HCMV (Bhat and Anderson 2007).

While transcytosis plays a key role in the mechanisms of entry into trophoblasts during viral infections, it has recently

been found that membrane lipids are also involved in this process. Many viruses use these biomolecules as platforms for the recruitment of receptors, formation of replication complexes, assembly of viral factories or as sites to promote viral egress (Garcia-Cordero et al. 2014; Martin-Acebes, Vazquez-Calvo and Saiz 2016; Snyder and Danthi 2016). Microdomains known as lipid rafts are recruited to certain zones of the infected cell, forming complex systems that actively participate in the different stages of the viral life cycle.

CBV uses different mechanisms to infect polarized cells, wherein components of lipid rafts play a relevant role (Bozym et al. 2010). Delorme-Axford and collaborators determined that CBV uses lipid rafts during entry into trophoblasts; the study demonstrated the presence of receptors for CBV in the apical and basolateral zones in the BeWo cell line. Additionally, it was observed that this virus uses a pathway that involves lipid rafts independent of clathrin, caveolin-1 and dynamin II. This finding breaks the dogma of the entry process in cells permissive to CBV as these receptors are essential for CBV infection. The access of CBV to trophoblasts is inhibited by the use of drugs that disassemble lipid rafts; however, this does not happen when clathrin, caveolin and dynamin expression is inhibited, suggesting that both mechanisms contribute to CBV entry (Fig. 2A). The Src tyrosine kinase family also plays a key role in the entry of CBV into trophoblasts, as viral entry decreases when Src is pharmacologically inhibited (Delorme-Axford et al. 2013). Future studies should focus on the interaction of cell factors that allow viral entry through the maternal-fetal interface.

PATHOGENIC MECHANISMS IN TROPHOBLASTS DURING VIRAL INFECTIONS

For successful infection, viruses have developed several strategies to promote an ideal microenvironment in order to maintain viral persistence and optimize their infection cycle (Amara and Mercer 2015; Harak and Lohmann 2015; Vijaykrishna, Mukerji and Smith 2015). Several studies have reported the mechanisms via which viruses infecting the maternal-fetal interface can promote a series of pathogenic processes; these processes greatly depend on the expression of specific viral genes whose products are key players in the replication and efficient dissemination of the viruses (Singh et al. 2012; Aldo et al. 2016). For the purpose of this minireview, these pathogenic mechanisms will be classified with respect to the following: (i) modulation of cell apoptosis; (ii) impact of the immune response; and (iii) mechanisms underlying invasion and vascular damage during viral infection.

MODULATION OF CELL APOPTOSIS DURING VIRAL INFECTION OF PLACENTAL CELLS

Programmed cell death or apoptosis is a regulatory process that aids in fine-tuning for elimination of damaged or infected cells. This process occurs in trophoblasts of normal placentas throughout pregnancy; apoptosis occurs more frequently at the end of pregnancy than in the first trimester. Additionally, an increased incidence of apoptosis has been noted in pregnancies complicated by preeclampsia or infections. The viruses manipulate the host's mechanisms of cell death, which define the correct time for activating or stopping the cellular apoptotic machinery, thereby resulting in increase in viral replication or dissemination of viruses to new permissive cells (Danthi 2016).

Two pathways have been described for apoptosis induction in mammalian cells: the extrinsic and intrinsic pathways. The extrinsic pathway involves the activation of plasma membrane death receptors by extracellular signals (death ligands) that in turn induce the assembly of the death-inducing complex, eventually leading to caspase 8 and 10 activation. In contrast, the intrinsic pathway is activated through intracellular signals that act directly on the mitochondria, promoting a shift in its membrane permeability and the release of mitochondrial mediators that induce the assembly of a caspase-activating complex known as the apoptosome. The death-inducing complex and apoptosome induce the activation of effector caspases 3 and 7, which cleave substrates critical for promoting death (Galluzzi et al. 2008).

Many of the viral agents that infect the maternal-fetal interface can either promote or inhibit the apoptotic mechanisms of their host cells. Infection with human herpes virus 8 promotes an apoptotic response in a placental histoculture system (Di Stefano et al. 2008). The activation of apoptosis in HHV-8-infected placental tissue may result in obstetric complications that are associated with fetal growth alterations, preterm birth, congenital abnormalities and abortion (Fig. 3); however, the mechanism through which HHV-8 promotes placental apoptosis is not clear (Gaye-Diallo et al. 2001; Sarmati et al. 2003).

Apoptosis induction is one of the pathogenic mechanisms involved in HPV-16 infection (Bermudez-Morales et al. 2009). HPV infection in the placenta could occur early during pregnancy. In some studies, placental tissues from gestational weeks 11 and 13 were recovered transabdominally to avoid contamination with HPV-infected cervical cells; however, viral DNA was still detected in these placenta samples (Weyn et al. 2011). The presence of HPV in the cervix and placenta has been found to be associated with spontaneous abortion and spontaneous delivery (Ambühl et al. 2016). This may be explained by the fact that HPV-16 induces apoptosis in a first-trimester trophoblast cell line (HTR-8/SV); Gomez et al. (2008) showed that trophoblasts transfected with a plasmid containing the HPV-16 genome had a higher apoptotic rate than those transfected with an empty plasmid. Furthermore, HPV-16 E6 and E7 oncoproteins promote cell death on transfection in trophoblasts, an effect that is more evident in the case of E7 (You et al. 2002). The pro-apoptotic role of E7 has been linked to its interaction with retinoblastoma protein (Fig. 3), which promotes the dissociation of retinoblastoma protein from the transcription factor E2F complex, in turn inducing the degradation of the protein through the proteasomal pathway and the activation of E2F to activate apoptosis (Barbosa et al. 1990; Boyer, Wazer and Band 1996). In another study, the role of the E5, E6 and E7 oncoproteins was evaluated in BeWo cells transfected with these sequences (Boulenouar et al. 2010). The oncoproteins had negative effects on growth and cellular adhesion and increased the migratory and invasive properties of trophoblasts. This phenomenon will be discussed in more detail in later sections. This study showed that the E5 oncoprotein had an important cytotoxic effect on the transfected BeWo cells because of its effects on cellular adhesion. Additionally, the toxic effect of E5 is promoted by its activity as a viroporin, a hydrophobic protein that oligomerizes in the host cell membrane to form a hydrophilic pore that can alter cell permeability (Nieto-Torres et al. 2015). Keratinocytes transfected with the E5 sequence promote osmotic stress (Fig. 3), resulting in apoptosis (Wetherill et al. 2012). Further studies involving primary culture trophoblasts or choriocarcinoma cell lines are required to elucidate the molecular mechanism underlying apoptosis induction by HPV-16 oncoproteins that could be associated with the induction of spontaneous abortions.

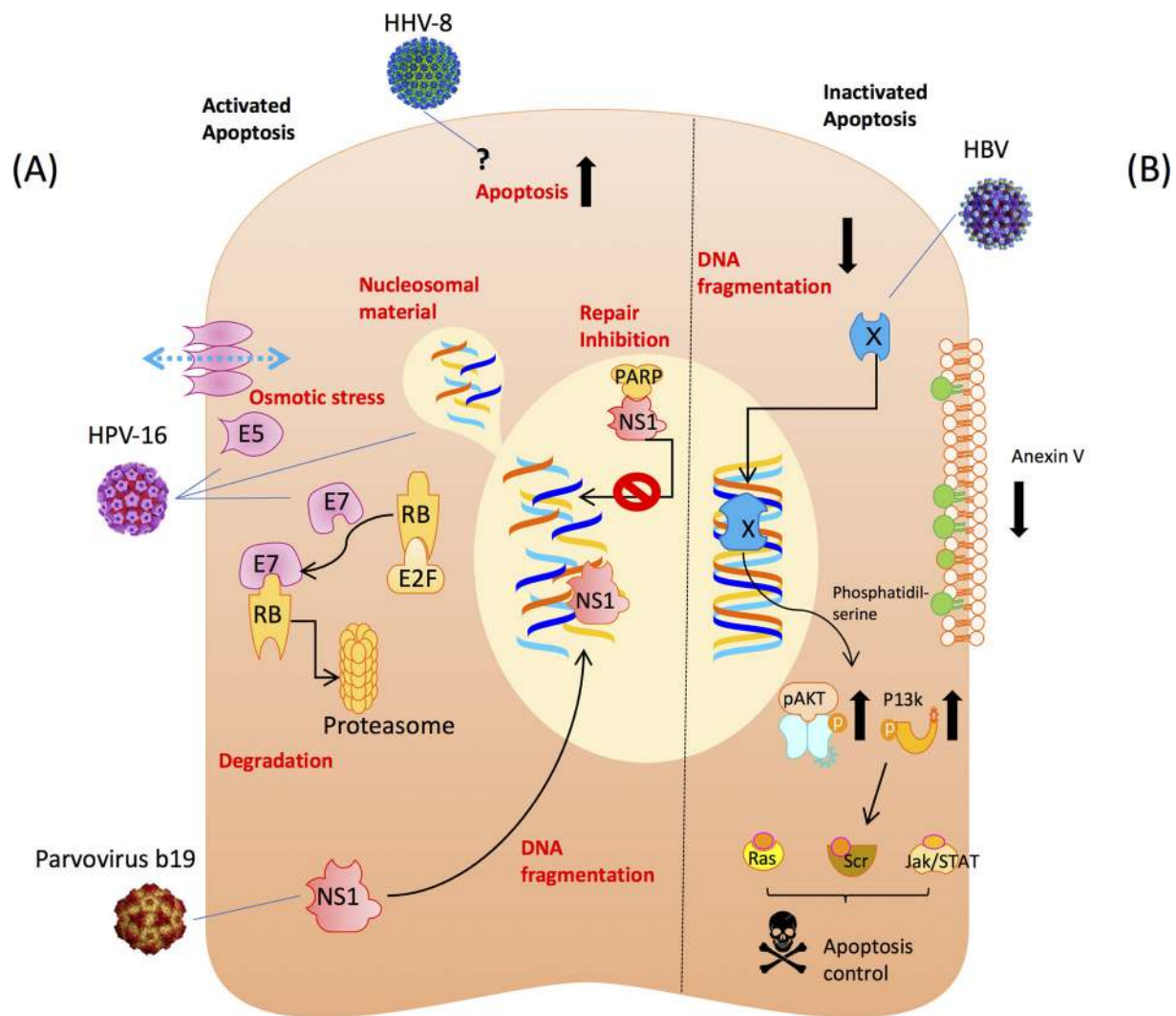


Figure 3. Regulation of apoptosis in trophoblast during viral infection. (A) Induction of apoptosis in trophoblast by HHV-8, HPV-16 and parvovirus B19. The HHV-8 induces DNA fragmentation and releases nucleosomal material in cytoplasm; the HPV-16 through oncoprotein E7 hijacks the RB protein and promotes its degradation by proteasome. E5 oncoprotein of HPV, has function like viroporin these protein generates pores in membranes to result in stress osmotic and promoting the apoptosis process. NS1 protein of parvovirus activates the apoptosis by nuclear translocation of this protein and its association with DNA cellular and PARP protein. (B) Inhibition of apoptosis during trophoblast infection with HBV. This virus regulates apoptosis through protein X; this protein translocates to nucleus the infected cell and promotes the overexpression of molecules such as AKT and PI3K; this protein activates others proteins like cyclin D1 p-Smad-2 and 3, which are ways that could control the cell death in trophoblast infected with HBV.

Apoptosis induction in placental tissue infected with human parvovirus B19 occurs through caspase 3 activation (Jordan and Butchko 2002). It has been proposed that the NS1 viral protein, which possesses NTPase, helicase and transcriptional factor activities, can bind to host DNA to promote genome damage (Momoeda et al. 1994). NS1 can be associated with DNA in a covalent manner, promoting distortion of the double helix and generating cleavages in a single strand, which are signals for initiating the apoptotic process. NS1 can inhibit the DNA repair pathways by associating with poly (ADP-ribose) polymerase protein (Fig. 3), which, together with DNA damage, promotes apoptosis (Poole et al. 2011). To date, it is not known whether trophoblasts or any other components of the placenta express NS1 protein during parvovirus B19 infection and if this protein is the main inducer of apoptosis during placental infection.

It is clear that many viruses induce apoptosis in the host cell to stimulate viral dissemination in the late stages of infection; however, many of these pathogens develop strategies to inhibit

cell death in the early stages of the infection cycle, thus allowing for increased viral replication (Richard and Tulasne 2012). There have been studies regarding this phenomenon in viruses that infect the maternal-fetal interface. In the trophoblast cell line JEG-3, HBV infection was found to induce early and late apoptosis in low percentages of cells (Fig. 3), when analyzed with specific apoptotic markers, that is annexin V and DNA fragmentation, respectively (Bai et al. 2013). Additional studies have demonstrated that HBV X protein (HBx) plays an important role during infection by modulating cellular pathways related to proliferation or apoptosis (Wang, Studach and Andrisani 2011). The HBx antigen has been detected in the cytoplasm and nucleus of HBV-infected trophoblast cells (Fig. 3), and its presence in the cytoplasm is correlated with high phosphatidylinositol-3-kinase (PI3K) expression, which might be involved in the process of resistance to apoptosis in placental tissue (Bai et al. 2012; Wang et al. 2016). In addition, HBx protein could be involved in apoptosis inhibition through the Smad signaling pathway; when

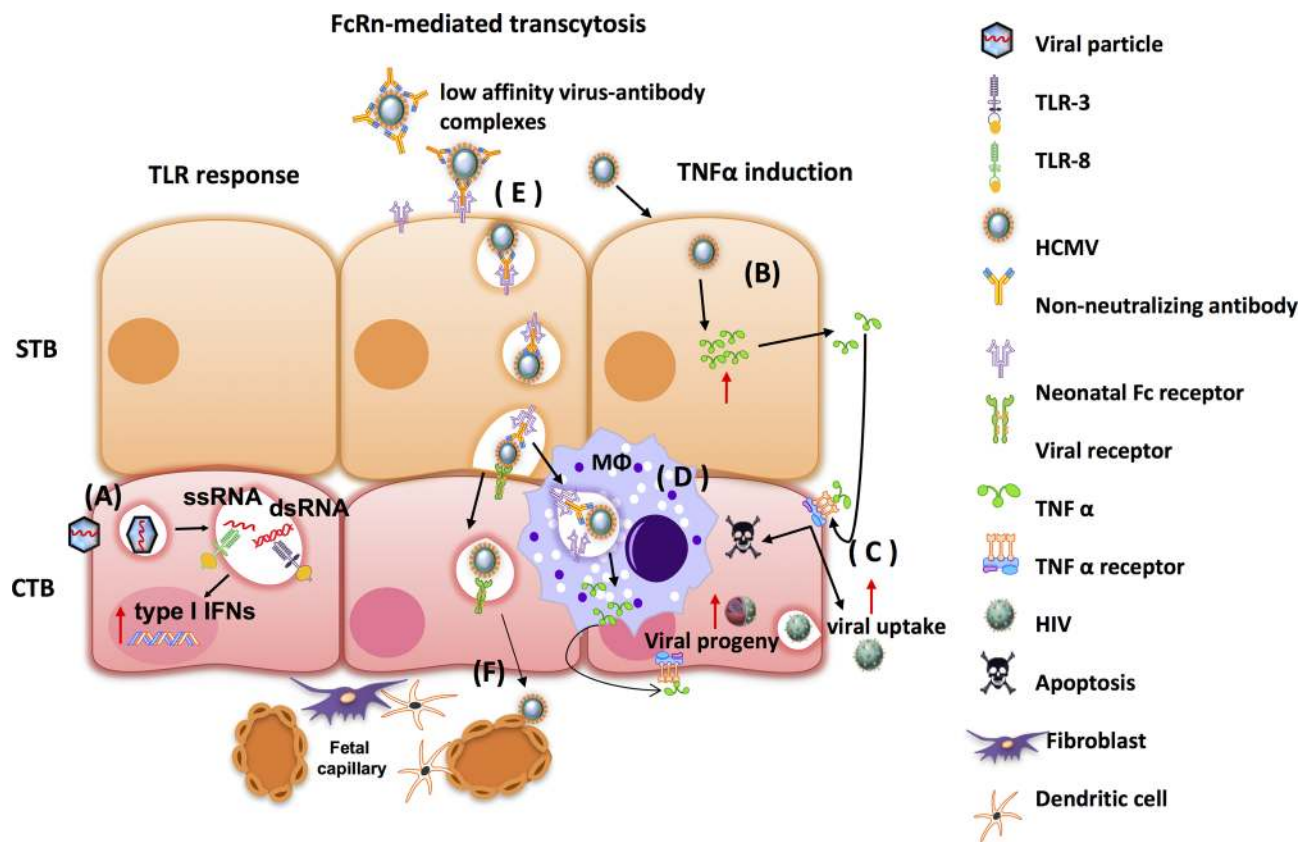


Figure 4. Trophoblast immune response in a viral infection. (A) Single-strand RNA (ssRNA) or double-strand RNA (dsRNA), through TLR-8 and TLR-3, respectively, activated type I interferon pro-inflammatory response. (B) CTBs and STBs infected with HCMV can induce the expression and secretion of TNF- α . (C) Paracrine effect of TNF- α promotes the apoptotic mechanisms or a suitable environment for vertical infection. (D) Interaction between the macrophage (M ϕ) and the infected STB stimulates TNF- α secretion, which in turn acts on the infected STB activating viral replication. (E) Low-affinity virus-antibody complexes experience a process of transcytosis in the STB and can be transported to the basal membrane and establish contact with CTBs or captured by M ϕ placental. (F) Focal infection can extend to stromal fibroblasts, capillaries and fetal leukocytes.

HTR-8 cells were transfected with HBx protein, p-Smad2 and p-Smad3 (Fig. 3), which are important regulators of proliferation and apoptosis, were upregulated (Cui et al. 2015). The HBx protein is a promoter of proliferation in other cellular systems, for example, when oval cells were transfected with HBx plasmid, cyclin D1 overexpression was noted. The use of a specific inhibitor of phosphatidylinositol-3-kinase and MEK was found to inhibit AKT and ERK activation and decrease cyclin D1 proliferation and expression in HBx-transfected oval cells (Fig. 3), suggesting the participation of this mechanism in the effect of HBx on proliferation (Wang et al. 2014). More studies involving trophoblast systems are required to identify the mechanism underlying the effect of HBx protein during infection in the placenta.

IMPACT OF THE IMMUNE RESPONSE ON VIRAL INFECTION IN TROPHOBLASTS

The placenta has been considered a barrier to prevent the crossing of infectious agents from the maternal to the fetal circulation. In recent years, trophoblasts have been identified as important players in immune response regulation in the case of severe infection at the maternal-fetal interface (Koga et al. 2014; Giugliano et al. 2015). Viruses have developed evolutionary mechanisms to allow their escape from detection by the immune system or to control the immune response, which in turn leads to an altered immune response associated with

pathogenic processes in the viral life cycle (Wujcicka, Wilczynski and Nowakowska 2014).

A type I interferon-related pro-inflammatory response has been reported to be activated in *in vitro* models of first-trimester trophoblasts transfected with single- or double-stranded RNA (Aldo et al. 2010; Potter et al. 2015). This response is activated by the participation of receptors of the innate immune system (Fig. 4), such as Toll-like receptor-8 and Toll-like receptor-3, which are widely expressed in different components of the maternal-fetal interface (Kumar et al. 2016). Besides this pro-inflammatory response, synthetic RNAs promote a caspase-dependent apoptotic process, wherein pro-apoptotic proteins such as Bid and Bax are activated; additionally, XIAPs proteins, which are canonical apoptosis inhibitors, are inactivated. All this strongly suggests that trophoblasts that have been stimulated by viral genetic material promote paracrine and autocrine mediators, which in turn are responsible for apoptosis induction (Aldo et al. 2010). These studies provide the first evidence regarding the mechanism through which viral infections can compromise the integrity and function of trophoblasts, thus allowing the dissemination of the virus to other cells at the maternal-fetal interface or in the fetal circulation.

Several studies using viral models such as HCMV, herpes virus, HIV-1, influenza and, most recently, ZIKV, have demonstrated that the immune response at the maternal-fetal interface is directed towards a pro-inflammatory state, which in turn disturbs the structural and functional conditions of the human

placenta (Muller et al. 2010; Weisblum et al. 2015, 2017; Johnson and Chakraborty 2016). CTBs and STBs infected with HCMV express and secrete tumor necrosis factor (TNF)- α , a cytokine that promotes activation of the apoptotic mechanisms in uninfected cells in a paracrine manner. Consistent with these results, the apoptotic effect was found to be inhibited through treatment with a neutralizing antibody against TNF- α . The early genes IE1-72 and IE2-86 of HCMV induced the same phenomenon of promoting TNF- α expression and secretion when transfected into trophoblasts. Transfected trophoblasts with HCMV genes can induce paracrine apoptosis in untransfected cells through TNF- α secretion (Chan et al. 2002). These data suggest that HCMV infection stimulates the immune response in cells from the maternal-fetal interface via its viral proteins, compromising the viability of this structure and favoring the process of infection.

Another component of the immune response that is involved in viral infection at the maternal-fetal interface is the secretion of certain cytokines that act as soluble mediators controlling diverse cellular functions during gestation (Kato, Yamamoto and Chishima 2017). Studies performed using JAR or JEGIII cells during HBV infection have demonstrated that these trophoblast cell lines are more susceptible to HBV when incubated with TNF- α , as evidenced by increased viral uptake and increase in viral antigens, than on infection alone (Li et al. 2007). This would suggest that TNF- α favors a suitable environment for vertical infection. This phenomenon has also been identified in trophoblasts isolated from the placentas of women infected with HIV-1, wherein increased TNF- α expression was correlated with increase in the transcription of the viral *gag* gene (Lee et al. 1997). TNF- α can participate in the reactivation of latent HIV-1 infection in STBs. Interaction between macrophages and infected STBs seems to stimulate TNF- α secretion, which in turn acts on infected STBs, activating viral replication and production (Bacsi et al. 2001). The mechanism through which TNF- α promotes increase in the production of viral progeny has not been entirely elucidated (Fig. 4); however, it has been suggested that TNF- α can induce the transcription of viral genes through activation of nuclear factor κ B. The fact that nuclear factor κ B can associate directly with the long terminal repeat region of the viral genome might explain the effect of TNF- α observed during placental infection with HIV-1 (Duh et al. 1989). Future studies should investigate whether nuclear factor κ B has the same effect in trophoblasts infected with HIV-1.

Humoral immunity during pregnancy plays an important role as a protective mechanism against different pathogens, as passive transference occurs from the maternal to fetal circulation. The placenta has been reported to participate in this process of passive immunity transference through STBs that form the first layer that comes into contact with maternal blood (Faucette et al. 2015). The transport of these antibodies through the STB system is mediated by a well-defined process. STBs express Fc receptors on their membranes, which interact with the Fc fraction of the antibodies found in maternal circulation; transcytosis through STBs allows translocation of the antibodies towards fetal circulation (Fuchs and Ellinger 2004).

As mentioned earlier, transcytosis also plays an important role in the pathogenesis of viral infections. Antibody-dependent enhancement (ADE) is a process through which viral particles form complexes with antibodies with low or null neutralizing activity; these complexes may be captured by cells expressing Fc receptors on the outer surface of their membranes. These complexes are later endocytosed into the cytoplasm where the null activity of the antibodies allows the escape of the viral parti-

cles from the endocytic pathway and their release into the cytoplasm, resulting in an infective cycle (Taylor et al. 2015).

ADE can also occur during pregnancy (Parruti et al. 2013). The antibodies generated during primary infection with HCMV were found to possess protective activity against secondary infection; additionally, during the pregnancy of women who were pre-exposed to the virus, the antibodies could neutralize the infection and therefore, fetal damage was not evident (Parruti et al. 2013). Maternal antibodies specific for HCMV and with high neutralizing activity can form complexes with viral particles, which in turn are transported through different cellular components of the maternal-fetal interface, wherein no active infection processes are observed. However, a study involving women with low titres of anti-HCMV antibodies showed the presence of viral antigens in decidual cells, trophoblasts and the endothelium. This demonstrates the protective role of antibodies during vertical infection with HCMV (Pereira et al. 2003). Low-affinity virus-antibody complexes undergo transcytosis in the STBs of the floating villi during HCMV infection. In the STBs, these complexes can be transported to the basal membrane and later come into contact with CTBs that express receptors for viral entry (Fig. 4). These complexes can also be endocytosed by placental macrophages that also express the FcR, promoting focal infection that can extend to stromal fibroblasts, capillaries and fetal leukocytes. Both events result in vertical infection (Maidji et al. 2006).

A health problem that has currently gained importance is the increase in the incidence of ZIKV infection and the resulting perinatal complications. Like DENV, this pathogen belongs to the *Flavivirus* genus. The high homology between the structural proteins of these two viruses, specifically the envelope protein, could be associated with ADE. Therefore, during infection with DENV, the immune response of the host can induce cross-reactive antibodies against other members of the genus, like ZIKV. Studies focusing on the serological cross-reactivity between these two pathogens have shown that DENV-reactive sera may promote ADE for ZIKV. This finding was first obtained in the human myeloid cell line U937, which is resistant to infection with DENV in the absence of ADE. In particular, U937 became permissive to ZIKV, when the virus was pre-incubated with a pool of convalescent sera for DENV. These results indicate the possibility that pre-existent immunity to DENV results in increased ZIKV replication, which could be related to the epidemics and pathological effects currently noted in the Americas (Dejnirattisai et al. 2016; Priyamvada et al. 2016). Future studies should focus on the evaluation of ADE during transplacental infection with ZIKV.

MECHANISMS UNDERLYING TISSUE DAMAGE AND ALTERED TROPHOBLAST INVASION DURING VIRAL INFECTION

The infiltration process of EVT's allows for invasion of the uterine wall and the remodeling of the spiral arteries within the endometrium and myometrium, producing a hybrid maternal-fetal vasculature necessary for the maintenance of pregnancy. Improper invasion of trophoblasts is associated with alterations in the blood flow and oxygen and nutrient supply to the developing fetus, resulting in dysfunction of the placenta and complications during pregnancy (Warner et al. 2012).

Viruses may affect the ability of trophoblasts to migrate and invade uterine tissue. HCMV is one of the most studied pathogens *in utero* in this field of study. HCMV infection inhibits

the proliferation and invasion of an EVT cell line (SGHPL-4), probably because of reduction in the secretion of matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, two key enzymes in the migration and invasion of EVTs (LaMarca et al. 2006). Importantly, HCMV infection has also been studied in villos explants and EVT primary cultures obtained from women who tested negative for HCMV IgM in peripheral blood and who decided to interrupt their pregnancy during the first trimester (5 to 10 weeks) (Liu et al. 2011a,b). In these models too, HCMV infection inhibited the invasive capacity of EVTs, which was associated with reduction of MMP-2, MMP-9 and c-erbB-2 protein levels and loss of MMP-2 and MMP-9 activity (Liu et al. 2011a,b, 2015). Liu et al. (2015) proposed that this effect could be due to an HCMV-mediated increase in the levels of transforming growth factor- β 1, a molecule that can inhibit the expression of both MMPs. Furthermore, they found that HCMV infection downregulates the expression of Smad-7, the main negative regulator of transforming growth factor- β 1, which could enhance the inhibitory effect on MMPs (Liu et al. 2015). Other proposed mechanisms to explain the ability of HCMV to modulate the migration and invasion of trophoblasts contemplate its ability to alter the expression or functionality of receptors and signaling molecules that are important in the regulation of these processes, such as cytokines and their receptors. In particular, HCMV infection causes decrease in the protein levels of c-erbB-2, a common receptor of EVT related to invasion, and integrin α 1 β 1, which participates in the invasion process (Fisher et al. 2000; Liu et al. 2011a,b, 2015). Furthermore, HCMV infection has been found to induce the transcription of peroxisome proliferator-activated receptor gamma in primary EVTs and in a cell line derived from EVT (HIPEC), favoring the expression of key genes for viral replication (IE2) and significantly affecting the invasive capacity of these cells (Rauwel et al. 2010).

In the case of cytokines, HCMV infection induces reduction in the secretion of CXCL12 and increases the expression and membrane recruitment of CXCR4 and CXCR7 receptors, which are important for migration and invasion capacity, in the SGHPL-4 EVT model. Functionally, compared to uninfected cells, this EVT model infected with HCMV exhibits lesser capacity for migration and invasion in response to a CXCL-12 gradient (Warner et al. 2012). In addition, HCMV infection induces the production of other cytokines such as interleukin-10 in CTBs isolated from first-trimester placentas. In contrast, HCMV infection induces the production of other cytokines such as interleukin-10 (IL-10) in CTBs isolated from first-trimester placentas. CMV infection reduces the activity of MMP-2 and MMP-9 in fibroblasts and vascular endothelium cell lines. Particularly, IL-10 human and its viral analogue cmvIL-10 reduced the migration of endothelial cells in 'wound healing' assays and also decreased the invasive capacity of CTBs in matrigel assays (Yamamoto-Tabata et al. 2004). All these phenomena induced by HCMV infection could affect the ability of trophoblasts to remodel the uterine vasculature. Furthermore, *in vitro*, uterine microvascular endothelial cells infected with HCMV transmit the infection to invasive CTBs, suggesting a role for the uterine vasculature in virus transmission (Maidji et al. 2002).

Besides these *in vitro* models, an *in vivo* study involving human placental explants surgically transferred to the renal parenchyma of severe combined immunodeficient mice showed that HCMV infection reduces the invasive capacity of CTBs, deregulates lymphangiogenesis and leads to deficient remodeling of the renal vasculature. CTBs infected with HCMV express vascular endothelial growth factor-A, vascular endothelial growth factor-C and increase production of vascular

endothelial growth factor-C and basic-fibroblast growth factors, which stimulate the proliferation of lymphatic endothelial cells *in vitro*. The production of these lymphangiogenic factors induced by HCMV could explain the abnormal lymphatic endothelial cell proliferation and the deregulation of the lymphangiogenesis (Tabata et al. 2012).

A similar effect on trophoblast invasion has been found in response to adenovirus infection; however, the associated mechanisms differ. EVTs that were isolated from third-trimester placenta, infected with adenovirus, and co-cultured with lymphocytes from the decidua showed increase in the number of apoptotic nuclei. On the basis of these data, it has been proposed that adenovirus infection and/or the associated immune response could induce apoptosis of EVT cells. This would have a negative impact on the process of placental invasion (Koi et al. 2001).

Besides these direct effects on EVTs, adenoviruses could play an important role during co-infections of placental cells by facilitating the replication of other pathogens like adeno-associated virus 2 (AAV-2). Research performed using a transformed EVT cell line (HTR-8/Svneo) susceptible to AAV-2 infection, in the presence or absence of adenovirus, has shown that co-infection reduces the invasion of the extracellular matrix and has cytopathic effects. However, infection with AAV-2 alone induces these effects and also favors clear reduction in the capacity of HTR-8/SVneo cells to adhere to fibronectin-covered plates. This would mean that the infection with AAV-2 could affect the ability of EVTs to invade uterine vessels, resulting in complications during pregnancy. Consistent with this proposal, AAV-2 DNA has been found in trophoblasts isolated from patients with severe preeclampsia (Arechavaleta-Velasco et al. 2006).

These regulatory effects on the processes of adhesion, migration and invasion of trophoblasts are also exerted by HPV-16 oncoproteins. The BeWo cell line transfected with the HPV-16 oncoprotein E5 showed decreased adhesion to plastic substrates and endometrial cell lines (RL-95 and HEC1-A) (Boulenouar et al. 2010). Similarly, compared to parental cells, the 3A trophoblast cell line transduced with E6 and E6/7 showed decrease in adhesion to RL-95 and HEC cells (You et al. 2002). Compared to transfected control cells, BeWo cells transfected with E5 oncoprotein showed higher migration and invasion capacity; this increase was greater in the case of transfection with E6/E7 oncoproteins. A possible molecular mechanism that would explain these phenotypes in trophoblastic cells is that transfection with E5, E6/E7 or both oncoproteins decreases the levels of E-cadherin, a key molecule in cell adhesion (Boulenouar et al. 2010).

Expression of the complete form and the two truncated variants of HBx stimulates the proliferation, reduces apoptosis and increases the migration capacity of the HTR-8/SVneo cell line. Mechanistically, transfection of HBx promotes increase in the expression and induces higher levels of the phosphorylated forms of Smad-2 and Smad-3, as well as decrease in the protein levels of E-cadherin and higher protein levels of mesenchymal markers, such as vimentin and N-cadherin (Fig. 5); this could explain the higher invasive capacity of this EVT model in response to HBx expression (Cui et al. 2015).

CONCLUSION

The study of viral infections during pregnancy is a currently popular and interesting topic. The discovery of the potential teratogenic effect of ZIKV on development in humans has focused research back on processes related to the ability of viruses to infect the maternal-fetal interface. Throughout evolution,

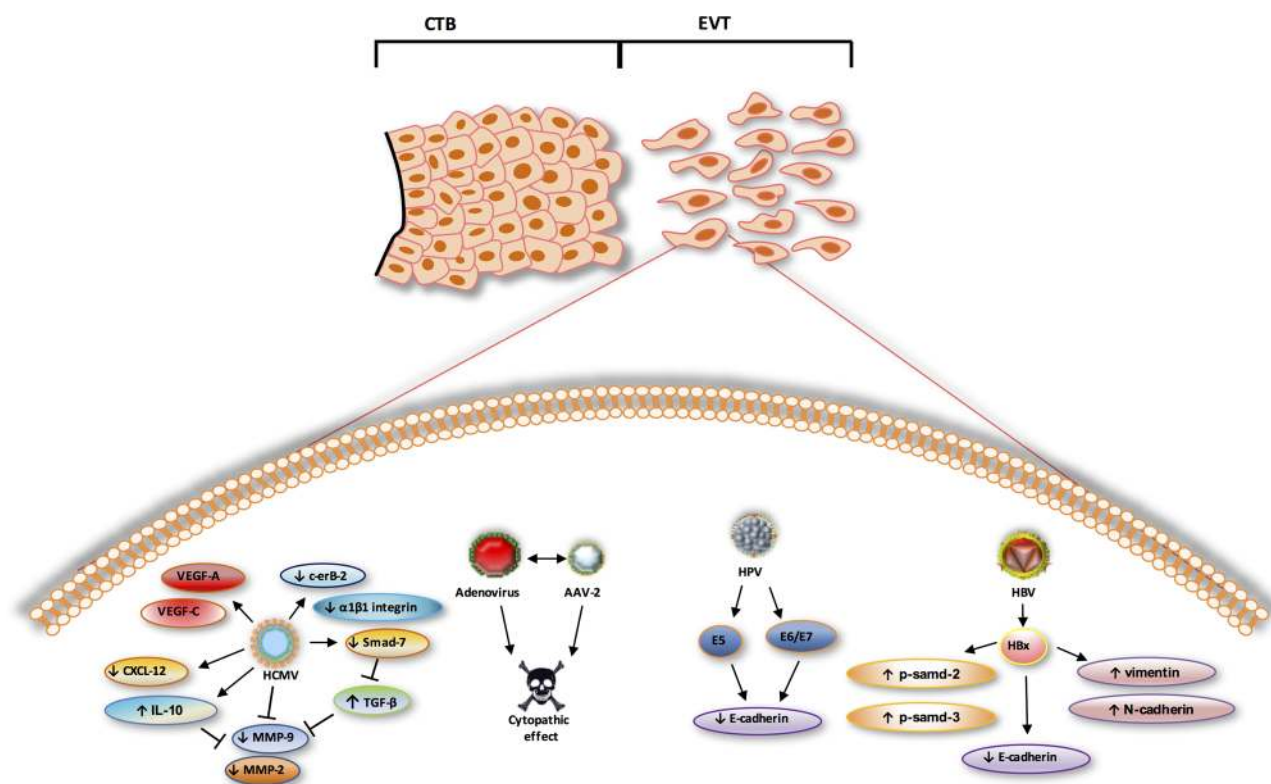


Figure 5. Effects of viral infection during trophoblast invasion. Trophoblast cells infected with cytomegalovirus (HCMV), adenovirus (AAV-2), HPV and HBV have distinct effects in molecules and process related with trophoblast invasion. HCMV, adenovirus and AAV-2 induce for different mechanisms, low protein and activity levels of MMP-2 and MMP-9, and also, cytopathic effects that produce low invasion. In contrast, HPV and HBV through viral proteins E5, E6/E7 and HBV X protein, respectively, induce a reduction of protein levels of E-cadherin and high invasion. Also HBV X protein increase mesenchymal markers such as vimentin and N-cadherin.

the host cell and diverse viruses have been engaged in a constant battle for viral subsistence or infection resolution. These pathogens develop different mechanisms to control certain cellular processes. The trophoblast is an important component of the placenta; different viruses try to enter trophoblasts and manipulate its machinery to generate viral factories that would help spread the infection to the fetal circulation.

In this minireview, we have described a series of events in the trophoblast that are key for the establishment of the viral cycle; these include various cellular mechanisms that viruses use to obtain access into cells and other mechanisms that are involved in the pathogenic processes of viral infections, such as apoptosis, activation of the immune response and alterations in cell invasion. However, other cell types within this microenvironment may be susceptible to viral infection. Vertical infection with some viruses such as HCMV at the maternal–fetal interface has been studied in detail. Studies on emerging viruses such as ZIKV could help provide models for obtaining knowledge regarding molecular mechanisms underlying viral interaction with the placenta and might help explain the pathogenesis of different disorders observed in virus-infected pregnant women. This minireview provides an overall view of placental infection in the case of many viruses and the main pathogenic mechanisms involved, which would be valuable for identifying common mechanisms and processes of viruses and help study infection by unexplored viruses such as chikungunya and respiratory syncytial virus at the maternal–fetal interface.

Future studies focusing on the cross-talk between trophoblasts and other placental cell types will be useful for elucidating any new mechanisms discovered in relation with the

vertical transmission process. Understanding these mechanisms will be of great importance for developing antiviral therapies or diagnostic strategies to control or eradicate viruses that infect the placenta and thereby to achieve proper completion of the gestation process.

ACKNOWLEDGEMENTS

We would like to thank Sofía Carrilo-Halfon for her suggestions regarding the writing style in the support in the comments in the correction of manuscript.

FUNDING

This work was supported by the National Council for Science and Technology CONACYT (CB-2015-01-255007 to M.L.J.); the Instituto Nacional de Perinatología ‘Espinosa de los Reyes’ (212250-3210-21007-03-15 to M.L.J.); received fellowships from CONACYT (to L.D.G.G.).

Conflict of interest. None declared.

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