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Review

Neuroteratogenic Viruses and Lessons for Zika Virus Models

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The Centers for Disease Control and Prevention has confirmed that Zika virus (ZIKV) causes congenital microcephaly. ZIKV now joins five other neuroteratogenic (NT) viruses in humans and ZIKV research is in its infancy. In addition, there is only one other NT human arbovirus (Venezuelan equine encephalitis virus), which is also poorly understood. But further insight into ZIKV can be found by evaluating arboviruses in domestic animals, of which there are at least seven NT viruses, three of which have been well studied. Here we review two key anatomical structures involved in modeling transplacental NT virus transmission: the placenta and the fetal blood–brain barrier. We then survey major research findings regarding transmission of NT viruses for guidance in establishing a mouse model of Zika disease that is crucial for a better understanding of ZIKV transmission and pathogenesis.

Trends

A substantial body of scientific information exists on transplacental transmission (TPT) of naturally occurring neuroteratogenic (NT) viruses, including mouse models of Japanese encephalitis virus TPT.

An ideal mouse model of Zika viral disease will demonstrate vector and sexual transmission, TPT, and productive infection of the fetal brain in a maximally immunocompetent host. Microcephaly is a nonspecific defect seen in many viral and noninfectious diseases.

Each virus and host has a gestational teratogenic window of time during which infection of the brain can result in birth defects.

To infect the fetal brain, viruses must breach the placenta and fetal blood–brain barrier.

Potential target cells in the brain are stem/progenitor cells, neurons, oligodendrocytes, astrocytes, microglia, and endothelial cells.

Neuroteratogenic Viruses and the Emergence of Zika Virus (ZIKV)

Although ZIKV-associated **microcephaly** (see Glossary) has recently garnered much attention due to more than 1000 cases associated with the 2015 outbreak beginning in Brazil[i], there are many precedents of fetal infection resulting in defective neurologic development. The most common infectious neuroteratogens are quickly remembered using the mnemonic TORCH (*Toxoplasma*, Others, Rubella, Cytomegalovirus, and Herpes simplex) [1,2]. NT viruses include both RNA and DNA viruses from multiple phylogenies with diverse modes of transmission (Table 1, Key Table). However, following maternal infection, all NT viruses are transmitted to the fetus transplacentally [3–8]. Transplacental transmission (TPT) is to be distinguished from other modes of vertical transmission from mother to child, which include intrapartum contact with body fluids and mucous membranes at the time of birth, postpartum contact, and via ingestion of milk. For each virus–host pair, there is a window of gestational time, usually within the first half of pregnancy (see below), during which TPT can produce developmental defects [1–5,9–11]. For purposes of this review, this segment of gestation will be referred to as the teratogenic window.

Microcephaly is just one of many central nervous system (CNS) developmental defects that can result from fetal viral infection [3,5,9,12]. Fetal brain development is a dynamic process [1,12–14] involving a highly coordinated migration and maturation of several cell types (Table 2). Viral infection can arrest neuroblast migration and result in agyria (note: agyria is a normal feature of mouse brain). Cerebrospinal fluid (CSF), produced by ependymal cells, constantly flows through the brain's ventricles, and disruption of fluid production and drainage can also impact development. Most commonly, a net positive CSF balance results in hydrocephalus. The ultimate manifestation of one or more defects depends on the tropism, timing, and severity of viral injury. Severe inflammation [1,15] and the residual effects of tissue injury can also cause developmental defects beyond initial viral cytopathic effects. For instance, dystrophic calcification can result in intracranial calcifications as seen in ZIKV cases [16–20].

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Currently the exact mechanisms by which ZIKV infects the fetal CNS are unknown, and the challenge to conquer fetal Zika disease would appear daunting: a virus, circulating with immunologically similar viruses in the same vector space [18], is introduced into the maternal host and traverses both the placenta and fetal blood–brain barriers to establish infection in an immunologically privileged site [8,21] of the genetically dissimilar fetus and causes developmental defects against the time-sensitive backdrop of fetal development. However, progress made against congenital Rubella syndrome [1,2] and congenital varicella syndrome [22] are examples of viral diseases which once posed great threats to fetal health, including microcephaly, but which are now relatively rare due to the development of effective vaccines and public health measures. In developing animal models of NT virus infection, priority should be placed on finding models that produce TPT and productive fetal brain infection over exact replication of microcephaly. Further development of mouse models, the most manipulable *in vivo* mammalian systems, will provide invaluable tools in understanding and preventing the pathogenesis of ZIKV disease.

Modeling Transplacental NT Viral Infection in Mice

The key events in TPT of a NT virus include maternal infection and viremia, followed by virus crossing the placenta and infection of the fetal brain [6–8,23]. While pregnant women are suspected to be more susceptible to viral infection [24], and researchers are well accustomed to modeling adult infection and viremia, to most virologists breach across the placenta and into the fetal brain are new territories which merit an introduction.

Placenta

The placenta constitutes the physical and immunologic barrier between the maternal and fetal circulations, and therefore the principal barrier between the viremic mother and the naïve fetus [6,8]. The reader is referred to an excellent recent review of placental structure and defenses in humans and animals [7]. Because human and mouse chorionic villi are separated from maternal blood only by trophoblasts, their placentas are categorized as hemochorial (Figure 1). Further, human and mouse placentas are subcategorized as hemomonochorial and hemotrichorial, with the two circulations separated by one and three layers of trophoblasts in human and mouse, respectively [7,25,26]. Pig and ruminant placentas are epitheliochorial, meaning that the chorionic epithelium is separated from maternal blood by the uterine epithelium, connective tissue and endothelium (Table 3[ii]).

At first glance it would appear that more layers between maternal and fetal circulations would be more prohibitive to TPT. Evidence for this postulate comes in the form of cytomegaloviruses: human cytomegalovirus (HCMV) is transmitted transplacentally whereas mouse CMV (MCMV) is not [23]. However, porcine CMV is also transmitted transplacentally despite an epitheliochorial placenta. There are numerous other examples of related viruses across host species which demonstrate that layering is but one of many factors determining placental permeability and permissiveness [4,5,12].

In the human placenta there are two environments where a virus might gain entry to the fetal blood (Figure 1B,C) [7,8,25]. Proximally, the placental (chorionic) villi are bathed in and separated from maternal blood by a multinucleate syncytium of trophoblasts. Distally, mononuclear extravillous trophoblasts invade the decidua and intermingle with maternal mesenchymal cells and leukocytes.

Innate defenses are thought to be emphasized over cell-mediated immunity in the placenta because strong adaptive immunity is not conducive to the placenta's role of preventing immune rejection of the semi-allogenic fetus [6–8]. The syncytium has many 'un-epithelial' properties as compared to epithelia that line other tissues. In particular, there are no intercellular junctions

Glossary

Abortion: the term abortion is used selectively in the human context to distinguish elective procedures from spontaneous fetal death. In animals the distinction is rarely needed, and abortion is used as an umbrella term to signify death and expulsion of the fetus.
Agyria/lissencephaly: in humans, the loss of the brain's normal gyri and sulci. This is a normal feature of the mouse brain.
Arthrogryposis: congenital fixation of a joint in an extended or flexed position. In congenital neurologic disease this often results from loss of nervous input.
Chorion: the outermost membrane surrounding an embryo.
Cyst: fluid-filled cavity lined by an epithelium.
Dysplasia: abnormal growth or development.
Dystrophic calcification: mineralization occurring at sites of chronically damaged tissue.
Encephalopathy: any disease of the brain.
Hydranencephaly: near to complete loss of cerebral cortical tissue with filling of the remaining cavity by fluid.
Hydrocephalus: increase in the amount of cerebrospinal fluid within the cranial cavity that is usually accompanied by expansion of the cerebral ventricles and atrophy of the brain.
Hypoplasia: decreased organ size resulting from arrested development.
Microcephaly: undersized head. Virtually synonymous with microencephaly, which refers to an undersized brain.
Micromyelia: undersized spinal cord.
Microphthalmia: undersized eye or eyes.
Mummification: drying and shriveling of the dead fetus.
Neuroteratogen: an agent that commonly disrupts development of the central nervous system, although the peripheral nervous system can also be affected.
Porencephaly: presence of abnormal cavities in the brain.
Syncytiotrophoblast: a multinucleate single cell, formed from the fusion of individual trophoblasts, which covers the placenta.
Trophoblast: the epithelium that covers the placenta.
Ventriculomegaly: enlargement of the brain's ventricles.