Teratogenic effects of the Zika virus and the role of the placenta

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The mechanism by which the Zika virus can cause fetal microcephaly is not known. Reports indicate that Zika is able to evade the normal immunoprotective responses of the placenta. Microcephaly has genetic causes, some associated with maternal exposures including radiation, tobacco smoke, alcohol, and viruses. Two hypotheses regarding the role of the placenta are possible: one is that the placenta directly conveys the Zika virus to the early embryo or fetus. Alternatively, the placenta itself might be mounting a response to the exposure; this response might be contributing to or causing the brain defect. This distinction is crucial to the diagnosis of fetuses at risk and the design of therapeutic strategies to prevent Zika-induced teratogenesis.

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Introduction

Over the last 6 months, thousands of microcephalic babies have been delivered in Brazil, the country that is experiencing the highest Zika virus infection rates worldwide. Given the apparent pronounced effects of this vector-borne viral pathogen on the developing fetus, there is a rush to ascertain whether and how Zika virus might be causing microcephaly, as well as the need to address concerns regarding potential biases in the reporting of cases, or historic under-reporting of cases in Brazil and in other Zika virus-affected countries. Knowledge of mechanism could lead us to early detection methods and therapeutic responses to this new apparent teratogen and enhance preparedness for the next viral epidemic. We make a case for epidemiologists, virologists, pathologists, obstetricians, toxicologists, and developmental biologists to rigorously consider and test the role of the placenta as a mediator of the viral exposure and the cause of the fetal brain defects associated with Zika virus infection.

Direct transfer hypothesis

One possibility is that the virus has neurotropic properties and, via the placenta, is directly accessing and damaging the developing brain. This hypothesis implies the presence of the virus within the embryo at the earliest developmental stages of the cerebral cortex. However, in this early period, the embryo or fetus is fairly well shielded from maternal circulation. Maternal blood flow into the placenta only begins at 10 weeks gestation (figure).[1] The routes of entry for the virus would be uterine gland secretions, leakage through the trophoblastic plugs that block maternal blood flow, or diffusion of preconceptional viral concentrations into the amniotic and yolk sacs as they form. Once the virus reaches the trophoblast barrier, the virus as part of an immunocomplex with non-neutralising antibodies could be carried through the placenta with the help of Fc gamma receptors.[2,3] However, this process is less likely to be the case before 16 weeks.[4,5] Similar to the dengue virus,[6,7] Zika virus might work through the endoplasmic reticulum of the trophoblast to become a sort of cargo of placental exosomes. Those exosomes might then be targeting embryonic or fetal neuroepithelium. The virus could be causing localised reactions at the interface of the placenta that can allow free virus to pass through.[8] Zika virus might also be transmitted through semen, potentially giving the virus access to the early embryo.[9]

Tissue samples at this stage are not yet available to test these assumptions directly. Most of the signs of infection in women delivering microcephalic babies in Brazil are reported from 8–16 weeks gestation (Marques ETA, unpublished),[9] which could mean that the virus is reaching the fetus at a later stage in brain development, and possibly precisely at this point when maternal blood flow into the placenta begins. A recent case report presents novel cross-sectional evidence that the cause of microcephaly in a fetus was direct transfer.[10] The fetus was exposed to maternal Zika virus infection late in the first trimester (based on maternal symptoms) and the authors measured higher viral titres in the brain tissue at autopsy relative to other tissues. In two cases, the viral DNA was measured in amniotic fluid at 28 weeks, but not in maternal serum or urine.[11] Additional evidence for direct transfer and brain damage by Zika virus is the detection of IgM against viral antigen (but not the viral mRNA) in the cerebral spinal fluid of 30 of the 31 samples analysed of the babies born with microcephaly (Marques ETA, unpublished). Although still sparse, these data are crucial towards an understanding of the teratogenic mechanism of Zika virus. Hopefully, additional data will continue to provide answers to questions central to a complete understanding of the relationship between Zika virus and microcephaly: does the virus reach the fetal brain in all cases? Can this process be measured within the developmental timeframe that matters most for the development of primary microcephaly?

Because these measurements have been made late in or at the end of pregnancy, whether the virus is able to cross the placenta in the early period, which is most relevant to the risk of microcephaly, remains unclear. The ultimate proof would be a positively correlated metric of virus exposure in maternal and fetal tissues that coincides with the timing of the fetal defect. Alternatively, a cohort analysis (currently ongoing in Brazil) can statistically confirm the temporal ordering of prenatal maternal infection, fetal brain development, and increased

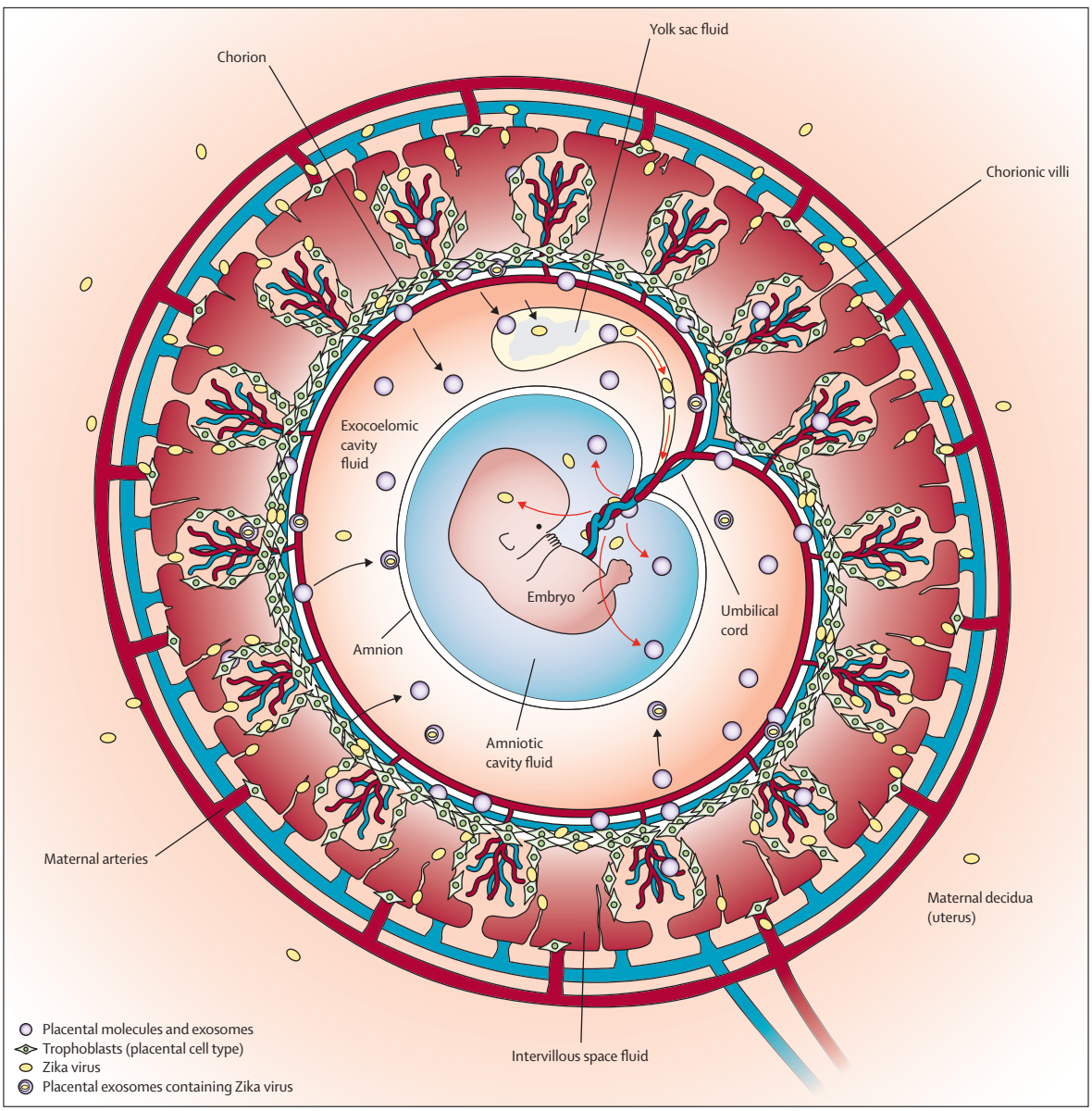

Figure: **The gestational sac in the first trimester before the onset of maternal–placental blood flow**
The placenta consists of the chorion and the chorionic villi which encircle the embryo and carry out synthesis and secretion of molecules that can enter into the embryo. Different scenarios include: direct transfer of free virus through the trophoblast layers, placental exosome-mediated transfer, or minimal to no transfer.

risk of delivering a microcephalic infant. In-vitro models in which embryonic neuronal cells are directly exposed to Zika virus might generate useful information. However, these cells might also misrepresent conditions during early pregnancy in vivo, in which the virus must traverse fluid and tissue layers to reach the developing brain.

Interestingly, no information to date points to global effects of Zika virus on placental function such as smaller birth size (excluding the head), reduced trophoblast invasion (higher incidences of pre-eclampsia or growth restriction), or preterm birth—all of which are tightly linked to the health and function of the placenta. Again, this observation suggests that the virus works through a more specific molecular pathway instead of outright destruction of the placenta and fetus.

The pathology of placentas infected with cytomegalovirus might offer some foresights while data for Zika virus placental pathology evolves.[12,13] The most frequent symptom in placentas infected with cytomegalovirus was chronic villitis, or wide-scale inflammation of the cell layers of the chorionic villi. The degree of placental inflammation has been correlated with the severity of the fetal effects that include microcephaly.[12,13] The placenta, through a microRNA-

mediated response, might be blocking or enhancing replication of the Zika virus as has been shown with other viruses.[14]

Placental mediation hypothesis

An alternative and potential complement to the previous hypothesis of direct viral effect is that the placental response is the main cause of the brain defect. If the infected pregnancy is not spontaneously miscarried, then the virus is probably disrupting molecular synthesis in the outer layers of the placenta (figure). This change might happen before 10 weeks, when primary microcephaly occurs. Disruption of placental signals to the developing brain might cause or contribute to microcephaly. A model for this type of effect has been shown in the case of murine herpesvirus, in which the fetal effects occurred in the absence of the virus.[15] The investigators offered an explanation that the placental response invoked a change in the profile of inflammatory markers within fetal organs. Whether the fetus itself expressed the inflammatory molecules, or if these molecules were delivered from the placenta, is not yet clear.

Primary microcephaly, which appears to be the dominant type of microcephaly described in reports from Brazil (Marques ETA, unpublished), originates during early neurogenesis. The main features are fewer neurons at birth, simpler gyral surfaces, and smaller brains.[16–19] Microcephaly has various biological causes; Zika virus might be correlated with a specific type but evidence to definitively know is not yet available. Without or prior to making contact with the embryo proper, the virus might be able to perturb the synthesis or secretion of molecules (ie, proteins, neuropeptides, non-coding RNAs, or cytokines) within the placental chorionic villi. The chorionic villi have greater exposure to maternal blood than the early embryo, which is embedded within two fluid sacs and shielded by two membranes from maternal (but not placental) circulation. The perturbation in the synthesis and secretion of placental molecules by Zika virus itself or by some other process as a result of Zika virus infection might be a key component of virally induced fetal defects. One theory and modest data suggest that the placenta synthesises and secretes molecules that are essential for normal fetal brain development.[20] Although largely unexplored, evolutionary hypotheses might provide a theoretical basis for linking placental functions (and dysfunction) to brain development.

Our group can conceptualise two scenarios that support the idea of placental mediation of Zika virus-induced microcephaly. In one scenario, a general pro-inflammatory response of the placenta might be disrupting embryonic brain development. This idea might unify the diverse maternal exposures linked to higher risk of microcephaly (eg, radiation exposure, tobacco smoke, cocaine use, cytomegalovirus, alcohol consumption, or α-haemolytic streptococci).[21–25]

In another scenario, specific molecules or pathways synthesised by the early placenta are being disrupted, such as the microcephaly genes (ie, *MCPH1-12*, *CEP63*, and *CASC5*). Mutations in these genes have been causally related to microcephaly.[16,18,19] The simultaneous under or over expression of these genes in the placenta and the fetus (ie, deficient *vs* toxic levels of a particular protein) might contribute to fetal defects. Even though the expression of the microcephaly proteins has been characterised as intracellular, their placental analogues might be packaged and secreted within placental exosomes—an important mechanism for intercellular communication.[26]

Two places should be studied to identify candidate molecules in the placental and fetal tissue of Zika virus-infected pregnancies. The first is the medical literature on genetic causes of microcephaly.[18,19,27] Are these same genes causally related to microcephaly in the case of viral infection? If so, how and in which cell type are these genes being disrupted as a result of Zika virus infection? This finding could mean that the placenta, being fetal tissue, expresses the same genes but under different conditions and for different reasons. Or this finding could indicate some placental participation in these early phases of embryonic or fetal development.

The second is the set of molecules causally related to Zika virus transfer across the epidermis.[28] The trophoblast, like the epidermis, is an epithelial cell type. Hence, analogies might exist between genes involved in the response of the epidermis (ie, *AXL*, *DDX58*, *IFIH1*, and *MX1*) and the placental response to the virus. Inquiry into this area might uncover shared mechanisms for receptor-mediated transmission, pattern recognition, and interferon stimulation. The chorionic villi are considered to be like the skin of the placenta. These villi are the primary interface between components of maternal blood and fetal circulation.

Discussion

Why does it matter if the apparent teratogenic effects of Zika virus are placentally or directly mediated? As more data are collected, we might find that either or both hypotheses prove true depending on the timing of exposure and the stage of brain development.

The placental mediation hypothesis holds appeal as we can easily and non-invasively measure an early placental response, whereas we cannot easily access the embryo. The placenta is an effective broadcaster of information on placental and fetal exposures and developmental consequences.[29] Secreted placental molecules and vesicles can be measured in maternal circulation and used by clinicians to diagnose a fetus at risk. Real-time monitoring of changes in concentrations of secreted molecules over the first trimester, linked to imaging of the morphology of the gestational sac, could establish temporality and provide clues as to how these relationships shift before and after the onset of maternal–placental circulation.

Similarly, understanding any contribution of placental inputs to the teratogenic effect might clear the path for developing pharmacological methods to block teratogenesis. In the case of West Nile virus (a mosquito-transmitted flavivirus like Zika virus), treatments to pharmacologically stimulate autophagy were effective in reducing the neurotoxic effects in non-pregnant individuals.[30]

While the world awaits the epidemiology and pathology of this unique viral infection to help to explain the current epidemic in Brazil, now is the time to update our thinking and approaches to studying teratology and the role of the placenta. Doing so might provide large scientific rewards for this outbreak as well as future similar epidemics.

Contributors

JJA conducted the literature search and drafted the manuscript. ETAM contributed unpublished data, participated in the discussion of ideas, and helped with the writing. AC contributed to the discussion of ideas and helped with the writing. RHB contributed to the discussion of ideas and the writing.

Declaration of interests

We declare no competing interests.

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