Pregnancy, the placenta and Zika virus (ZIKV) infection

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Zika virus (ZIKV) infections have been recognised in Africa and Asia since 1940. The virus is in the family *Flaviviridae* and genus *Flavivirus*, along with Dengue, Japanese encephalitis virus, Tick borne encephalitis, West Nile virus, and Yellow fever virus. These viruses share biological characteristics of an envelope, icosahedral nucleocapsid, and a non-segmented, positive sense, single-strand RNA genome of ~10 kb encoding three structural proteins (capsid C premembrane/membrane PrM/M, envelope E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). ZIKV has three known genotypes; the West African (Nigerian cluster), East African (MR766 prototype cluster), and Asian strains. Virus sequencing from the most recent South American outbreak suggests this virus is related to the 2013 French Polynesian isolates of Asian lineage.

ZIKV like other flaviviruses is arthropod-borne (arbovirus), with more recent evidence for sexual transmission, persistent presence in semen[1], and higher rates of acquisition due to a higher reproductive number than Dengue virus (DENV)[2]. Infection with ZIKV is usually asymptomatic (~80% of cases) or causes mild disease similar to less severe DENV. However, ZIKV has emerged as a major public health threat globally due largely to substantial recent outbreaks in areas of large gatherings, and observed association with fetal neurological damage including microcephaly in the Americas and elsewhere (reviewed in Marrs *et al.*[1]). Countries involved in the most recent outbreaks are summarised in the associated paper here. As a result of the risk to pregnant women, Australian public health authorities (and those in many other countries) recommend pregnant women defer travel to high risk countries. However, if exposure is likely, these women should prevent mosquito bites, have their sexual partners avoid mosquito bites, and post exposure avoid pregnancy for 8 weeks (summarised in Marrs *et al.*[1]), or possibly longer.

Clinical outcomes of mother to child transmission and diagnostic difficulties

Mother to child transmission (MTCT) of ZIKV has been documented via placental infection and damage, with increasing evidence of fetal ZIKV microcephaly. The number of infected mothers, compared with number of infected fetuses (i.e. rate of MTCT), is unclear, although in one Brazilian study, of 88 pregnant women with rash before 38 weeks gestation, 82% had ZIKV and 12/42 (27%) had fetal abnormalities on ultrasound compared with 0/16 women without ZIKV[3]. However, this is likely a significant overestimate due to the method of collection, the nature of the clinic and the lack of confirmed transmission on amniocentesis. A case control study of association between ZIKV and microcephaly showed ZIKV present in mothers of 24/32 cases of microcephaly compared with 39/61 mothers of controls ($p = 0.12$), and that in the babies, 13/32 with microcephaly compared with 0/16 of the controls had ZIKV infection[4]. These rates compare with rates of MTCT in maternal cytomegalovirus (CMV) infection of 32% in primary infection and 1.4% during reactivation[5], and for rubella of 80% to 25%, depending upon gestation[6]. Effects on the fetus for all these infections depend upon many factors, including maternal immunity, gestation of infection, and viral characteristics.

Identification of mothers infected with ZIKV is predominantly via symptoms, serology, and molecular testing of the acutely infected person. Diagnosis is confounded by the low rate of symptoms (in ~20% of adults), technical difficulties with serology cross-reactivity, and the brief period of viraemia in some infections. Serology diagnostic problems occur due to co-circulation of other flaviviruses (particularly Dengue virus) in ZIKV affected areas. Cross-reactivity between ZIKV and Dengue virus occurs[7], falsely negative tests for ZIKV may result if high levels of antibody are present to other flaviruses (such as occurs following vaccination for Yellow fever virus), and acute ZIKV infection may result in false positive Dengue NS1 antigen tests[8], further confusing diagnosis. Molecular testing using nucleic acid tests such as PCR is definitive if positive, although the duration of viraemia makes identification difficult when combined with low rates of symptomatic infection.

A major concern is whether MTCT occurs in ZIKV-infected asymptomatic women resulting in unexpected fetal damage. This occurs in murine models where ZIKV tropism for cells at the maternal-fetal interface is the likely source of transplacental transmission[9], and is consistent with human cell studies *in vitro*[10]. Prolonged maternal viraemia, and excretion of ZIKV in urine for 5–6 weeks following infection provides opportunities for improved diagnosis[11], but also the possibility of continuing risk of ZIKV transmission either to other adults or MTCT during asymptomatic phases of an infected mother[1]. ZIKV has been found in breast milk in three case reports of mothers infected <3 days from delivery[12], although MTCT transmission via breast milk has not been documented.

Placental and fetal infection

Most microcephaly is thought to arise from first trimester (T1) infection, although sampling difficulties occur with the high rate of asymptomatic infection. ZIKV has been detected in fetal brain tissue from microcephalic infants, in amniotic fluid taken from mothers of affected infants[13], and from central nervous system tissue of affected microcephalic infants[14]. These are mainly observational data with minimal controls, albeit with autopsy and ultrasound data being consistent with microcephaly resulting from ZIKV infection during pregnancy[15]. ZIKV has been known to be neurotropic in animals for 60 years, with more recent murine experiments demonstrating replication in embryonic brain targeting neural progenitor cells, with consequent cell cycle arrest, apoptosis and inhibited neural progenitor cell differentiation[9,16]. This is presumed to result in the microcephalic phenotype via neuronal cell death[16]. This is consistent with observations that African ZIKV strains infect neural precursor cells in murine models (summarised in Klase *et al.*[17]).

Mother to child transmission (MTCT) studies often use models from T2 or T3 placentae, which differ from T1 placentae in structure, cell components and surface markers. Studies of infection of explanted placentae in other viral infections such as with CMV show neonatal neural malformation and intra uterine death may be caused partly through Th1 cytokine-induced placental damage[18,19]. The placenta is a complex organ that changes significantly over pregnancy, and comprises some unique cells with differential susceptibility to viral infection (Figure 1). ZIKV infects isolated placental primary cells and human placentae cultured *ex vivo*, with mid pregnancy (T2) chorionic villi (cytotrophoblasts, endothelial cells, fibroblasts, Hofbauer cells) and amniochorionic membranes (amnion epithelial cells, trophoblast progenitors) infected[10]. As MTCT requires virus to traverse the placenta, the role of trophoblasts (either as differentiated syncytiotrophoblasts or cytotrophoblasts) is likely to be key, similar to the key role they have for MTCT

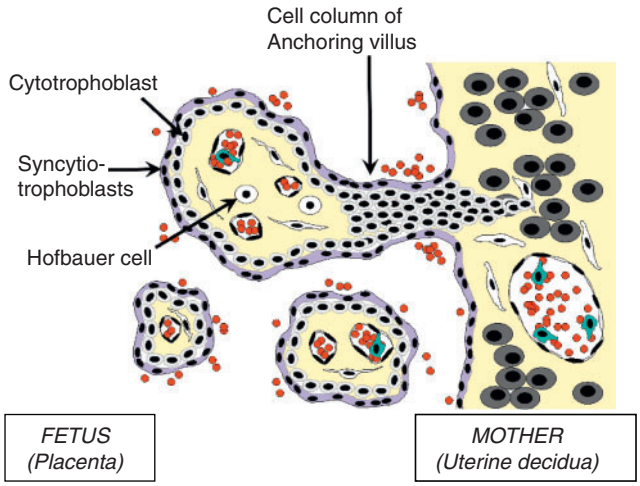

Figure 1. Possible sites of Zika virus infection of the human placenta. Cytotrophoblasts (CTB) form the inner layer of villi, fuse into multinucleated syncytiotrophoblasts, or give rise to extravillous trophoblasts (EVT), which invade and migrate into maternal uterine decidua (that is from left to right in the figure). Viruses infect different cell types, with ZIKV shown in *ex vivo* explants to infect CTB, endothelial cells, fibroblasts, and Hofbauer macrophage-like cells in the villus core on the uterine decidual side (Tabata *et al.*[10]).

of other viruses[18]. Placental inflammatory response to ZIKV may be important in fetal neurological pathology, although this remains to be proven in humans.

Early gestation (T1) infection with ZIKV has been associated with miscarriage, intrauterine growth restriction, and microcephaly[14], and although causation is likely, it is still to be proven. These changes result from direct infection of the fetal neuronal tissue, although placental infection may contribute to the more generalised fetal pathology as occurs with other viruses causing similar fetal pathology, possibly through virus-induced cell cycle dysregulation[20]. The presence of receptors and cell entry cofactors on these cells (Axl, Tyro3, TIM1) which are known also to be bound by other flaviviruses (DENV – Tyro3, Axl, Mertk) suggest a common mechanism of entry may exist[21]. These receptor tyrosine kinases are from a family known to clear apoptosed cells and interact with the innate immune system. Interventions that prevent ZIKV binding to these may provide therapeutics that can be trialled in mouse models or human placental explant models where reduced placental damage may reduce fetal injury[9].

Future studies

ZIKV infection remains a disease clinically of either no symptoms, or relatively mild presentation with fever, myalgia, eye pain, and/or fatigue associated with a maculopapular rash. The major complication of fetal injury, particularly microcephaly and death *in utero*, need to be addressed with further research. Good murine and human placental explant models exist[18], and candidate targets for ZIKV cell binding inhibition have been identified[10]. Vaccines for

related flaviviruses are now licensed in some countries (DENV – the live recombinant Denvaxia from Sanofi Pasteur) or undergoing trials. If continued spread of ZIKV occurs either within currently infected countries, or to other naïve populations, enhanced vaccine development needs to be considered, as suggested by some commentators. If so, such a vaccine will need to prevent MTCT and address the issue of cytokine/immune-dependent injury to the fetus, transplacental transmission of ZIKV[10,18], with the potential to significantly reduce the risk of congenital ZIKV abnormalities, as has occurred with the successful use of vaccines for rubella virus. Finally, all sources of ZIKV transmission to pregnant women should be avoided, including via blood products, as these may be infected despite being from asymptomatic individuals[22].

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Biography

Professor William Rawlinson, AM, is Director of Serology and Virology Division (SAViD), Director or the NSW State Organ and Tissue Donor screening laboratory, Director of a State Reference Laboratory for HIV, and Deputy Chair Serology Quality Assurance Program (QAP) RCPAQAP. He is a clinician scientist recognised internationally for translational research into congenital malformation of infectious causes, particularly with cytomegalovirus (CMV). His work in emerging infections include inaugural Chair of the Australian Biosecurity Quality Assurance Program (QAP) RCPAQAP, which collaborates nationally and internationally with the WHO to provide proficiency testing for biothreats and emerging pathogens, including Zika virus.

Read more about Zika in the article **The voyages of Zika virus** by Derek Gatherer on page 206.