HHS Public Access

Author manuscript

Am J Reprod Immunol. Author manuscript; available in PMC 2018 February 01.

Published in final edited form as:

Am J Reprod Immunol. 2017 February ; 77(2): . doi:10.1111/aji.12613.

Zika Virus Infection of Hofbauer Cells

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Abstract

Recent studies have linked antenatalinfection with Zika virus (ZIKV) with major adverse fetal and neonatal outcomes, including microcephaly. There is a growing consensus for the existence of a congenital Zika syndrome (CZS). Previous studies have indicated that non-placental macrophages play a key role in the replication of Dengue virus (DENV), a closely related flavivirus. Since the placenta provides the conduit for vertical transmission of certain viruses, and placental Hofbauer cells (HBCs) are fetal-placental macrophages located adjacent to fetal capillaries, it is not surprising that several recent studies have examined infection of HBCs by ZIKV. In this review we describe congenital abnormalities associated with ZIKV infection, the role of HBCs in the placental response to infection, and evidence for the susceptibility of HBCs to ZIKV infection. We conclude that HBCs may contribute to the spread of ZIKV in placenta and promote vertical transmission of ZIKV, ultimately compromising fetal and neonatal development and function. Current evidence strongly suggests that further studies are warranted to dissect the specific molecular mechanism through which ZIKV infects HBCs and its potential impact on the development of CZS.

Keywords

Hofbauer cells; Zika Virus; congenital Zika syndrome; placenta

Prenatal infection of ZIKV causes congenital abnormalities

Recent observational studies have demonstrated causality of antenatal ZIKV infection and adverse pregnancy effects ranging from poor obstetrical outcomes to congenital fetal anomalies.[1-3] Fetal musculoskeletal abnormalities are among the signs associated with first and second trimester antenatal infection.[1] Microcephaly was the initial serious finding, starting the recognition of the ever-evolving congenital Zika syndrome (CZS).[4] Since, nervous system abnormalities such as hearing loss, seizures, intracranial calcifications and

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brain deficiencies have also been added as signs of CZS.[1-3, 5, 6] A confirmed ZIKV antenatal infection without any of these abnormalities likely occurred in the third trimester, and is considered to be 'mild'. However, as the number of infections rise and these children age, new associations such as ocular dysfunction and developmental delays have become correlated to these late infections, suggesting that the placenta is vulnerable to ZIKV at any stage of fetal development.[7, 8]

The human placenta has innate defenses that must be evaded or infiltrated for a virus to cause such severe outcomes in a short inoculation period.[9-11] Unlike all other members of the Flavivirus genus, ZIKV infection is capable of this feat, and compromises the integrity of the maternal-fetal barrier. Early cases exhibited intra-placental calcifications, fibrin deposits and sclerosis, all of which are indicative of a fetus with poor uteroplacental flow, and at increased risk of adverse outcomes.[1, 3, 6, 12, 13] Miscarriage, abruption and stillbirth – outcomes that can be due to placental dysfunction – have been linked to ZIKV infections.[3, 14, 15] Identifying the mechanism by which ZIKV is able to disrupt the host immune system is critical to possible prevention and treatment modalities.

The role of Hofbauer cells and evidence for their microbial infection in adverse pregnancy outcomes

HBCs are large, pleiomorphic, highly vacuolated macrophages which appear on the 18th day of gestation and are present until term.[16] They are located beneath the syncytium and adjacent to fetal capillaries,[17] a site that is critical for the protection of the fetus against microbes migrating from the mother to the fetus. Since HBCs are observed prior to the appearance of a fetal circulation, they are likely derived from villous stem cells early in pregnancy, and thus can be viewed as the first "fetal" immune cells.[17-19] Later in pregnancy they arise from recruited fetal monocytes.[17-19] Macrophages have been traditionally classified as M1 (pro-inflammatory with increased expression of IL-1β, TNF-α, CD11b, and CD40) or M2 (anti-inflammatory with increased expression of IL-10, TGF-β, CD163 and FR-β). HBCs are M2 macrophages,[20, 21] supporting their role in angiogenesis and development.[22, 23] HBCs assist in development of the placenta by secreting sprouty proteins, which regulate villous branching, as well as attracting fibroblasts needed for support via a paracrine mechanism.[23] Blood vessels are formed in the stroma following the release of angiogenic factors such as vascular endothelial growth factor by HBCs.[24, 25] However, the utility of M1/M2 classification is now questioned as plasticity of the macrophage phenotype and response is recognized.[26, 27] Our recent findings strongly support such plasticity since HBCs, while expressing M2 markers, produce a robust pro-inflammatory cytokine response to bacterial lipopolysaccharide (LPS) compared to other placental cell types.[28] Our studies also revealed a 3-fold focal increase in HBCs in pregnancies with histological chorioamnionitis [HCA, polymorphonuclear leukocytes in the umbilical cord (funisitis), chorionic plate, or fetal membranes].[29] Conversely, we documented reduced HBC numbers in pregnancies with preeclampsia (PE).[30]

Adverse neonatal outcomes including cerebral palsy are associated with preterm birth and HCA when accompanied by fetal inflammatory response syndrome (FIRS, a multisystemic

microbial invasion of the fetus with the hallmark presentation of funisitis).[31-33] *In situ* RT-PCR revealed an infectious agent was present in 46 out of the 60 placentas from newborns with respiratory distress and severe neurological sequelae.[34] Multiple infectious agents (bacteria, coxsackie virus, cytomegalovirus, and herpes simplex virus) were localized primarily to HBCs and syncytiotrophoblasts.[34] Similar results were obtained when placentas were examined from 33 cases with significant neurodevelopmental delay.[35] A recent report indicated that DC-SIGN (an alternative HIV receptor) promoter variants expressed in HBCs correlated with mother-to-child transmission (MTCT) of HIV-1.[36] Of note, the presence of bacterial infection in HCA increased the risk of MTCT of HIV[37]. Thus, HBCs may be vulnerable to certain viral infections and facilitate vertical transmission. It has long been recognized that the syncytiotrophoblast undergoes a continuous process of damage and repair across pregnancy,[38-40] thereby potentially providing a portal for trans-syncytial movement of maternal virus in the intervillous space to HBCs.

Evidence for HBC susceptibility to ZIKV infection

Dengue virus (DENV), a closely related flavivirus, is well documented to primarily infect phagocytic cells (e.g. dendritic cells, macrophages) upon insertion of the virus into the skin via the mosquito proboscis.[41-43] These phagocytic cells, which become productively infected, migrate throughout the body and in turn promote dissemination of virus infection. It is therefore conceivable that productive infection of phagocytic cells within the placenta, and specifically placental macrophages/HBCs, could potentially serve as a mode for vertical transmission of ZIKV. Several studies have recently demonstrated the susceptibility of HBCs to ZIKV infection in isolated cultures[44, 45] and placental explants.[45-47]

Primary cultures of HBCs isolated and purified from term human placentas have been found to be productively infected upon exposure to multiple strains of ZIKV including two pre-epidemic strains: prototype Uganda/African strain MR766[44, 45] and FSS13025 the Cambodian/Asian isolate from 2010,[45] and two post-epidemic American-strains isolated from Mexico,[45] and Puerto Rico.[44] These findings indicate that the permissiveness of HBCs to ZIKV is not strain-specific. Although donor specific differences in kinetics and magnitude of virus replication were observed within one study,[44] they were not determined to be particularly evident within the other.[45]. Quicke et al. further showed that type I interferon (IFN-α) and pro-inflammatory cytokines (IL-6, MCP-1 and IP-10) were produced in response to ZIKV replication and virus replication resulted in a modest immune activation of cultured HBCs for the presentation of antigens.[44] Since type I and II IFNs are important for controlling ZIKV trans-placental infection, these findings may suggest that HBCs, at least initially, may mount a protective response against the virus.[48]

As HBCs reside below the syncytiotrophoblast within the placental villi, ZIKV must initially breach the maternal-fetal barrier prior to promoting fetal infection. In order to determine whether HBCs are susceptible within the context of tissue, first-trimester (8 weeks)[47] and full-term[45, 47] human placental tissue explants were infected *ex vivo* with both pre-epidemic (MR766 and FSS13025)[45, 47] and post-epidemic (Nicaragua isolate) strains.[47] Consistent with the studies using primary cultures of HBCs, HBCs in explants were infected by ZIKV in all cases as evidenced by the co-localization of virus antigen and macrophage markers,

CD163[45] or CD68.[47] These findings implicate HBCs as targets for ZIKV replication within the placenta throughout pregnancy. Although more conclusive studies are needed, a recent report found that TIM1 may be a potential cofactor for ZIKV infection of cells of the choronic villous including HBCs.[47]

Lastly, utilizing placental tissue retrieved from a miscarriage of a confirmed ZIKV-infected mother, a Brazilian case study found evidence of chronic placentitis characterized by chronic villous inflammation, edema, and trophoblastic epithelium lesions.[46] There was an apparent increase in amount of villous HBCs, stromal lymphocytic cells, and histocytes within the intervillous space when compared to normal, uninfected villous tissue.[46] Immunohistochemistry and morphological assessment localized ZIKV antigen to HBCs.[46] These *in vivo* analyses, coupled with *in vitro* experiments using placental explants and primary cultures of HBCs, provide strong evidence for a role of HBCs in ZIKV infection of the human placenta.

Putative functions of HBC infection in ZIKV spread

Challenge of isolated, primary human placental macrophage cultures signified that HBCs are capable of a productive ZIKV infection, whereas determining the susceptibility of these cells in explant studies indicated that HBCs could serve as replication targets upon exposure to virus. The discovery of ZIKV-infected HBCs within the placenta from a naturally acquired human infection which resulted in fetal demise confirms the cellular tropism of ZIKV for HBCs. Further, as infection of phagocytic cells has been associated with the dissemination of the closely related, DENV, the consistent cellular tropism of ZIKV for phagocytic cells of the placenta suggests this particular cell type could play an analogous role in the dissemination of ZIKV within the fetal compartment.

The proximity of chorionic villi HBCs to umbilical blood vessels, suggests simple virus amplification within HBCs could serve as a source of virus for dissemination through fetal blood to reach the neural progenitor cells with infection ultimately resulting in CZS. Additionally, as HBCs could serve as continuous ZIKV reservoirs within the fetal compartment, the immune tolerant environment of the placenta could further contribute to the permissive phenotype of HBCs to ZIKV infection. Furthermore, as M2 characterized macrophages, HBCs have also been found to be motile,[22] the putative migratory activity of this cell type may spread ZIKV throughout the placenta. In addition, based on their perivascular location, HBCs are uniquely positioned to facilitate vertical transmission of ZIKV ultimately compromising the development and function of fetal neural progenitor brain cells. Deciphering the specific role of HBCs in the dissemination of ZIKV is key to advancing our understanding of adverse fetal and neonatal outcomes in pregnancy complications associated with ZIKV infection. Potentially, manipulation of these cells could also lead to new prevention and treatment modalities for those at risk, or showing signs of CZS.

Acknowledgments

The sources of funding are: 5T32 AI007019-40 (K.A.J.), R01AI121183 from NIAID (V.M.A.) and an Albert McKern Scholar Award (S.G.). E. F. is an Investigator with the Howard Hughes Medical Institute.

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