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Chakraborty and Suthar Awarded U01 Grant

Mother-to-child transmission (MTCT) of HIV-1 and other viruses remains a significant global health concern. In 2015, an estimated 150,000 new HIV-1 infections occurred worldwide in exposed infants; most occurring during labor and delivery or through breastfeeding [1]. The risk of infection in the womb (in-utero transmission), however, is less than 7%; so that even in the absence of virologic suppression with maternal antiretroviral therapy, over 90% of HIV-1-exposed fetuses are 'naturally' protected from infection.

Protection from vertical transmission has been noted for other pathogens, including human cytomegalovirus [2], hepatitis C [3], and herpes simplex virus [4]. These observations suggest the placenta has evolved innate and adaptive mechanisms that restrict establishment of viral infection. Defining correlates that offset or promote in-utero transmission during on -going exposure will contribute to our understanding of viral evasion, and can contribute to the development of effective therapies.

Chakraborty Team

Drs. Johnson and Chakraborty, and colleagues have previously characterized the role of placental macrophages (Hofbauer cells) and T central memory cells (TCM) in cord blood during infection with HIV and HCMV [5-7]. Hofbauer cells appear to be key mediators of in-utero transmission of HIV-1, constitutively expressing elevated concentrations of regulatory cytokines, which inhibit HIV-1 replication in vitro [5]. Hofbauer cells appear to sequester HIV-1 within intracellular compartments that can be accessed by HIV-1-specific antibodies, which may offset MTCT [6]. Hofbauer also produce type I interferons and a host of antiviral factors, which may be pivotal in control of viral infections. In contrast, HIV-1/HCMV co-infection may promote inflammation, chronic villitis, and trophoblast damage, providing potential HIV-1 access into CD4+ CCR5+ target cells including Hofbauer cells and TCM cells [7]. These data suggest that the placenta exhibits a variety of mechanisms to limit HIV-1 replication, yet viral-induced activation may override this protection to facilitate in-utero HIV-1 transmission.

Zika virus (ZIKV) is a mosquito-borne flavivirus that has recently emerged in the Americas and is a pathogen of significant public health concern. ZIKV was first isolated in Uganda in 1947 [8] and remained dormant in Africa and Asia for decades, with sporadic outbreaks characterized by a mild self-limiting disease in humans [9]. ZIKV is transmitted by mosquito bites, sexual contact, and blood transfusion [10]. ZIKV can also be vertically transmitted from an infected mother to the developing fetus in utero, in some cases resulting in adverse pregnancy outcomes, which include spontaneous abortion and fetal brain abnormalities and microcephaly, associated with delayed or arrested brain development. The greatest risk of serious fetal sequelae is associated with ZIKV infection early in pregnancy, suggesting enhanced tropism for placental cells during the first- and

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second-trimester [11, 12]. The mechanism by which ZIKV establishes infection in the placenta and developing fetus is poorly understood.

In 2016, Drs. Suthar and Chakraborty teamed up to study ZIKV within the placenta. ZIKV antigen has been detected in chronic villi, specifically within placental macrophages or HCs and histiocytes from women who gave birth to infants with microcephaly or had active ZIKV infection during pregnancy [13, 14]. ZIKV RNA has also been isolated from placental tissue in humans and from pregnant mice infected with ZIKV [14-16]. Through in vitro infection studies, the Suthar and Chakraborty laboratories observed that primary human Hofbauer cells, isolated from full-term placentae, were permissive to ZIKV infection [17]. Following infection, Hofbauer cells were found to produce type I IFN and pro-inflammatory cytokines, suggesting that these placental macrophages are capable of mounting an antiviral response to ZIKV infection. Indeed, we observed transcriptional upregulation of the retinoic acid-inducible gene I (RIG-I)-like receptors (RLR) and antiviral effector genes. Our studies were the first to show that Hofbauer cells are permissive for ZIKV infection in the placenta. However, several unanswered questions remain regarding the interaction of ZIKV, and potentially other flaviviruses, with these Hofbauer cells.

Suthar Team

Drs. Suthar and Dr. Chakraborty were recently awarded a U01 grant from the National Institutes of Health to study how macrophages regulate immunity to ZIKV infection at the maternal-fetal interface. This grant is focused on addressing two critical areas: 1) The placental microenvironment is dynamic, with changes in immune cells, growth factors, and development of maternal-fetal blood exchange during the course of gestation. Despite the discovery of Hofbauer cells over 100 years ago [18], we still only have a rudimentary understanding of the molecular and regulatory changes that occur to these cells during the course of pregnancy. In the first part of this grant this team will focus on determining the dynamics of immune regulation by placental macrophages during pregnancy; and 2) The innate immune response is the first line of defense against an invading pathogen. The RLR, STING and type I IFN signaling are essential for host restriction of viral replication and control of flavivirus infection [19-22]. To the best of our knowledge, these innate immune signaling pathways have not yet been studied in placental macrophages, nor has there been any study determining the dynamics/potency of these antiviral signaling pathways in this cell population during gestation. A reduced ability to mount cell autonomous antiviral immune responses would directly impair blocking of viral replication. Thus, in the second part of this grant, this team focuses on how do placental macrophages control ZIKV infection and whether antiviral potential is dynamic within these cells during the course of pregnancy.

This project brings together a complementary set of expertise and long-standing collaborations between basic researchers and clinicians within the Emory University School of Medicine. Dr. Suthar is an Assistant Professor of Pediatrics with extensive expertise in flavivirus biology, innate immunity, and viral pathogenesis. Dr. Chakraborty is a clinician-scientist and Professor of Pediatrics with a long-standing interest in understanding perinatal infections particularly trans-placental HIV-1 transmission. Dr. Erica Johnson is a Senior Research Associate in Dr. Chakraborty's lab and an expert on viral infections at the maternal-fetal interface. Dr. Augustine Rajakumar is an Assistant Professor of Obstetrics and Gynecology with over 20 years of research experience investigating placental development in healthy and pathologic pregnancies. Dr. Lisa Haddad is. Medical Director of the Atlanta Women's Center (AWC) and an established physician-scientist, serving as Assistant Professor of Obstetrics and Gynecology at Emory University.

Overall, this team is seeking to elucidate mechanisms of innate immune control within the placenta and develop a deeper conceptual understanding of anti-ZIKV responses during pregnancy. These studies have a strong likelihood of providing a better understanding of the dynamics of the immune

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Recent Publication Highlights

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Correlated Fluorescence Microscopy And Cryo-Electron Tomography Of Virus-Infected Or Transfected Mammalian Cells

CM Hampton, JD Strauss, Z Ke, RS Dillard, JE Hammonds, E Alonas, TM Desai, M Marin, RE Storms, F Leon, GB Melikyan, PJ Santangelo, PW Spearman, and ER Wright. Nature Protocols. 2017: 12 (1): 150-167. PMID: 27977021; PMCID: PMC5385890

Cheri Hampton, PhD (left)
Elizabeth Wright, PhD (right)

Correlative light and electron microscopy (CLEM) combines spatiotemporal information from fluorescence light microscopy (fLM) with high-resolution structural data from cryo-electron tomography (cryo-ET). These technologies provide opportunities to bridge knowledge gaps between cell biology, cell physiology, virology, and structural biology. To successfully follow an individual virus interacting with a cell across multiple imaging platforms while in it's most native state, i.e., without chemical fixatives, we used vitirified (cryo-immobilized) samples in a newly developed liquid nitrogen-cooled fluorescent microscope stage. In this study, we described our protocol for correlated cryo-fLM, cryo-electron microscopy (cryo-EM), and cryo-ET (i.e., cryo-CLEM) of virus-infected or transfected mammalian cells. Mammalian-derived cells were cultured directly on EM substrates, using optimized conditions that ensured that the adherent cells were able to spread thinly across the substrate and were not physically disrupted. The cells were then imaged by live-cell fLM and then flash-frozen (vitrified) at approximately -180°C to preserve cell and virus structure in the native-state. The frozen-hydrated specimens were then imaged under cryo-conditions by cryo-fLM to take advantage of fluorescence labeling for locating points of interest in or on the cells. Once mapped, the frozen specimens were transferred to the cryo-transmission electron microscope (cryo-TEM) and imaged. In the course of this study, we resolved sites of respiratory syncytial virus (RSV) assembly, HIV-1 particle restriction by tetherin, and psuedotyped HIV-1 endocytosis and fusion. Cryo-CLEM strategies have the potential to assist in our identifying and resolving the structures associated with the macromolecular machinery, organelles, and smaller complexes that maintain cellular homeostasis as well as their disruption due to disease. The further integration and development of CLEM imaging technologies will be essential for broader structural investigations of human cells and more complex differentiated cell networks.

-Submitted by the authors, please find the article here: https://www.ncbi.nlm.nih.gov/pubmed/27977021

Integrating tuberculosis screening in Kenyan Prevention of Mother-To-Child Transmission programs

LM Cranmer, A Langat, K Ronen, CJ McGrath, SM LaCourse, J Pintye, B Odeny, B Singa, A Katana, L Nganga, J Kinuthia, G John-Stewart. Int J Tuberc Lung Dis. 2017 Mar 1;21(3):256-262. PMID: 28225335

Lisa Cranmer, MD

Maternal tuberculosis during pregnancy or postpartum results in poor maternal and infant outcomes, including infant low-birth weight, prematurity, and maternal and neonatal deaths. HIV-infected mothers in particular have high risk of TB co-infection during pregnancy and the postpartum period. The World Health Organization recommends TB symptom screening for all HIV-infected individuals, but integration of TB screening into routine maternal and child health program has not been widely implemented.

Nested within a national survey in Kenya of mother-infant pairs attending infant immunization visits, we performed maternal postpartum TB symptom screening and found a high proportion of HIV-infected mothers with TB symptoms (33%). Women who had a positive

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symptom screen were more likely to have an infant with HIV infection (p=0.02) and infant non-specific TB symptoms. A substantive proportion of mothers also reported recent TB exposure (11%), but few mothers who had been exposed to TB had received isoniazid preventive therapy (15%). Our results suggest that integration of maternal TB symptom screening into child immunization visits is feasible and may direct important TB/HIV treatment and prevention interventions for mothers and their children.

-Submitted by the authors, please find the article here: https://www.ncbi.nlm.nih.gov/pubmed/27354515

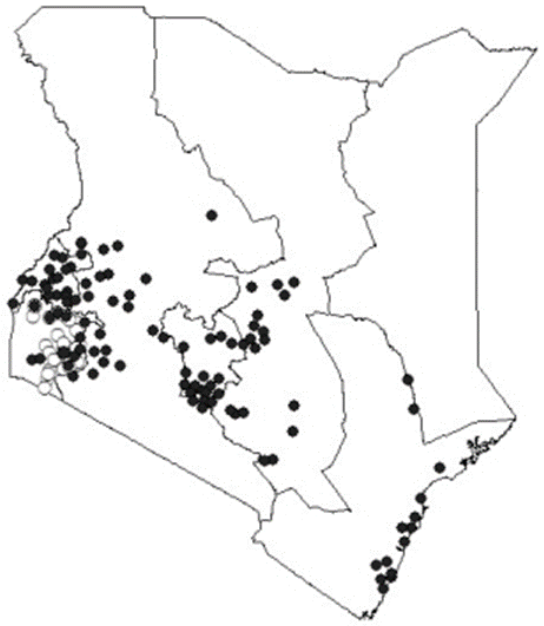

Figure 1 (right). Maternal and child health clinics surveyed across Kenya. Black circles indicate the National PMTCT Survey and white circles indicate the PMTCT-Nyanza Survey, focused on an area with high HIV prevalence.

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response to ZIKV infection within the maternal-fetal compartment. This team hopes that their findings will provide insight for the development of antiviral therapeutics and vaccines to thwart the ongoing ZIKV epidemic in the Americas.

-Submitted by Rana Chakraborty, MD, PhD & Mehul Suthar, PhD

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October 4, 2017 in the Health Sciences Research Building Auditorium, with Keynote Speakers:

- *Mark Slifka, PhD from Oregon Health Sciences*
- *Hans Langedijk, PhD from Johnson & Johnson (Netherlands)*

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