Subject: NOTE NEW TIME: Dissertation Defense - Samson Omole, PhD Biosciences
Date: Tuesday, July 2, 2024 at 1:43:09 PM Eastern Daylight Time
From: SSB Faculty List on behalf of Diane St. Germain
To: SSB-FACULTY-LIST-L@LISTSERV.GMU.EDU

NOTE NEW TIME

Dissertation Defense Announcement
To: The George Mason University Community

Candidate: Samson Omole

Program: PhD Biosciences

Date: Monday, July 15, 2024

Time: 1:00 PM Eastern Time (US and Canada)

Location: Location: In Person, IABR Conference Room #1004
And Via Zoom
Join Zoom Meeting:

https://gmu.zoom.us/j/93701568741?pwd=DnxcKXINK8bNYSqL5ytAWPR00aPnqu.1

Meeting ID: 937 0156 8741
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One tap mobile
+13017158592,,93701568741#,,,,*692552# US (Washington DC)
+12678310333,,93701568741#,,,,*692552# US (Philadelphia)

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+1 301 715 8592 US (Washington DC)
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Passcode: 692552
Find your local number: https://gmu.zoom.us/u/ac2nDYSgkY

Join by SIP
93701568741@zoomcrc.com

Committee chair: Dr. Ramin M. Hakami

Committee members: Dr. Alessandra Luchini, Dr. Yuntao Wu, and Dr. Massimo Caputi

Title: "Characterization of RIG-I Inducing RNA Species in Small Extracellular Vesicles (sEV) from Cells Infected with the Cytoplasmic RNA virus Rift Valley Fever Virus (RVFV)"

Abstract:

RVFV is a zoonotic pathogenic RNA virus belonging to the family Phenuiviridae genus Phlebovirus. It is a cytoplasmic single-stranded RNA virus, and the genome is made of three segments designated as Large (L), Medium (M), and Small (S) based on their relative sizes. RVFV is the causative agent of Rift Valley Fever (RVF) disease for which there is no approved vaccine or effective therapy. The virus is mainly transmitted by Aedes and Culex mosquitos. According to a recent report from the World Health Organization (W.H.O.), from 2000 to 2016, there have been more than 4,600 cases and 957 deaths due to RVFV infections, with a case fatality rate of more than 20% in non-endemic areas. Symptoms may vary from a mild, flu-like syndrome to ocular, encephalitic, or hemorrhagic syndromes. Considering the high economic and public health impacts of RVFV, NIAID has classified RVFV as a pathogen of highest concern (Category A). Interferon-B response is the primary innate antiviral defense against RVFV, and it is also known that RVFV viral genome activates IFN-B via RIG-I. Previously, we showed that small extracellular vesicles (sEV) derived from RVFV-infected cells - designated as EXi-RVFV - strongly activate interferon beta (IFN-B) in recipient cells via the RIG-I pathway, culminating in induction of autophagy to eliminate the virus should the cell encounter RVFV and the virus gain cell entry. Because the RVFV viral genome has been shown to selectively activate RIG-I, and viral RNA genome segments are packaged within the EXi-RVFV, we hypothesize that EXi-associated RIG-I induction occurs through the function of packaged viral RNA. To address this hypothesis, we first developed a new sEV purification method to significantly scale up sEV recovery, enabling us to obtain sufficient EXi-associated RNA for RNAseq analysis in order to identify the RIG-I activating species. Our results showed that the new purification method based on a combination of UF, SEC, and DUG increased recovery of functional sEV amounts by 100 fold and allowed obtaining sufficient sEV-associated RNA to perform RNAseq analysis. For purification of sEV-associated RNA, we used the best performing kit based on our comparison of several purification methods. Analysis of purified EXi-associated RNA showed that it activates IFN-B via the RIG-I pathway, and that enrichment for either large or small RNA does not show any difference in the level of activation. We have also shown co-localization of RIG-I and EXi-associated RNA. For this study, following treatment of cells with fluorescently-labeled EXi-associated RNA, cellular RIG-I was fluorescently labeled using anti-RIG-I antibody and immunofluorescence analysis was performed to demonstrate co-localization. As a corollary to these studies, we also analyzed separately each of the three RVFV RNA genome segments, and also each of the three anti-genome segments, for their ability to induce IFN-B activation. T7 promoter-based vectors expressing separately each of the

RVFV RNA genome and anti-genome segments were used for in-vitro transcription of each of the segments. Our results showed that, similar to EXi-associated RNA, all the genome and anti-genome segments activate IFN-B via the RIG-I pathway. Our biochemical characterization demonstrated that RVFV RNA genome sequences and the EXi-associated RNA species that activate RIG-I form double-stranded structures and carry 5' end phosphate groups. We have also completed RNAseq of purified sEV-associated RNA and future studies will focus on bioinformatic analysis of the RNAseq data as part of our effort to identify the RIG-I activating species. These results provide insights into the immune regulation functions of EXi-associated RNA and RNA genomic segments and provide information that can be utilized for development of novel countermeasures against RVF and also other virus infections for which type I IFN response is a critical aspect of host response.

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