









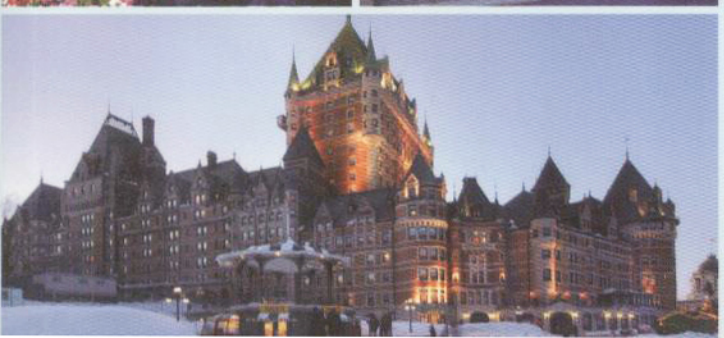

2010 Keystone Symposia
Meeting Abstract Book

Cell Biology of Virus Entry, Replication and Pathogenesis

Scientific Organizers:
Margaret Kielian, Peter Sarnow,
Terence S. Dermody and Paul D. Bieniasz

Sponsored by
Santaris Pharma A/S

February 16-21, 2010
Sagebrush Inn and Conference Center
Taos, New Mexico, USA

KEYSTONE SYMPOSIA™
on Molecular and Cellular Biology
Accelerating Life Science Discovery

225 Control of Toll-like receptor and cytosolic receptor responses by an HSV virulence factor promotes viral pathogenesis

Yijie Ma[1], Dustin Verpooten[1], Huali Jin[1], Zongdi Feng[1], Zhipeng Yan[1], Tibor Valyi-Nagy[2] and Bin He[1]
[1]Dept of Microbiology and Immunology;
[2]Dept of Pathology, University of Illinois, Chicago, IL, USA, 60612

Herpes simplex virus 1 (HSV) infection triggers Toll-like receptor (TLR) and cytosolic receptor dependent innate immune responses. Modulation of such responses by HSV is a complex process involving multiple viral factors. The $\gamma_1$34.5 protein, an HSV virulence factor, subverts the innate response mediated by cytosolic receptor dsRNA-dependent protein kinase(PKR) by redirecting protein phosphatase 1(PP1) to dephosphorylate the α subunit of translation initiation factor 2 (eIF2α). However, its precise role in HSV pathogenesis remains incompletely understood. We recently screened for HSV inhibitors by systematically knocking out HSV genes. The results revealed a previously unrecognized role of $\gamma_1$34.5 where it negatively modulated a cellular target TANK-binding kinase 1(TBK1), a key component that activates interferon regulatory factor 3 (IRF3) and cytokine expression. Unlike wild type virus, the $\gamma_1$34.5 null mutant induced IRF3 activation and the expression of ISG54, ISG56 and type I interferon. Additionally, this mutant replicated efficiently in TBK1-/- cells but not in TBK1+/+ cells. Hence, TBK1 restricts viral replication in the absence of $\gamma_1$34.5 expression. When expressed in mammalian cells, $\gamma_1$34.5 alone associated with TBK1 and inhibited the TBK1-IRF3 complex formation, resulting in a block of IRF3 activation. Furthermore, $\gamma_1$34.5 inhibited the induction of ISG56 and IFN-β by adaptor molecules TRIF, RIG-I, and MDA5, suggesting that $\gamma_1$34.5 interrupts TLR3, RIG-I/MDA5 mediated innate defenses. The function of $\gamma_1$34.5 to antagonize TBK1 is not colocalized with that to counteract the PKR response. Using a mouse ocular infection model, we further demonstrated that $\gamma_1$34.5 suppressed type I interferon production in dendritic cells, which paralleled with HSV replication in vivo. Remarkably, selective perturbation of either anti-TBK1 or anti-PKR function drastically impaired viral replication in cell cultures. Consistently, the mutant viruses bearing either defect were unable to spread from the peripheral tissues to the central nervous system, suggesting that both functions are non-redundantly required for HSV neuroinvasion. These data suggest that the coordinate action of HSV $\gamma_1$34.5 to modulate innate immune responses innervating from TLR and cytosolic receptor pathways is crucial for viral pathogenesis.

This work was supported in part by a NIH grant AI081711.

226 Ancient and recurrent evolution of the antiviral gene fusion *TRIMCyp* in primates

Ray Malfavon-Borja[1],Michael Emerman2[2,3] and Harmit S. Malik[1,2]
[1]Department of Genome Sciences, University of Washington, Seattle, WA 98195;
[2]Divisions of Basic Sciences; and [3]Human Biology, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, PO Box 19024, WA 98109

The discovery of the *TRIM5 - CyclophilinA* (*TRIMCyp*) gene fusion in some primates revealed a new class of antiviral factor. The fusion of *TRIM5* effector domains with the capsid binding ability of *CyclophilinA* (*CypA*) was found to generate a protein active in the defense against viral pathogens, such as lentiviruses like HIV-1. *TRIMCyp* has been identified in owl monkeys and macaques, and was revealed to be the result of independent *CypA* retrotranspositions into or downstream the *TRIM5* gene, respectively. An in silico analysis of the rhesus genome revealed that their *TRIM5* loci encodes two additional *CypA* retrogenes downstream of *TRIM5*, in addition to the *CypA* retrogene that generates *TRIMCyp*. We performed a screen of the three *CypA* retrogenes, labeled according to their proximity to *TRIM5*, on a panel of primate genomic DNA. CypA1, the most proximal *CypA* retrogene, was expected to be present sporadically in the macaque lineage, and was only found in a single macaque species. CypA2 was present throughout Old World monkeys and in some Hominoids. Moreover, CypA2 was found to be active in the expression of a *TRIMCyp* gene fusion in several Old World monkeys, though premature stop codons observed in CypA2 suggests that the encoded *TRIMCyp* transcript would not encode a full length *TRIMCyp* protein. CypA3, the *CypA* retrogene located farthest from *TRIM5*, was only found in Old World monkeys. These findings reveal an ancient, yet inactive *TRIMCyp* encoded by Old World monkeys that has been lost in most extant Hominoids. This further highlights the remarkable degree of convergent evolution that has led to *TRIMCyp* evolving and being used multiple times in primate evolution.

ARRA Fellowship F31-AI084620

227 Analysis of *in vivo* dynamics of influenza virus infection using a GFP reporter virus

Balaji Manicassamy[1], Santhakumar Manicassamy[2], Alan Belicha-Villanueva[1], Guisseppe Pisanelli[1,3], Bali Pulendran[2] and Adolfo García-Sastre[1]
[1]Department of Microbiology, Mount Sinai School of Medicine, New York, NY
[2]Emory Vaccine Center, Emory University, Atlanta, GA
[3]Department of Pathology and Animal Health, School of Biotechnological Sciences, University of Naples "Federico II", Naples, Italy

Influenza A virus is being extensively studied due to its major impact in human and animal health. However, the dynamics of influenza virus infection and the cell types infected in vivo are poorly understood. These characteristics are not easy to determine partly because currently there is no replication-competent virus expressing a fluorescent reporter gene. Here, we report the generation of a complete influenza virus carrying a GFP reporter gene in the NS segment of its genome (NS1-GFP virus). NS1-GFP virus replicates efficiently in cell culture and shows pathogenicity in mice at levels similar to parental virus. We have analyzed the *in vivo* dynamics of influenza infection progression in mice by flow cytometry and whole organ imaging of infected lungs. Using flow cytometric analysis of infected lungs, apart from epithelial cells, we find antigen presenting cells like CD11c+, CD11b+ CD11c+, CD11b+ and B cells to be GFP positive. In addition, NK cells are susceptible to influenza infection. Whole organ imaging of lungs show that influenza infection starts in the respiratory tract in areas closer to large conducting airways and with time spreads to deeper sections of the lungs. We have also tested the effects of oseltamivir and amantadine on the kinetics and in vivo infection progression in mice and find interesting differences in the effects of these antivirals. Treatment with oseltamivir dramatically reduces influenza infection in all cell types, whereas, interestingly, amantadine treatment blocks infection in a cell type specific manner.

228 Activation of p38 Mitogen Activated Protein kinase via a MyD88-dependent pathway activates virus entry trafficking

David Marchant, Gurpreet K. Singhera, Jonathan Boyd, Soraya Utokaparch, Zongshu Luo, Xioaning Si, Delbert R. Dorscheid, Bruce M McManus and Richard G Hegele
James Hogg Research Laboratories,
Providence Heart + Lung Institute,
Department of Pathology and Laboratory Medicine,
University of British Columbia, Room 166-Burrard Building,
St. Paul's Hospital, 1081 Burrard Street Vancouver,
British Columbia, Canada, V6Z-1Y6

Respiratory viruses continue to exert a heavy toll of morbidity and mortality worldwide. There is the lingering threat of an avian influenza pandemic, an H1N1 influenza pandemic is underway and hospitalisations due to Respiratory Syncytial Virus (RSV) are ever increasing. However there are few specific treatments for RSV infection and many influenza isolates are now resistant to amantidine and Tamiflu™. We screened a panel of small-molecule kinase inhibitors to determine the most pertinent host cell kinase during virus replication. We identified p38 and ERK Mitogen Activated Protein (MAP) kinases as the most sensitive targets, such that inhibition of these kinases resulted in significant inhibition of replication of viruses from Adeno, Paramyxo, Picorna, and Orthomyxo virus families. MAP kinase activation is biphasic and we present evidence that early activation of p38 is required to activate virus entry trafficking and internalization. Using antibody neutralization studies and experiments that incorporate cells from gene knock-out (KO) animals our data suggest that virus interaction with host-cell toll like receptor type 4 (TLR4) activates p38-mediated virus internalization via a MyD88-dependent signaling pathway. The dependence of virus entry trafficking upon p38 activation is demonstrated via confocal microscopy of single virus particles and 3D reconstruction. Finally we demonstrate the promise of p38 inhibition as an effective antiviral approach, *in vivo*. Therefore p38 MAP kinase inhibition may be an efficacious and broad antiviral approach by targeting virus entry.