2 (ii) : Viral Fundamental Mechanisms

Session Chair

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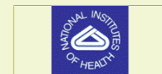
Session Introduction

Title: HIV-1 Latency in Memory T cells
Vincente Planelles, University of Utah, USA

Title: Vpu is responsible for HIV-1 evasion of natural killer cells
Edward Barker, Rush University Medical Center, USA

Title: RelA participates in the regulation of the coupling between HCV RNA replication and HCV translation in huh7.5.1 cells
Lumin Zhang, National Institutes of Health, USA



Title: Role of SNARE proteins in HIV-1 assembly and release
Anjali Joshi, Texas Tech University Health Sciences Center, USA

Title: Discovery and analysis of cellular elongation factors that are critical for HIV-1 reverse transcription
David Harrich, Queensland Institute of Medical Research, Australia

Title: SAMHD1 enzymatic activity toward non-canonical nucleotides
Sarah Amie, Rochester University, USA

Title: P-body components LSM1, GW182, DDX3, DDX6 and XRN1 are recruited to WNV replication sites and positively regulate viral replication
Harendra S. Chahar, Texas Tech University Health Sciences Center, USA

Title: Differential expression/stability of core protein during HCV infection and its effect on viral life cycle
Muhammad Sohail Afzal, National University of Science & Technology, Pakistan

Title: Influence of pathogen-associated molecular patterns on HIV-1 latency
Alberto Bosque, University of Utah School of Medicine, USA

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HIV-1 Latency in Memory T cells

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Background: Central memory, CD4+ T lymphocytes (TCM) harbor the majority of latent HIV-1 proviruses in vivo. We have developed a latency assay based on cultured TCM cells. We have so far identified two signaling pathways that can reactivate latent viruses in cultured TCM: antigenic stimulation and incubation with IL-2+IL-7. Antigenic stimulation reactivates virtually all latently infected cells, and successfully depletes the reservoir. Despite the potency of antigenic stimulation, anti-CD3/CD28 treatment has been shown to exert deleterious effects in the host. In contrast, IL-2+IL-7 incubation reactivates one tenth of the latently infected population, while inducing homeostatic cell proliferation. Consequently, cells are able to proliferate in response to IL-2+IL-7 stimulation, in the absence of viral reactivation, thus propagating latent proviruses through mitosis. Thus, other signaling pathways need to be identified. This work describes a third pathway that we have recently identified, in which massive viral reactivation is achieved with minimal T cell activation.

Methods: We have used a high-throughput variation of our published assay of viral latency in TCM, and identified novel candidate compounds that reactivate latent HIV-1. The biological properties of candidate compounds from the screen were further investigated in order to examine their ability to induce activation markers (CD25 and CD69) and proliferation. This was done in parallel with antigenic stimulation and homeostatic proliferation inducers as a comparison.

Results: We have identified a compound ("C7") which, when incubated with latently infected TCM cells at nanomolar concentrations, displays viral reactivation ability that is about 80% of that with anti-CD3/CD28. The activation profile of the C7-treated cells was indistinguishable from that of untreated cells as evidenced by a lack of increase in the expression of CD25 and CD69. C7 induced proliferation although at much lower levels than anti-CD3/CD28 treatment did.

Conclusions: We demonstrate the existence of compounds that can reactivate latent HIV in TCM cells with comparable efficiency to antigenic stimulation, but with very limited or no ability to induce the expression of activation markers. These results demonstrate that signaling pathways exist, which can be specifically lead to activation of latent proviruses in primary cells. Key signaling elements controlling these pathways should be considered as novel targets.

Biography

Vicente Planelles obtained his Ph.D. from the University of California at Davis in 1991 and then conducted postdoctoral studies at UCLA until 1995. Between 1996 and 2002 he was Assistant Professor at the University of Rochester. In 2002 he became Associate Professor at the University of Utah School of Medicine, and in 2008 he became Professor of Microbiology and Immunology. He has published more thatn 80 papers and reviews on many aspects of HIV-1 and related lentiviruses.

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Vpu is responsible for HIV-1 evasion of natural killer cells

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Natural killer (NK) cells are recognized as being crucial in the defense against viruses. NK cells interaction with virus-infected cells ultimately leads to the death of the virus-infected cells. However, HIV-infected cells are refractory to lysis by NK cells. We have found that resistance to lysis is not due to the failure of HIV to activate NK cells but rather to HIV's ability to prevent NK cell release of its lytic granules. These outcomes are the result of the combined action of viral proteins that both lead to NK activation but ultimately act to suppress the lytic function of NK cells. The key findings from our laboratories, demonstrate that: 1) HIV-1 Nef down modulates HLA-A and –B and 2) HIV-1 Vpr induces ligands to NK cell activation receptor NKG2D. Both of these events lead to NK cell activation. However, HIV-1 Vpu counters NK cells' ability to degranulate by down modulation of NTB-A a homotypic ligand to the NK cell coactivation receptor, NTB-A. NTB-A on the infected cells is critical for eliciting NK cell cytolytic response because degranulation requires both simultaneous engagement of activation with coactivation receptors. Vpu acts to prevent NTB-A surface expression by retention of NTB-A within the trans-Golgi network of the infected cell. Vpu actions on NTB-A are independent of Vpu's activity against CD4. Vpu acts on NTB-A in a similar fashion as Vpu mediated suppression of the host cell innate factor tetherin/BST-2. The ultimate goal will be to use the knowledge gained from studies on Vpu-NTB-A interactions to devise novel therapeutic approaches aimed at rendering HIV-infected cells sensitive to NK cell killing.

Biography

Ed Barker has completed his Ph.D. at the University of Illinois at Chicago and postdoctoral studies from University of California at San Francisco. Currently, he is an Associate Professor in the Department of Immunology and Microbiology at Rush University Medical Center in Chicago. Ed is working on his sixth year of an NIH grant to study how HIV evades natural killer cells.

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RelA participates in the regulation of the coupling between HCV RNA replication and HCV translation in huh7.5.1 cells

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Hepatitis C virus, HCV, is a positive-strand RNA. After released into cytoplasm, HCV RNA severs as a template for the viral translation and transcription. Therefore, the coupling between the viral translation and transcription plays an important role in the modulation of HCV replication. In order to escape the systemic surveillance, HCV develops a complexity strategy to coordinate these two processes. Of these, host factors have been implicated in involvement in the regulation of this adjustment. Although Huh7.5.1 cells is permissive for the study of HCV1a replication in culture system, a low viral production still restricts explore in the understanding of HCV living cycle. Recent studies suggest that a sustained NF-kB activation is a major factor for the impediment of viral replication. To further clarify the role of NF-kB in the HCV replication, we used shRNA to inhibit the activation of RelA. Intriguingly, we found that RelA silencing remarkably suppressed HCV IRES mediated translation. The expressions of viral proteins were also inhibited. Inversely, RelA silencing improved the production of HCV. The further investigation showed that this enhancement was mediated by the increment of HCV RNA replication through the inhibition of interferon beta response. In summary, our results suggest that RelA may participate in the coupling of HCV1a RNA translation and HCV1a RNA transcription, and so regulate HCV1a replication.

Biography

Lumin Zhang has completed his Ph.D at the age of 32 years from Nagoya University. Now, he is a visiting fellow in National Institutes of Health.

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Role of SNARE proteins in HIV-1 assembly and release

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Retrovirus assembly is a complex process that requires the orchestrated participation of viral components and host-cell factors. The concerted movement of different viral proteins to specific sites in the plasma membrane allows for virus particle assembly and ultimately budding and maturation of infectious virions. The soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins constitute the minimal machinery that catalyzes the fusion of intracellular vesicles with the plasma membrane, thus regulating protein trafficking. Using siRNA and dominant negative approaches we demonstrate here that generalized disruption of the host SNARE machinery results in a significant reduction in human immunodeficiency virus type 1 (HIV-1) and equine infectious anemia virus particle production. Further analysis of the mechanism involved revealed a defect at the level of HIV-1 Gag localization to the plasma membrane. Our findings demonstrate for the first time a role of SNARE proteins in HIV-1 assembly and release, likely by affecting cellular trafficking pathways required for Gag transport and association with the plasma membrane.

Biography

Dr. Anjali Joshi is a Research Instructor in the Department of Biomedical Science at Texas Tech University Health Sciences Center. She pursued her PhD in Feline Immunodeficiency virus from North Carolina State University, Raleigh, USA. Immediately after completing her PhD, she received four years of post doctoral training in the Lab of Dr. Eric Freed, Head of the viral assembly section at the National Cancer Institute, Frederick. At NCI, she worked on various aspects of retrovirus assembly including the role of cellular factors in this pathway, role of viral domains in determining the site and process of assembly and several basic aspects of cellular trafficking pathways and retrovirus biology.

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Discovery and analysis of cellular elongation factors that are critical for HIV-1 reverse transcription

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Using protein fractionation combined with an in vitro endogenous reverse transcription assay and mass spectrometry, we identified a cellular protein complex composed of subunits of eukaryotic elongation factor 1 (eEF1) that was able to stimulate late steps of HIV-1 reverse transcription in vitro. Further studies showed that the eEF1A and eEF1G subunits of eEF1 are important components of the HIV-1 reverse transcription complex (RTC) as evidenced by: (a) co-localization of eEF1G and eEF1A with reverse transcriptase (RT) in HIV-1 infected cells; (b) co-purification eEF1 subunits with RTC isolated from infected cells; (c) markedly reduced HIV-1 reverse transcription when eEF1A or eEF1G were down regulated by siRNA. Protein- protein interaction between recombinant RT and eEF1 complex subunits isolated from HEK293T cells was investigated by surface plasmon resonance. Both RT51 and 66 subunits were able to bind to eEF1A with a Kd of 5.4 nM and 1.9 nM, but not to eEF1B, eEF1D or eEF1G. Recent experiments indicate that eEF1A is a major RT binding protein and most likely a mediator of eEF1A complex and HIV RTC interaction as knock-down of eEF1A expression in cells by siRNA treatment resulted in significantly reduced binding of HIV RT to proteins in the cell lysate. Binding to RT could be restored by over-expression of exogenous eEF1A by plasmid transfection. The interaction of RT and eEF1A are refined by mapping the binding domains within the two proteins. The role of RT-eEF1A interaction in HIV reverse transcription represents a potential new target for anti-HIV drug screening.

Biography

David is an Australian Research Council Future Fellow and Group Leader of Molecular Virology at the Queensland Institute of Medical Research. After completing a PhD at UCLA in 1994 in Experimental Pathology of HIV-1 with Prof. Richard Gaynor, he undertook is post-doctoral studies at the University of Texas Southwestern Medical Centre under an NIH Fellowship in immunology and virology. He moved to Australia in 1997 to lead a lab in the National Centre of HIV Virology Research investigating HIV reverse transcription. He has published 50 peer-reviewed paper and serves on the Editorial board of several leading journals including PLoSONE.

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SAMHD1 enzymatic activity toward non-canonical nucleotides

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Macrophages are able to maintain a long-lived viral reservoir of HIV. Although HIV-1 is able to infect macrophages, it does so at a much slower rate compared to $CD4^+$ T cells. This is due to their low dNTP pools, which are needed as substrates for HIV reverse transcriptase (RT) to synthesize viral DNA. It has recently been shown that the low dNTP levels found in macrophages are not just the result of maintaining a static lifecycle, but the result of a newly discovered myeloid specific host restriction factor, SAMHD1, which is able to hydrolyze dNTPs into nucleosides. Another mechanism of host restriction in macrophages is the high level of dUTP (58 fold higher than TTP), which is frequently misincorporated by RT; however HIV-1 has evolved to package host nuclear uracil DNA glycosylase (UNG2) to remove dUMPs from its genome. This mechanism was not adopted by HIV-2. Instead, HIV-2 has a separate anti-restriction capability, which allows it to replicate efficiently in macrophages. HIV-2 evolved the accessory protein Vpx to direct proteasomal degradation of SAMHD1 thereby increasing dNTP pools. We hypothesize that degradation of SAMHD1 will reduce the concentration disparity of dUTP and TTP during viral replication. Therefore, we predict that SAMHD1, which is only present in macrophages infected by HIV-1, is able to selectively decrease the levels of canonical dNTPs and not non-canonical dUTP resulting in the virus to evolve separate antiviral activities from HIV-2 to counteract uracilation of its' genome.

Biography

Sarah Amie is in the process of completing her PhD at the University of Rochester. She is in the Microbiology Immunology department under the advisement of Dr. Baek Kim. Her research has focused on the incorporation of ribonucleotides by HIV-1 reverse transcriptase in macrophages and the implications of their persistence in viral DNA. She also has been trying to elucidate the activity of SAMHD1, an HIV-1 host restriction factor in myeloid cells, on non-canonical nucleotides.

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P-body components LSM1, GW182, DDX3, DDX6 and XRN1 are recruited to WNV replication sites and positively regulate viral replication

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In mammalian cells, proteins involved in mRNA silencing and degradation localize to discrete cytoplasmic foci called processing or P-bodies. These include Sm-like protein LSM1, GW182, 5'-3' exonuclease XRN1, dead box RNA helicase DDX3 etc. West Nile Virus (WNV) infection causes depletion of P-bodies. However, how this P-body depletion occurs and what happens to the P body proteins is not known. WNV infected HeLa cells were analyzed for P-bodies by immunostaining, for protein levels by western blotting. Transient knockdown of P body components was achieved by specific siRNA transfection, WNV replication was measured by FACS analysis and qRTPCR. In WNV infected cells 36 hPI, complete depletion of LSM1, GW182, DDX3 and XRN1 P-bodies was observed but levels of these proteins remained equal in uninfected and infected cells. On the other hand, we found that many P-body components including LSM1, GW182, DDX3, DDX6 and XRN1, but not others like DCP1 and EDC4 are recruited to the viral replication sites as evidenced by their colocalization at perinuclear region with viral NS3. Kinetic studies suggest that the component proteins are first released from P-bodies in response to WNV infection within 12 h post infection, followed by recruitment to the viral replication sites by 24-36 h post infection. These data suggests that in response to WNV infection p body components relocalize to WNV replication complexes and this in-turn might cause depletion of P-bodies. Silencing of the recruited proteins individually with siRNA interfered with viral replication to varying extents suggesting their collective requirement for efficient viral replication. Thus, the P-body proteins might provide novel drug targets for inhibiting viral infection.

Biography

Harendra S Chahar completed his Ph.D from All India Institute of Medical Sciences, New Delhi India-2011. He works in the Center of Excellence in Infectious Disease Research at Texas Tech University, El Paso, Texas in Dr. Manjunath Swamy's group. Their primary area of interest is to understand RNAi mechanism in special reference to flaviviruses and develop novel therapeutics.

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Differential expression/stability of core protein during HCV infection and its effect on viral life cycle

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HCV core protein plays a critical role in viral assembly as a structural component as well as a regulator of assembly site formation. Core recruits replication complexes and envelope glycoproteins to the vicinity of cytosolic lipid droplets.

In current study, we observed a differential core protein expression/stability during infectious HCV life cycle. We analyzed the expression kinetics of HCV proteins in a single viral cycle assay using a CD81-negative Huh-7 derivative cell line. It was observed that most viral proteins accumulated with a half maximal value at 25 to 27 hours post-electroporation in this system. In contrast, the half maximal accumulation of core was reached at 33 hours post-electroporation, indicating a 6-to-8-hour delay in core expression, compared to other HCV proteins. The delay in core expression was confirmed in infected Huh-7 cells using an immunofluorescence-based assay.

Our results showed an increase in core expression during late step of the viral life cycle. core was found to turn over with a half-life of approximately 90 minutes when measured at early time points of HCV infection, or in heterologous expression systems. Strikingly, there was a ten-fold increase of core half-life over the course of infection, whereas other viral proteins half-lives were not increased by more than two times.

As core protein stabilized itself during viral life cycle, to check the effect of this differential core turnover during viral life cycle, core protein was expressed at different concentration and viral replication was quantified. Core protein down regulates the viral replication in concentration/stability dependent manner. As core recruits replication complex on lipid droplets (LDs) for viral assembly, the effect of differential core stability on viral assembly was observed by expressing core in stable cell line expressing sub-genomic replicon for different time points. Our results showed that core protein at later time points after stabilized by itself, more efficiently recruits replication complex to LDs, most probable viral assembly sites.

Altogether, these results indicate that core is an unstable protein, which is stabilized when expressed at higher expression levels. In the course of HCV infection, this stabilization down regulate the HCV replication, and recruits HCV replication complex more efficiently on LDs, and shift the viral life cycle from replication to assembly. This delayed core expression may constitute a mechanism participating in the regulation of the HCV life cycle.

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Influence of pathogen-associated molecular patterns on HIV-1 latency

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Pathogen-associated molecular patterns (PAMPs) are molecules present on microbes and are recognized by cells of the innate immune system to activate innate immune responses and protect the host form infections. PAMPS are recognized by Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs). CD4 T cells have been shown to express TLRs, however the effects of PAMP recognition and its downstream signaling on the HIV-1 latent reservoir are largely unknown. We have developed a system whereby naive cells from the periphery of healthy donors are induced to undergo normal development ex vivo in the presence of select cytokine cocktails or antigenic stimulation through CD3/CD28. These cells are infected while in the activated state, and return to quiescence as central memory cells (TCM). Infection of these ex-vivo generated memory cells leads to latency with a high frequency (about 90% of infections) and leads to a polyclonal population of integrated viruses. Using this paradigm, we have explored the influence of PAMPs on HIV-1 latency in cultured TCM.

We have found that the triacetylated lipopeptide Pam3CSK4, a TLR2 agonist, can reactivate latently infected cultured TCM cells. Interestingly, other tested TLR2 agonists, such as the diacetylated lipopeptides Pam2CSK4 or FSL-1; the yeast cell wall glucan Zymosan or the heat-killed Listeria monocytogenes failed to induce reactivation of latent viruses. Moreover, LPS and Poly(I:C)LMW/LyoVecTM, TLR4 and RIG-I/MDA-5 ligands, respectively, reactivated latent HIV only in a subset of human blood donors. The mechanisms behind these differences are under investigation.

Biography

Dr. Alberto Bosque is a Research Assistant Professor in the Department of Pathology at the University of Utah. He completed his Ph.D in Human Immunology at the University of Zaragoza, Spain. After completing his Ph.D, he undertook his postdoctoral training at University of Utah School of Medicine. In 2011, he became Research Assistant Professor at the Division of Microbiology and Immunology at the University of Utah. He has published more than 20 papers in reputed international journals, in the areas of apoptosis, autoimmunity and HIV-1 latency.

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