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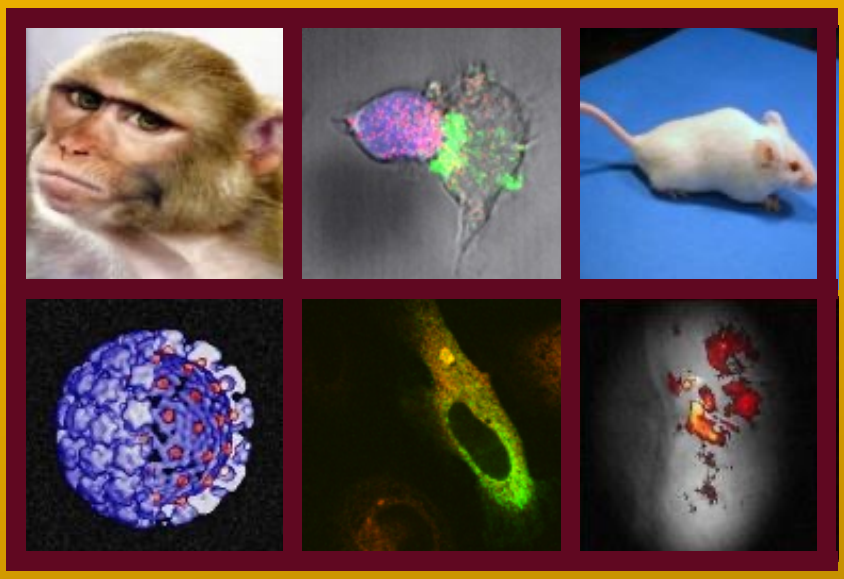

Building 45 Natcher Conference Center
Room E1/E2

Friday December 9, 2022

8:45am - 5:00pm

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HIV vaccine candidate efficacy mediated by cyclic AMP-dependent efferocytosis and V2-specific ADCC

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Vaccination with the HIV clades B/A/E recombinant canarypox–derived vector (ALVAC) and bivalent clade AE/B gp120-envelope proteins in alum significantly reduced the risk of HIV acquisition in the RV144 HIV Phase III vaccine trial in Thailand. Both RV144 and pre-clinical studies in macaques identified antibodies to SIV/HIV variable region 2 (V2), CD4 cells, and Antibody Dependent Cell Cytotoxicity (ADCC) as correlates of risk of HIV/SIV acquisition, highlighting this animal model's relevance to HIV in humans.

The development of an effective vaccine against HIV acquisition would be facilitated by in-depth understanding of the innate and adaptive responses to vaccination. Due to the reproducible identification of $CD14^+$ monocytes as a correlate of reduced risk of infection of the DNA/ALVAC/gp120/alum vaccine, and its durability, we hypothesized that the vaccine efficacy could be linked to epigenetic reprogramming of these innate cells. To test this hypothesis, we conducted two studies in macaques immunized with DNA/ALVAC/gp120/alum regimen and exposed to intravaginal challenges with low doses of SIV_{mac251}. Samples collected prior to and following vaccination were analyzed with different canonical (ADCC, flowcytometry, luminex and $CD14^+$ efferocytosis of apoptotic neutrophils) and multiomics (RNA-, and ATAC-sequencing) analyses. The analyses confirmed the central role of ADCC, and particularly the V2-specific one, in decreasing the risk of acquisition. In addition, we found that, efferocytosis, a cyclic AMP (cAMP)-dependent process of $CD14^+$ monocytes that clear engulfed apoptotic cells, is a novel correlate of reduced risk of SIV_{mac251} acquisition that complements V2-ADCC. The vaccine-induced modification of the chromatin accessibility to cAMP response element-binding protein 1 (*CREB1*) within $CD14^+$ monocytes was linked to miR-139-5p expression and to the level of the V2-specific ADCC. Moreover, the analyses showed that the engagement of the CCL2/CCR2 axis and tolerogenic dendritic cells producing IL-10 (DC-10) is central to vaccine efficacy. These data posit that epigenetic reprogramming in $CD14^+$ cells and its effect on efferocytosis, through the prompt and effective removal of apoptotic infected cells, contributes to vaccine efficacy by decreasing inflammation and maintaining tissue homeostasis.

Presenter: Massimiliano Bissa, Staff Scientist

Mechanism of Pomalidomide-Induced Immune Marker Upregulation in EBV-Positive Lymphomas and Therapeutic Implications

David A. Davis, Hannah K. Jaeger, Ashwin Nair, Prabha Shrestha, Alexandra Stream, Amulya Yaparla and Robert Yarchoan
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Abstract
Kaposi sarcoma herpesvirus (KSHV) and Epstein Barr virus (EBV) downregulate immune surface markers allowing these viruses to avoid immune recognition. Pomalidomide (Pom) is an immunomodulator that functions by interacting with cereblon, an E3-ubiquitin ligase. We previously showed Pom could increase expression of immune surface molecules in tumors caused by KSHV and/or EBV, such as primary effusion lymphoma (PEL) and certain non-Hodgkin lymphomas. Here, we explored the mechanism by which Pom leads to these effects in EBV-infected cells. Pom induced an increase in mRNA expression for B7-2/CD86 in EBV-infected lymphoma cells. Pom-induced increases in protein and surface expression for ICAM-1 and B7-2 and an increase in mRNA of B7-2 were substantially blunted in EBV-infected Daudi cells made resistant to Pom-induced cytotoxicity, which showed decreased levels of cereblon. This suggests that Pom likely initiates the upregulation of these markers by interacting with cereblon. Interestingly, Pom increased the proinflammatory cytokines IP-10 and MIP1α/β in EBV infected cells and these cytokines have been shown to mediate tumor necrosis of Burkitts lymphoma tumors. Previous work by others demonstrated that MIP1α/β can induce the phosphoinositide 3-kinase (PI3K)/AKT pathway in myeloma cells supporting a possible role for this pathway in Pom's effects. Indeed, inhibitors of PI3K (LY294002 and idelalisib) blocked AKT-Ser phosphorylation and Pom-induced B7-2 surface expression. PU.1, which is expressed in EBV-infected cells is a downstream target for AKT and has previously been shown to upregulate B7-2 in dendritic cells. Pom treatment led to an increase in PU.1 levels and binding on the B7-2 promoter based on ChIP analysis. These data indicate that, in EBV-infected cells, Pom acts through cereblon to activate the PI3K/AKT/PU.1 pathway leading to upregulation of B7-2 mRNA and surface protein expression. The increase in immune recognition in addition to the increases in proinflammatory cytokines upon Pom treatment suggests Pom may be useful in the treatment of EBV-positive lymphomas.

Presenter: David A. Davis, Staff Scientist

Potent Dual Block to HIV-1 Infection using Lentiviral Vectors Expressing Vif-Resistant APOBEC3G and Fusion Inhibitor Peptide mC46

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Abstract

Currently, an estimated 38 million individuals world-wide are living with HIV-1. Although combination antiretroviral therapy has been extremely effective in controlling viral replication and extending the life span of infected individuals, it is expensive and can lead to health complications, non-compliance, and emergence of drug-resistant HIV-1 variants. Therefore, gene therapy strategies that effectively inhibit HIV-1 replication are needed to reduce the requirement for life-long antiviral therapy and potentially achieve a functional cure. *Ex vivo* genetic modifications of autologous T cells or CD34+ hematopoietic stem and progenitor cells (HSPCs) provides a viable approach to deliver CD4+ T cells that are resistant to infection or inhibit further spread of HIV-1 infection.

We previously designed self-activating lentiviral vectors that efficiently delivered and expressed a Vif-resistant mutant of host restriction factor APOBEC3G (A3G-D128K) to T cells, which potently inhibited HIV-1 replication and spread with no detectable virus. Here, we developed vectors that express A3G-D128K, plus membrane-associated fusion inhibitor peptide mC46 which blocks HIV-1 entry, and O^6-methylguanine-DNA-methyltransferase (MGMT) for *in vivo* selection of transduced CD34+ HSPCs.

MGMT-selected T cell lines MT4, CEM and PM1 that expressed A3G-D128K (with or without mC46) potently inhibited NL4-3 infection for at least 45 days post infection with no detectable viral replication. Expression of mC46 was sufficient to block HIV-1 infection >80% in a single cycle infectivity assay. Importantly, expression of mC46 provided a selective growth advantage to the A3G-D128K modified T cells in the presence of replication competent virus. This combinational approach to first block HIV-1 entry with mC46, and then block any breakthrough infection with A3G-D128K could provide an effective gene therapy treatment and a potential functional cure for HIV-1 infection.

Presesenter: Krista A. Delviks-Frankenberry, Staff Scientist

Expression of Human Endogenous Retroviruses due to Defects in Chromatin Remodeling in Clear Cell Meningioma

Tara Doucet-O'Hare[1,2]*, Jared Rosenblum[1]*, Danielle Dang[1], John Heiss[3], Dorian McGavern[4], Winson Ho[5], Ashish H. Shah[6], Alexander Vortmeyer[7], and Zhengping Zhuang[1†]

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Abstract:
Clear cell meningioma (CCM), a malignant tumor with a bimodal age distribution, is characterized by altered gene expression due to defects in SMARCE1, part of the chromatin remodeling complex. We recently discovered that a similar defect in SMARCB1 led to aberrant expression of HML-2 human endogenous retrovirus (HERV-K) envelope protein (env), and maintenance of pluripotency critical for tumorigenesis in atypical teratoid rhabdoid tumor (AT/RT). HERV expression is critical for early development of tissues which includes formation of syncytia; however, it is typically downregulated in mature differentiated tissues. We hypothesized expression of HERVs due to defects in chromatin remodeling acquired early in development is critical to tumorigenesis in both CCM and AT/RT. We found increased expression of HERVs in multi-nucleated syncytial tumor initiating cells in CCM samples (n=8) and in neural stem cells with knock-down of SMARCE1. We observed expression of OCT4, a marker of cell stemness, significantly correlated with HML-2 env expression ($p<0.033$, Pearson's $R=0.7461$, $n=8$). In addition, we found expression of CD63, a marker for exosomes, in the vesicles expressing HML-2 env, in all samples tested (n=6). Finally, we observed transcription of Syncytin-1 (n=8) and protein expression of Syncytin-2 (n=1), genes of retroviral origin which, in addition to HML-2 env, facilitate formation of multinucleated cells. These findings suggest either the aberrant persistent expression or reactivation of HERVs due to SMARCE1 loss may be a critical step in oncogenic transformation in early developmental tumors with chromatin remodeling defects. Biallelic loss of SMARCB1, another chromatin remodeling protein, can lead to different tumors depending on the timing and cell lineage in which it occurs. Due to the bimodal distribution in age of CCM patients and our findings, we believe both persistent activation or reactivation of HERVs may be critical to tumorigenesis in tumors with chromatin remodeling defects.

Presenter: Tara Doucet-O'Hare, Research Fellow, Neuro-Oncology Branch, Zhuang Lab

Alternative splicing and circRNA biogenesis driven by alpha and gamma herpesviruses

Dremel SE[1], Koparde VN[2,3], Arbuckle JH[4], Kristie TM[4], Krug LT[1], Conrad NK[5], Ziegelbauer JM[1]

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5. Department of Microbiology, UT Southwestern Medical Center, Dallas, United States.

Abstract:
Circular RNAs (circRNA) are a novel class of noncoding RNA which form a continuous loop due to a 5' to 3' covalent linkage called a back-splice junction. Because circRNAs lack ends, they have low immunogenicity and extended half-lives. CircRNAs are generated through alternative splicing so they expand the number of gene products without extending genome size—a strategy advantageous to organisms with limited coding space. We performed circRNA profiling for Herpes Simplex Virus-1 (HSV-1), Kaposi sarcoma-associated herpesvirus (KSHV), and murine gammaherpesvirus 68 (MHV68). Using Illumina and Nanopore sequencing technologies and a custom bioinformatic pipeline we identified thousands of novel back-splicing variants with distinct expression profiles in lytic and latent infection. This included circRNAs derived from viral latency genes (circK12, circLAT). During lytic infection circRNAs tiled the viral genome, with a transcriptional density 4,500-fold greater than the host. We characterized cis- and trans-elements controlling back-splicing and found that lytic infection promoted rampant, sequence-independent back-splicing. Treatment with two distinct inhibitors of the major spliceosome had minimal impact on expression of viral circRNAs. We investigated how viral effectors exclusive to lytic infection may be promoting these back-splicing products. Drivers of late lytic transcription, including HSV-1 genome replication and KSHV TATA-binding protein (ORF24), promoted circRNA synthesis. Additionally, using eCLIP and Nascent (4SU) RNA-Seq, we determined that the KSHV RNA binding protein (ORF57) preferentially bound RNA fragments containing back-spliced junctions and promoted viral circRNA accumulation post-transcriptionally. Our work elucidates a unique splicing mechanism driven by late lytic replication and identifies a class of transcripts with potential to function in replication, persistence, or tumorigenesis.

Presenter: Sarah Dremel, CRTA Postdoctoral Fellow

Triple depletion of Monocytes, CD8 and NK results in the establishment of early and robust infection by HTLV-1 $_{p12KO}$ in macaques

Anna Gutowska[1], Sarkis Sarkis[1], Ramona Moles[1], Robyn Washington-Parks[1], Cynthia Pise-Masison[1,2], Genoveffa Franchini[1].

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Abstract

We have directly investigated the role of *orf-I* and host cell responses in primary HTLV-1A infection by depleting separately monocytes or NK or $CD8^+$ T-cells and exposing macaques to either HTLV-1A wild type (HTLV-1_{WT}) or to the HTLV-$1A_{p12KO}$ mutant, unable to infect replete animals because of a single point mutation in *orf-I* that inhibits its expression. However, the fully seroconverted animals have shown no symptoms or HTLV-1 associated diseases and infection in macaques was not as robust as that of HTLV-1 in humans. We hypothesized that a simultaneous depletion of monocytes, NK and CD8 T-cells would establish a robust infection and allow for a more thorough investigation of the role of innate and adaptive responses to viral infection, as well as of virus dissemination, and pathogenesis, which cannot be investigated *in vitro*. To test this, two groups of 5 animals each were depleted of CD8-T cells, NK cells and monocytes prior to exposure to either the HTLV-1_{WT} or HTLV-$_{p12KO}$ viruses.

Preliminary data shows that M-T807R1 antibody, and clodronate administration effectively depleted CD8+ cells, total NKG2A+ and monocytes subtypes respectively as expected. We found that the triple depletion of monocytes, CD8+ and NK cells accelerated seroconversion in all animals exposed to HTLV-1_{WT} and HTLV-1_{p12KO}, and all animals were positive by nested PCR for viral DNA and had detectable proviral load (PVL) in blood as soon as 3 weeks post inoculation. Double depletion of NK and CD8 cells and exposure to HTLV-1 in prior work resulted in seroconversion at one months and HTLV-1 PCR positivity in blood was not sustained.

We hope that with triple depletion we will be able to establish robust persistent infection that mirrors virus levels observed in HTLV-1 infected individuals, improving our ability to use this model for therapeutical interventions.

Presenter: Anna Gutowska, Postdoctoral Fellow

MUTATIONS OUTSIDE INTEGRASE LEAD TO HIGH-LEVEL RESISTANCE TO DOLUTEGRAVIR

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Abstract

HIV-1 drug resistance usually results from mutations in the viral genes targeted by antiretroviral (ARV) drugs. However, particularly in the case of integrase (IN) strand transfer inhibitors (INSTIs), virological failure in some individuals can occur in the absence of drug-target gene mutations, indicating that mutations outside the target genes can contribute to drug resistance. To examine the pathway(s) by which HIV-1 develops high-level resistance to the INSTI dolutegravir (DTG), we performed long-term passaging of HIV-1 over nearly one year with an escalating concentration of DTG. In a manner independent of viral isolate and coreceptor usage, HIV-1 became resistant to DTG by sequentially acquiring mutations in Env and Gag-nucleocapsid (NC) in the absence of resistance mutations in IN. The selected NC mutations clustered in the zinc-finger domain and conferred modest (3-5 fold) resistance to INSTIs. An Env mutant, 7XEnv, containing seven substitutions (V85A, S162K, R298K, Q363R, A541V, V693I and G825E) exhibited faster-than-WT replication and resistance to multiple classes of ARVs, with the fold resistance being markedly higher for INSTIs. Viral transmission of 7XEnv through cell-cell contact is about 3-fold more efficient than WT, resulting in a higher MOI and reduced sensitivity to DTG. Viral infection using VSV-G-pseudotyped viruses over a range of MOIs revealed that INSTIs are more readily overwhelmed by high MOI compared to RT inhibitors. These findings demonstrate that a combination of mutations in Env and NC can confer high-level resistance to INSTIs in the absence of IN mutations. The Env mutations overcome inhibition by INSTIs through increased MOI mediated by highly efficient cell-cell transfer. These results advance the understanding of how HIV-1 can evolve resistance to ARVs in the absence of mutations in drug-target genes and provide new insights into the contribution of cell-cell transfer to viral replication and drug resistance.

Presenter: Yuta Hikichi, Visiting Fellow

Loss of STAT3 in B cells Heightens Expression of Antiviral Genes and Reduces Latency of Murine Gammaherpesvirus 68 *in vivo*

Chad H. Hogan[1,2], Glennys V. Reynoso[3], Camille Khairallah[4], Heather D. Hickman[3], Brian Sheridan[4], Nancy C. Reich[4], Laurie T. Krug[2,4]

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Abstract

Constitutive activation of the host transcription factor STAT3 is a hallmark of cancers associated with the oncogenic gammaherpesviruses Epstein-Barr virus and Kaposi sarcoma herpesvirus. We utilize a murid pathogen, murine gammaherpesvirus 68 (MHV68) to investigate host determinants of gammaherpesvirus latency and immune control. We determined that loss of STAT3 in B cells of $CD19^{cre/wt}STAT3^{f/f}$ mice leads to a ten-fold reduction in peak latency. In addition, $CD19^{cre/wt}STAT3^{f/f}$ mice infected with MHV68 exhibit disorganized germinal centers (GC) and a heightened virus-specific CD8 T cell response 16 days post infection compared to WT ($STAT3^{f/f}$) littermates. To circumvent the immune dysfunction observed in the B cell STAT3 knockout (KO) mice and directly evaluate intrinsic roles for STAT3, we created a competitive model of infection by generating mixed bone marrow chimeras, reconstituted from $CD19^{cre/wt}STAT3^{f/f}TmRed^{stopf/f}$ and control $CD19^{cre/wt}$ mice. STAT3 KO B cells had a ten-fold reduction in latency compared to their WT counterparts in the same animal. This is the first demonstration of an intrinsic requirement for STAT3 to support gammaherpesvirus latency in the host. To define the STAT3-dependent transcriptional profile, we sorted for GC B cells with or without STAT3, and with or without MHV68-H2bYFP infection. RNAseq analysis comparing infected KO and WT cells revealed 677 differentially expressed host genes (316 downregulated and 362 upregulated), with no difference in viral gene expression. Gene set enrichment analysis identified significant hits in pathways involving apoptosis, cell cycle regulation, and interferon signaling. Thus, STAT3 promotes a gene signature conducive to latency, promoting B cell survival and proliferation, while counteracting interferon stimulated antiviral responses. STAT3-responsive host gene targets provide mechanistic insights into the role of STAT3 as a latency determinant in B cells for oncogenic gammaherpesviruses, and detail potential therapeutic targets for the prevention and treatment of virus-driven malignancies.

Presenter: Chad Hogan, CRTA Graduate Student Fellow

ELUCIDATING THE ROLE OF IP6 IN HIV-1 ASSEMBLY, MATURATION, AND INFECTION.

Alex Kleinpeter[1], Nadine Renner[2], Donna Mallery[2], Yanan Zhu[3], K.M. Rifat Faysal[4], Till Böcking[4], Adolfo Saiardi[5], Peijun Zhang[3], Leo James[2], Eric Freed[1]

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Abstract:
HIV-1 replication is dependent on the assembly of two metastable protein complexes. The immature Gag lattice (IGL) assembles at the plasma membrane of an infected cell, promoting the production of an immature virus particle. The viral protease then cleaves the Gag polyproteins that comprise the IGL, resulting in its disassembly. The liberated capsid protein (CA) then assembles into a conical structure called the capsid, which encloses and protects the viral genome and enzymes required for productive infection of a new cell. The conversion of an immature virus particle containing an IGL to a mature virion containing a capsid is called maturation. In recent years, the cellular metabolite inositol hexakisphosphate (IP6) has been identified as a key cofactor for HIV-1 replication. Importantly, IP6 binds, stabilizes, and promotes the assembly of both the IGL and the capsid, making the elucidation of its functions vital to a deeper understanding of HIV-1 assembly, maturation, and infection. Here we describe HIV-1 Gag mutants that assemble in an IP6 independent manner. Although competent for particle assembly, these mutants exhibit severe infectivity defects because they are unable to package IP6 during IGL assembly, leading to defects in capsid formation during maturation. These results support a model in which IP6 is specifically enriched into immature virus particles via IGL binding. IP6 is then released from its binding site upon Gag cleavage, allowing the newly liberated IP6 to promote capsid assembly, rendering the particle infectious. Importantly, our identification of a mutant that does not require IP6 for IGL assembly and virus particle production suggests that IP6 is not an intrinsic requirement for IGL assembly. Rather, the recruitment of IP6 by the IGL during particle production is principally a mechanism to ensure that IP6 is incorporated into particles to fulfill its indispensable function: promoting capsid assembly, maturation, and virion infectivity.

Presenter: Alex Kleinpeter, Postdoctoral Fellow, HIV Dynamics and Replication Program

A Bipartite NUP153 Motif Plays a Critical Role in Nuclear Import of HIV-1 Cores

Sushila Kumari,[a] Qi Shen,[b,c,d] Ryan C. Burdick,[a] Chaoyi Xu,[e] Sooin Jang,[f,g] Qiancheng Xiong,[c,d] Chunxiang Wu,[b] Swapnil C. Devarkar,[b] Taoran Tian,[d] Therese N. Tripler,[b] Yingxia Hu,[b] Shuai Yuan,[b] Joshua Temple,[b] Qingzhou Feng,[c,d] C. Patrick Lusk,[c,h] Alan N. Engelman,[f,g] Juan R. Perilla, [e] Chenxiang Lin,[c,d] Yong Xiong [b], and Vinay K. Pathak [a]

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Abstract

Recent studies from our lab show that human immunodeficiency virus type 1 (HIV-1) intact cores can cross the nuclear pore complex (NPC) and disassemble inside the nucleus. However, little is known about how the cone-shaped capsid interacts with the nucleoporins (NUPs) in the nuclear pore for traversing through the nuclear pore complex (NPC). A bipartite motif containing both canonical and noncanonical interaction modules was identified within the C-terminal 65 amino acids of NUP153. A previously unidentified triple-arginine (RRR) motif at NUP153 C-terminus targeted HIV-1 capsid strongly at the interface of tri-hexamers. However, how these motifs facilitate HIV-1 nuclear import was not known.

To examine the role of these motifs in nuclear import, we knocked down endogenous NUP153 in HeLa cells, expressed exogenous mRuby-tagged WT or mutant NUP153, and determined the effect of FTFG and RRR mutations on HIV-1 core nuclear import, association with NPCs, and infectivity. We observed that endogenous NUP153 was efficiently depleted and that expression of WT and mutant mRuby-NUP153 was robust. mRuby-NUP153 WT and mutants were primarily localized to the nuclear envelope and reversed the cytotoxicity induced by endogenous NUP153 depletion. Infectivity, nuclear import of viral cores labeled with green fluorescent protein (GFP), and stable association of the GFP-labeled viral cores with the NPCs were reduced ~2-fold in cells expressing the double mutant (FTFG>4A + RRR>3A) or single mutant (FTFG>4A or RRR>3A) NUP153 compared to cells expressing WT NUP153. Our results show that infectivity was reduced in mutant NUP153-expressing cells because of the decrease in nuclear import, which was likely due to reduced stable docking of the HIV-1 cores with the NPCs. The interaction of RRR and FTFG motif with HIV-1 CA is further confirmed by co-pelleting assay in the cells expressing mRuby-tagged WT or mutant NUP153. NUP153 Double mutant (FTFG>4A + RRR>3A) or single mutant (FTFG>4A or RRR>3A) are defective for binding to HIV-1 CA.

Overall, the studies show that HIV-1 core interactions with NUP153 FTFG and RRR motifs are critical for stable viral core docking with the nuclear envelope and nuclear import. In addition, these studies establish a cell-based assay to examine the role of NUP153 and other NPC proteins in HIV-1 nuclear import.

Presenter: Sushila Kumari

Innate Immune Correlates Of Cell- Associated HIV RNA And DNA During Long-Term Suppressive ART

Chuen-Yen Lau [1], Robert Gorelick [2], Thuy Nguyen[1], Matthew Adan[1],Jessica Earhart [1], Jeannette Higgins [2], Catherine Rehm, Robin Dewar [2], Ariana Savramis[1], Danielle Konlian[1] , Deborah McMahon [3], Anuradha Ganesan [4], Brian Luke [2], Frank Maldarelli [1], National Institutes Health

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Abstract:

Persistence and clonal expansion of HIV-infected cells during long-term antiretroviral therapy (ART) prevents HIV cure in persons living with HIV (PLWH). Replication- competent and -incompetent proviruses persist during ART, and both may contribute to HIV pathogenesis, but mechanisms driving their persistence remain unclear. To investigate the potential role of immune-activation in persistence of proviral populations, we analyzed cell-associated HIV RNA and DNA levels in the context of cellular immunophenotyping. Clinical information and peripheral blood mononuclear cells (PBMC) were obtained from PLWH in IRB-approved protocols undergoing ART for ≥ 3 years. Levels of cell-associated HIV LTR and *gag* RNA and DNA were measured by multiplexed droplet digital PCR. PBMC were analyzed in flow cytometry panels quantifying >20 lymphocyte subsets and activation markers. We analyzed clinical, immunophenotyping and virologic data with parametric and nonparametric methods to detect significant correlates and identify composite sets of parameters with highest Pearson correlation by multilinear regression. Participants (N=73, 11% female, average age 49.9 [range 20-70], median CD4 = 639 [range 250-1765] cells/μl at sampling) had undergone continuous ART for median 14 (range 3-29) y. Levels of HIV LTR DNA (median 2500 copies/million CD4 cells) varied over 100-fold (59.9-52508 copies/million CD4 cells) and were highly deleted in *gag.* Regression analyses revealed levels of proviruses were strongly associated with proportion of CD8+ memory cells, duration of infection, and nadir CD4+ count (Pearson r=0.57, p<0.001). HIV RNA data was available from 58/73; levels of cell- associated LTR RNA varied >100-fold (0.02 - 4.3 copies HIV LTR RNA/provirus). In best fit analyses, levels of LTR RNA/provirus were highly correlated in models composed of %CD15+CD56+ natural killer (NK) cells in PBMC and age (Pearson r=0.59, p<0.01).Levels of cell associated HIV LTR RNA were strongly associated with proportion of NK cells in peripheral blood, suggesting innate immune sensing of HIV RNA.

Presenter: Chuen-Yen Lau, Staff Scientist, Clinical Retrovirology Section

Kaposi sarcoma patient-derived xenografts as a preclinical model for evaluation of novel therapeutic strategies

Xiaofan Li[1], Zoë Weaver Ohler[2], Amanda Day[2], Laura Bassel[2], Ralph Mangusan[1], Kathryn Lurain[1], Robert Yarchoan[1], Ramya Ramaswami[1], and Laurie T. Krug[1]

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Abstract

Kaposi sarcoma (KS) is a malignancy defined by hyper-angiogenesis, inflammatory infiltrates, and endothelial cells infected with KSHV. KS is an AIDS-defining malignancy and a major cause of morbidity and mortality in people living with HIV globally. KS may be successfully treated with relapse is frequent. The exploration of novel therapies is hampered by the lack of a preclinical animal model. Here we report on the status of KSHV infected endothelial cells upon single passage of KS patient-derived xenografts (PDXs) in immunodeficient NOD SCID gamma mice (NSG). Sixteen KS biopsies (13 cutaneous KS and 3 gastrointestinal (GI) KS) were implanted into 36 NSG mice (2-4 recipients per biopsy), all KS PDXs were maintained in recipient mice for long periods that ranged from 103 to 272 days, until the experimental endpoint. 1) Thus far, KS-PDX tumor sizes have not increased in the first or second passage. However, of significant note, the cellularity increased in KS-PDX explants compared to input biopsies based on staining of the human-specific NUMA-1 marker. 2) KS histological features including hyper-angiogenesis and slit-like structures were recapitulated in KS-PDX examined. 3) Moreover, based on analysis of KSHV LANA as a percentage of human NUMA-1+ cells, LANA+ cells in KS-PDX explant samples were consistently and sometimes dramatically elevated compared to levels in the input biopsy. 4) The Ki67 proliferation marker and sporadic expression of viral IL-6 overlapped with LANA+ areas, and more importantly, immunofluorescence revealed that most Ki-67 positive cells were also LANA positive. Taken together, the successful maintenance of KSHV-infected endothelial cells with proliferative potential suggests the patient-derived cutaneous KS xenografts may serve as a pre-clinical model to test novel and patient-personalized therapies.

Presenter: Xiaofan Li, Ph.D., Research Fellow

PROVIRAL POPULATIONS PERSISTING IN GALT DURING LONG-TERM ANTIRETROVIRAL THERAPY ARE HIGHLY DELETED

E Madeen[1], T Nguyen[1], R Gorelick[2], ME Zipparo[1], L Adams[1], S Hill[1], M Adan[1,3], N. Wulan[1,4], CY Lau[1], F Maldarelli[1]

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Abstract:
Antiretroviral therapy (ART) controls but does not cure HIV infection, and HIV infected cells persist in blood and anatomic compartments even after long term antiretroviral therapy. Understanding persistence and clonal expansion of HIV-infected cells in tissue compartments is essential to developing ART-free HIV eradication and control strategies. In peripheral blood, the total level of proviruses remains stable over time while the relative proportion of all proviruses with deletions increases. The dynamics of HIV decay in tissues is not well understood. To investigate HIV proviral decay in lymphoid tissue we characterized levels of total and deleted proviruses in blood, ileum, and colon from HIV-infected individuals undergoing long-term ART.

Volunteers (N=6) undergoing suppressive ART for >3 years underwent concurrent phlebotomy and research colonoscopy for sampling of ileum and colon. Peripheral blood mononuclear cells (PBMC) were obtained by ficoll separation; single cell suspensions of colon and ileum were obtained using collagenase digestion procedures. HIV DNA was measured using multiplexed droplet digital PCR assay for HIV LTR and *gag*; CCR5 copy number was used to normalize HIV copy numbers per million host cells. Levels of HIV DNA species and LTR/*gag* ratios were analyzed using Pearson correlation and Wilcoxon non-parametric analyses.

Approximately 30 biopsies from each volunteer were obtained from ileum and colon. Levels of HIV LTR and *gag* DNA were detectable all individuals in PBMC; in contrast, a number of individuals had levels of HIV DNA below limit of quantification in ileum or colon. Proportion of deleted proviruses, measured by ratio of LTR/*gag*, was higher in ileum or in colon than in PBMC in many individuals. The relative loss of *gag*-containing proviruses in gut suggests a role for tissue-specific factors shaping the proviral landsacape.

Presenter: Erin Madeen, Cancer Prevention Fellow

Genome-wide regulation of KSHV RNA splicing by viral RNA-binding protein ORF57

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Abstract

We previously showed that Kaposi's sarcoma-associated herpesvirus (KSHV) ORF57 protein is a viral splicing factor promoting viral lytic gene expression. Using RNA-seq we compared the splicing profile of viral RNAs in KSHV-infected cells carrying a wild-type (WT) versus the cells containing an ORF57 knock-out (57KO) KSHV genome. We identified 269 RNA splicing events in the WT and 255 in the 57KO genome, including the splicing events spanning large parts of the viral genome and the production of vIRF4 circRNAs. No circRNA was detectable from the PAN region. We found that the 57KO alters the RNA splicing efficiency of targeted viral RNAs. The two most susceptible RNAs to ORF57 splicing regulation are the K15 RNA with eight exons and seven introns and the bicistronic RNA encoding both viral thymidylate synthase (ORF70) and membrane-associated E3-ubiquitin ligase (K3). ORF57 inhibits the splicing of both K15 introns 1 and 2. ORF70/K3 RNA bears two introns, of which the first intron is within the ORF70 coding region as an alternative intron and the second intron in the intergenic region between the ORF70 and K3 as a constitutive intron. In the WT cells expressing ORF57, most ORF70/K3 transcripts retain the first intron to maintain an intact ORF70 coding region. In contrast, in the 57KO cells, the first intron is substantially spliced out. Using a minigene comprising of ORF70/K3 locus, we further confirmed ORF57 regulation of ORF70/K3 RNA splicing, independently of other viral factors. By monitoring protein expression, we showed that ORF57-mediated retention of the first intron leads to the expression of full-length ORF70 protein. The absence of ORF57 promotes the first intron splicing and expression of K3 protein. Altogether, we conclude that ORF57 regulates alternative splicing of ORF70/K3 bicistronic RNA to control K3-mediated immune evasion and ORF70 participation in viral DNA replication in viral lytic infection.

Presenter: Vladimir Majerciak, Associate Scientist

Role of Monocytes, CTL and NK cells in primary HTLV-1 infection

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Abstract:

The immune responses that inhibit HTLV-1 infection are still unknown and their identification is critical for the development of an effective HTLV-1 vaccine. We investigated the impact of monocytes, NK cells, and $CD8^+$ T-cells in primary HTLV-1 infection by depleting cell subsets and exposing rhesus macaques to either HTLV-1 wild type ($HTLV\text{-}1_{WT}$) or to the $HTLV\text{-}1_{p12KO}$ mutant unable to infect replete animals due to the lack of expression of the viral gene, *orf-I*.

The *orf*-I encoded for the viral proteins p8/p12 which counteract cytotoxic NK and $CD8^+$ T-cells *in vitro* and favor viral DNA persistence in monocytes. Double NK and $CD8^+$ T-cells or CD8 depletion alone accelerated seroconversion in all animals exposed to $HTLV\text{-}1_{WT}$. In contrast, $HTLV\text{-}1_{p12KO}$ infectivity was fully restored only when NK cells were also depleted, demonstrating a critical role of NK cells in primary infection. Monocyte/macrophage depletion resulted in accelerated seroconversion in all animals exposed to $HTLV\text{-}1_{WT}$, but antibody titers to the virus were low and not sustained. Seroconversion did not occur in most animals exposed to $HTLV\text{-}1_{p12KO.}$ *In vitro* experiments in monocytes comparing $HTLV\text{-}1_{WT}$ and $HTLV\text{-}1_{p12KO}$ demonstrated that *orf-I* expression is associated with inhibition of inflammasome activation in primary cells, and decreased monocyte engulfment of infected cells.

Collectively, our data demonstrate a critical role for innate NK cells and a dual role of monocytes in primary HTLV-1 infection. On one hand, *orf-I* expression increases the chances of viral transmission, and on the other may protect the engulfed infected cells by modulating inflammasome activation. Once infection is established, *orf-I* expression may contribute to the chronic inflammation observed in patients by downmodulating monocyte engulfment. Lastly these data suggest that an effective vaccine needs to engage also innate responses able to promptly eradicate infected cells and avoid the establishment of a vicious cycle that leads to chronic inflammation.

Presenter: Ramona Moles, Postdoctoral Fellow

CAPSID-CPSF6 INTERACTIONS THAT REGULATE KINETICS OF HIV-1 NUCLEAR IMPORT

Mohamed Husen Munshi[1], Ryan C. Burdick[1], Shuang Guo[3], Kunio Nagashima[5], Xiaolin Wu[3], Stephen H. Hughes[4], Wei-Shau Hu[2], and Vinay K. Pathak[1, *]

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Abstract

A cure for acquired immunodeficiency syndrome (AIDS) remains a global challenge. Human immunodeficiency virus type 1 (HIV-1) must integrate its DNA into the host genome, which requires nuclear import of intact viral cores containing viral nucleic acid. HIV-1 cores, composed of viral capsid protein (CA), reside at the nuclear envelope (NE) for ~1.6 hours in contrast to cellular cargos, which are imported within milliseconds. Interaction of the viral cores at the NE with host nuclear protein cleavage and polyadenylation specificity factor 6 (CPSF6) is essential for nuclear import, but how the cores are imported through nuclear pore complexes (NPCs) is not understood. To identify the CA determinants important for nuclear import, we analyzed nuclear import kinetics of several CA mutants (M10I, M10V, and I15V) located at or near the capsid surface) by live-cell imaging of fluorescently labeled viral cores, and determined that the M10I and M10V mutants, but not the I15V mutant, exhibited a longer NE residence time (~2.8 hours) compared to WT (~1.6 hours).

To investigate the role of CPSF6 in nuclear import of the M10I mutant, we constructed HeLa cell lines that expressed low levels (~50% of HeLa cells) or high levels (~200% of HeLa cells) of CPSF6 and determined the nuclear import kinetics of WT and mutant viral cores. In the Low-CPSF6 cells, the M10I mutant was highly defective in nuclear import, whereas in the High-CPSF6 cells, the NE residence time defect was reversed and the M10I mutant was imported with WT kinetics. After nuclear import, the M10I CA mutant and WT showed preferential integration into speckle associated chromatin domains (SPADs) whereas A77V CA mutant, which does not bind to CPSF6, shows preferential integration into lamina associated chromatin domains (LADs). In Low-CPSF6 cells, both WT and M10I CA mutant were similar to A77V and exhibited preferred integration into LADs. These results suggest that the M10I mutant viral core has weaker affinity towards CPSF6 compared to the WT core. This defect is exacerbated in Low-CPSF6 cells and results in failure of nuclear import, whereas in High-CPSF6 cells the defect is overcome and the NE residence time is restored to WT levels. The M10I mutant had lower infectivity in primary activated CD4+ T cells, suggesting that the M10I mutant is defective for interaction with additional host factor(s) that are critical for nuclear import. In summary, we have identified a CA mutant with a novel phenotype of long NE residence time which can be modulated by the levels of CPSF6 in infected cells.

Presenter: Mohamed Husen Munshi

CHARACTERIZATION OF HIV INTACT AND NEAR-FULL LENGTH DEFECTIVE PROVIRAL POPULATIONS DURING LONG-TERM SUPPRESSION ON ANTIRETROVIRAL THERAPY

Thuy Nguyen[1], Lindsey Adams[1]*, Mary-Elizabeth Zipparo[1],Annie Glassey[1], Erin Madeen[1], Ulisses Santamaria[2], Catherine A Rehm[3], Jessica Earhart[1], Shawn Hill[1], Chuen-Yen Lau[1], Frank Maldarelli[1]

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Abstract:
The persistence of the HIV reservoir is a barrier to cure. Differential decay rate of replication-competent (intact) and incompetent (defective) proviruses during antiretroviral therapy (ART) suggests selective pressure of therapy and the immune system on the reservoir. To investigate the role of ART in shaping the landscape during ART, we performed a comparative analysis of intact and defective near-full length (NFL) proviruses prior to and following long-term ART.

Peripheral mononuclear blood cells were collected longitudinally from 9 participants at pretherapy (n=8) and multiple timepoints during ART (n=17, 0.5-20 years on ART). We sequenced single genomes of near full-length proviruses > 7kb (Illumina MiSeq). Viral population structure was analyzed using phylogenetic and population genetics analyses .

We obtained 950 NFL proviruses (348 intact, 602 defective) from participants. The median percentage of intact/total PCR-amplified proviruses was 17.56% at pretherapy and 1.63% during ART. A range of 0-12.50% of intact proviruses were present in identical sequences at pretherapy and 0-73.68% on ART. Relative levels of clonally expanded populations varied; 0-11.11% of the defective NFL were clonally expanded at pretherapy and 0-50% on ART. No significant differences in diversity were found between intact and defective populations at pretherapy vs long-term ART in any participant. Two participants harbored phylogenetically distinct intact populations between pretherapy and during ART.

We observed similar frequencies of identical sequences in intact and defective HIV proviruses at pretherapy and during ART. We did detect distinct intact and defective NFL proviral populations in longitudinal samples from pretherapy to long-term ART in several participants. Differential decay of intact and defective proviruses is not explained by either the composition of the viral genome or by ART duration alone, suggesting immune mediated mechanisms may contribute to their elimination.

Presenter: Thuy Nguyen, Clinicl Retrovirology Section, HIV Dynamics and Replication Program

Transient activation in HTLV-1-infected macaques treated with pomalidomide

Anna Gutowska[1], Katherine McKinnon[2], Sarkis Sarkis[1], Massimiliano Bissa[1], Ramona Moles[1], James D. Stamos[1], Mohammad Arif Rahman[1], Robyn Washington-Parks[1], David Davis[3], Robert Yarchoan[3], Genoveffa Franchini[1], Cynthia A. Pise-Masison[1]

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Abstract

HTLV-1 persists in the host despite a vigorous immune response that includes cytotoxic T cells (CTL) and natural killer (NK) cells, suggesting the virus has developed effective mechanisms to counteract host immune surveillance. We recently showed that *in vitro* treatment of HTLV-1-infected cells with the drug pomalidomide (Pom) increases surface expression of MHC-I, ICAM-1, and B7-2, and significantly increases the susceptibility of HTLV-1-infected cells to NK and CTL killing, in an effect dependent on viral and *orf-I* expression. We reasoned that by restoring cell surface expression of these molecules, Pom treatment has the potential to reduce virus burden by rendering infected cells susceptible to NK and CTL killing. We used the rhesus macaque model to determine if Pom treatment of infected individuals activates the host immune system and allows recognition and clearance of HTLV-1-infected cells. We administered Pom (0.2 mg/kg) orally to four HTLV-1-infected macaques over a 24 day period and collected blood, urine, and bone marrow samples throughout the study. Pom treatment caused immune activation in all four animals and a marked increase in proliferating $CD4^+$, $CD8^+$, and NK cells as measured by Ki-67^+ cells. Activation markers HLA-DR, CD11b, and CD69 also increased during treatment. While we detected an increased frequency of cells with a memory $CD8^+$ phenotype, we also found an increased frequency of cells with a Treg-like phenotype. Concomitant with immune activation, the frequency of detection of viral DNA and the HTLV-1-specific humoral response increased as well. In 3 of 4 animals, Pom treatment resulted in increased antibodies to HTLV-1 antigens as measured by western blot and p24Gag ELISA. Consistent with Pom inducing immune and HTLV-1 activation, in the urine of all animals we measured elevated markers of inflammation and leukotrienes LTB4 and LTE4. Despite an increase in plasma LTB4, no significant changes in plasma cytokine/chemokine levels were detected. In all cases, however, cellular populations, LTB4, and LTE4 decreased to baseline or lower levels two weeks after cessation of treatment. These results indicated that Pom treatment induces a transient HTLV-1-specific immune activation in infected individuals, but also suggest Pom may not be effective as a single-agent therapeutic.

Presenter: Cynthia Pise-Masison, Senior Associate Scientist

HIV vaccine candidate efficacy, augmented via enhanced NK and monocyte function and decreased T-cell activation, mediated by zinc protein inhibitor SAMT-247

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ABSTRACT

UNAIDS reports that 1.5 million people became newly infected in 2020, with 50% of them females. Approximately 5,000 women aged 15-24 become infected with HIV every week worldwide and, in sub-Saharan Africa, 6 out of 7 new HIV infections in occur in adolescents girls. cy. We developed a vaccine prime-boost approach, based on virus like particles (VLPs) with deleted V1 delivered by a DNA/ALVAC platforms and boosting with monomeric DV1gp120 in alum, that protects from SIV_{mac251} infection approximately 50% of macaques. Here, we tested whether combining the virucidal activity of a drug (SAMT-247) that targets the HIV/ SIV nucleocapsid protein, with vaccination augments protection against SIV_{mac251}. We immunized thirty-eight female macaques. Five weeks after the last immunization all animals were exposed to weekly intravaginal SIV_{mac251} challenges for a total of 14 week. Four hours prior to each challenge exposure, 20 of the vaccinated animals were treated vaginally with 0.8% SAMT-247 in HEC gel, and the remaining 18 animals with HEC gel only. Two additional groups of non-immunized animals (6 each) were treated either with SAMT-247/HEC gel or HEC gel at 4 hours prior to viral exposure as controls. All animals were challenged until infection was confirmed by nanodroplet PCR. Vaccine alone decreased the risk of virus acquisition by 65% (p=0.0074). Strikingly, the vaccine+SAMT-247 combination afforded a 92.7% reduction in the risk of virus acquisition when compared to controls ($p<0.0001$. The vaccine+SAMT-247 combination differed significantly from the vaccine-only group (p=0.006;) and protected from infection 16 of 20 animals (80%). AT the dose used, treatment with SAMT-247 alone did not significantly decrease the risk of virus acquisition. Vaccine efficacy results from the engagement by the CCR2/CCL2 anti-inflammatory axis (M2 monocytes) that orchestrates V2 antibody responses, recruitment of NKp44 IL-17^+ cells to mucosal sites and efferocytosis (see also Bissa et al. ; Stamos et al.: Silva de Castro et.al abstracts). We found here that SAMT-247 synergized with vaccination also by augmenting immune effectors of protection via cellular mobilization of zinc, a master regulator of immunity. Thus, delivery methods of SAMT-247 aimed to maintain effective drug concentrations in human vaginal mucosa, such as a controlled release of drug by intravaginal ring, combined with the DNA/ALVAC/gp120/alum vaccine regimen, may result in durable protection against HIV.

Presenter: Arif Rahman, Staff Scientist

Evaluating HIV-1 Gag kinetics with RNAs using switchSENSE technology

Constance Rink, Tomas Kroupa, Siddhartha A.K. Datta, Alan Rein

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Abstract

During HIV-1 virus assembly the genomic RNA (gRNA) is selected for packaging, despite the presence of a large excess of cellular mRNAs. gRNA is preferentially packaged because it contains the packaging signal Psi. Interestingly, Recombinant Gag protein binds with nearly equivalent affinity to RNAs with or without Psi under physiological salt conditions (Webb et al., RNA, 2013; Comas-Garcia et al., eLife, 2017). Since selective packaging could not be explained by special affinity of Gag for Psi, we hypothesized that binding to Psi leads to nucleation of assembly more efficiently than binding to other RNAs (Rein, Trends in Microbiol., 2019).

To evaluate this hypothesis, we have investigated the kinetics of Gag binding to different RNAs, using the switchSENSE DRX^2 instrument, which measures rates of association to and dissociation from immobilized RNAs. We measured the association rate of Gag binding in physiological salt buffer to four different RNAs (145nt): wild-type Psi; monomeric Psi; a Psi mutant in which multiple G residues, important in Gag binding, were replaced by A's; and scrambled RNA. We found that Gag binds to wild-type and monomeric Psi RNAs somewhat more rapidly than the other RNAs.

We then investigated if Gag binding kinetics could be localized to a region within Psi. We measured Gag association rates in physiological salt with RNAs comprised of either one or two of the three stem-loops of Psi. The RNA containing Psi stem-loops 2 and 3 exhibited significantly faster association rates than scrambled RNA, demonstrating the existence of differential Gag binding kinetics within Psi. In contrast, we observed no significant differences in rates of Gag binding to individual Psi stem-loops. Our data is all consistent with the hypothesis that Gag binding kinetics contributes to Gag selective packaging of viral RNA.

Presenter: Constance Rink, Postdoctoral Fellow

In vitro and *in vivo* infectivity of engineered HTLV-1A/C chimeric virus

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Abstract

HTLV-1 type C differs genetically from HTLV-1 type A, and is endemic in indigenous populations of Central Australia and Oceania. Both HTLV-1A and HTLV-1C are associated with T-cell Leukemia and TSP/HAM. However, HTLV-1C appears to be more frequently associated with life threatening lung manifestation than HTLV-1A. The contribution to the lung disease of virus genetic factors or co-morbidities and population genetic remains at present unclear. Differences between HTLV-1A and HTLV-1C include the lack of the translational initiation codon in *orf-I* and several amino acid changes in *orf-II* and *HBZ* that overlap with *orf-I.* Since HTLV-1A infection of macaques, a natural model of infection, requires *orf-I* expression to counteract *in vivo* cytotoxic NK and CD8+T-cells, we investigate whether HTLV-1C could expresses *orf-I* by alternative splicing. To do so we engineered two chimeric HTLV-1A/C molecular clones by inserting into the pAB HTLV-1A backbone either only the HTLV-1C *orf-I, II* genes (HTLV-1A/$C_{oI\text{-}II}$) or the *orf-I, II, III*, *IV* genes and the *3'LTR* (HTLV-1A/$C_{oI\text{-}L}$).

We found that all the un-spliced, singly and doubly spliced mRNAs identified in HTLV-1A are also expressed by both chimeric viruses. We found that in HTLV-1A/C *orf-I* is expressed via a doubly spliced mRNA that juxtaposes the first exon of *rex,* and its ATG in frame to *orf-I.* In HTLV-1C this *mRNA* encodes a 16KDA protein (p16). Furthermore, we demonstrated that both molecular clones can be produced by the chronically infected 729.6 B-cell line and can be transmitted to human cord blood and PBMC primary cells. Next, we tested the HTLV-1A/C infectivity in macaques and compared that to HTLV-1A, in replete conditions, or following depletion of NK, CD8 and monocyte. An understanding of the HTLV-1A/C infection consequences in macaques, if paired with equivalent results in humans, will provide a suitable animal model for testing approaches to treat and prevent inflammation associated with HTLV-1A and C.

Presenter: Sarkis Sarkis, Postdoctoral Fellow

Efficacy of Daratumumab Against Primary Effusion Lymphoma

Prabha Shrestha[1], Yana Astter[1], David A. Davis[1], Ting Zhou[2], Constance M. Yuan[2], Ramya Ramaswami[1], Hao-Wei Wang[2], Kathryn Lurain[1], Robert Yarchoan[1]

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Abstract:

Primary effusion lymphoma (PEL) is an aggressive non-Hodgkin lymphoma caused by Kaposi sarcoma-associated herpesvirus (KSHV). PEL has no standard therapy, and the median overall survival is poor with many patients experiencing refractory disease. PEL consists of plasmablast- like B cells that generally express CD38, the target of anti-tumor monoclonal antibody daratumumab, which is FDA-approved for use in multiple myeloma. Here, we assessed daratumumab's anti-tumor activity in PEL. We first performed flow cytometry on 8 patient- derived PEL cells and on 5 PEL-derived cell lines and each expressed varying levels of CD38.

Dara bound to CD38 on PEL cell surfaces with a saturating concentration of <1 μg/mL. Despite high CD38 expression, Dara did not induce human complement-dependent cytotoxicity (CDC) of PEL cell lines. Inhibiting two of the complement-inhibitory proteins (CIPs), CD55 and CD59, using neutralizing antibodies led to partial but significant increases in Dara-induced CDC of PEL cell lines suggesting that high levels of CIPs are at least partially responsible for the resistance of PEL cell lines to Dara-induced CDC. We then tested the ability of Dara to induce antibody- dependent cell-mediated cytotoxicity (ADCC) of PEL lines. Dara induced significant and dose- dependent increases in ADCC, particularly those lines with high CD38 levels. Two FDA- approved drugs, all trans-retinoic acid (ATRA) and pomalidomide, significantly increased surface CD38 levels in low-CD38 expressing PEL lines resulting in increased Dara-induced ADCC. Finally, we treated two patients with refractory PEL with Dara with or without pomalidomide and assessed their parameters on protocol 01-C-0038 (NCT00006518). One with leptomeningeal PEL had a complete response to Dara and pomalidomide. The other had improvement in performance status and resolution of malignant ascites with Dara alone. Taken together, these data support the exploration of the use of Dara in PEL either alone or in combination with ATRA or pomalidomide.

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Presenter: Prabha Shrestha, Staff Scientist

VLPs with V1 deleted envelope protect against SHIV infection

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Abstract:

We have previously demonstrated that vaccination with the DNA/ALVAC/gp120+Alum platforms delivering SIV envelope immunogens engineered by V1 deletion to favor the α-helix conformation of V2, was superior to wild type (WT) envelope immunogens in decreasing the risk of $SIVmac_{251}$ acquisition in macaques. In here we investigate whether a similarly engineered vaccine based on HIV also differed from WT-based envelope immunogens in immunogenicity and efficacy. We created a ΔV1 gp160 DNA vaccine by deleting V1 (ΔV1 gp160) in the envelope of HIV clade A/E (HIV A244) and expressed also the corresponding ΔV1 A244 gp120 protein in CHO cells. DV1 or WT gp160 combined with p57Gag (which generate Virus Lke Particles (VLP) DNA vaccine were given at week 0, 4, followed by two immunizations with ALVAC-HIV at week 8 and 12; one WT or ΔV1 HIV A244 gp120 protein boost in alum was given at week 12. Two months following the last immunization animals were exposed to 11 weekly low doses of SHIV 1157(QNE) Y173H intrarectally. The ΔV1 immunogens decreased the risk of SHIV 1157 (QNE) acquisition by 81% compared to controls, whereas the WT immunogens did not, recapitulating the results obtained with SIV-based vaccines. Analyses of immune response elicited by the HIV A244WT and A244ΔV1 immunogens revealed several differences. Immunization with DV1, elicited higher V2-specif ADCC, and more efficient efferocytosis, responses correlating with the decreased risk of virus acquisition also in the SIV model (see also Bissa et al. Abstract). Similarly, the frequency of mucosal CD14 + cells, IgG envelope specific B-cells (that correlated directly with V2-specific ADCC), CCR2+ and of CD73+ macrophages was higher in DV1 that WT immunized animals. The two immunization regimens differed also in the level of troglodytes, that was higher in WT immunized animals and correlated negatively with V2-specific ADCC. Thus, for reasons that are unclear at present, we conclude that V1 affects protective responses against HIV and further studies will be needed to address the mechanism(s).

Presenter: Isabela Silva de Castro, Research Fellow

The uracil-DNA Glycosylase of murine gammaherpesvirus 68 binds cognate viral replication factors independently of its catalytic domain

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Abstract

The human gammaherpesviruses Epstein-Barr virus (EBV) and Kaposi sarcoma herpesvirus (KSHV) are cancer-causing viruses. Each herpesvirus encodes enzymatically active homologues of host enzymes involved in nucleotide metabolism and DNA repair (viral Uracil-DNA glycosylase, vUNG). We use the murine gammaherpesvirus 68 (MHV68) as a model pathogen to inform lytic processes and pathogenesis of EBV and KSHV. Our lab demonstrated that an MHV68 vUNG knockout virus (vUNG.STOP) was deficient for viral replication *in-vivo* compared to the wildtype virus. In contrast, a mutant virus that expressed a catalytically inactive vUNG (vUNG.CM) had no replication defect. The discrepancy between the loss of fitness observed following infection with the vUNG.STOP virus and the replication of the vUNG.CM suggests that the vUNG has non-enzymatic properties that are critical for replication. The uracil-DNA glycosylases of other herpesviruses localize to viral replication compartments and interact with their respective viral DNA polymerase (vPOL) and viral DNA polymerase processivity factor (vPPF). I hypothesize that the MHV68 vUNG interacts with its cognate viral DNA replication factors, vPOL and vPPF, and that the enzymatic domain of vUNG is not required for this interaction. Immunofluorescence imaging demonstrated that vUNG has overlapping localization with both the vPOL and vPPF in viral replication compartments of the nucleus, independent of the vUNG catalytic domain. Co-immunoprecipitation following 293T transfection revealed that vUNG interacted with both the vPOL and vPPF independently of its catalytic domain. Last, the interaction of vUNG with vPOL and vPPF were validated in the context of lytic replication in infected fibroblasts by IP-mass spectrometry. We conclude that vUNG localizes and interacts with the vPOL and vPPF at viral replication compartments in an enzymatic independent manner. Our findings support a model wherein this complex is critical to repair uricylated viral DNA at nascent origins of replications to promote fitness in the host.

Presenter: Kyle R. Smith, CRTA PhD Candidate

Opposing effect on SIV_{mac251} acquisition of monoclonal antibodies recognizing SIV V2 in an α–helix or β-sheet conformation

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ABSTRACT

Antibodies to the variable region 2 (V2) of HIV are the primary correlates of decreased risk of HIV acquisition in the efficacious RV144 human vaccine trial and the equivalent SIV macaque model. Notably, the V2-specific monoclonal antibodies NCI05 and NCI09 recognize and bind to distinct conformations of overlapping V2 peptides adjacent to the main $\alpha_4\beta_7$ binding site; NCI05 targets an α-helical epitope while NCI09 targets a β-hairpin linear epitope. Here, we investigated the effects of passive administration of NCI05 and NCI09 on the per-challenge risk of SIV_{mac251} using mAbs cloned from the B-cells of a vaccinated macaque that resisted 22 low-dose intrarectal challenges with SIV_{mac251} for more than a year. Administration of NCI05 or NCI09 to macaques prior to infection did not decrease the risk of mucosal SIV_{mac251} acquisition compared to controls. However, NCI05 mucosal levels and ADCC correlated with delayed SIV_{mac251} acquisition, whereas NCI09 mucosal levels contributed to faster acquisition. NCI05 and NCI09 similarly inhibit gp120 binding to $\alpha_4\beta_7$, but only NCI05 inhibits V2 co-stimulation of $CD4^+$ cells, suggesting that antibodies to the V2 α-helix conformation contribute to vaccine efficacy, but are not sufficient to protect against virus acquisition. Concurrently, NCI09 more effectively mediates higher levels of ADCC, ADCP and trogocytosis, a monocyte function that contributes to immune evasion via the nibbling of cell surface antigens on infected cells. These data suggest that NCI05's superior ability to inhibit T-cell activation and NCI09's stronger induction of trogocytosis are at the basis of their divergent effects on the risk of SIV_{mac251} acquisition.

Presenter: James D. Stamos, Pre-doctoral CRTA Fellow

Viromes of bladder cancers of solid organ transplant recipients and people living with HIV reflect relative disease risk

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Abstract:

Solid organ transplant recipients (SOTR) are at a significantly increased risk for developing bladder cancer compared to the general population. Growing evidence points to a viral origin in a substantial fraction of these bladder tumors due to immune suppression and subsequent uncontrolled viral replication. However, people living with HIV (PLWH), who are at an increased risk for various virus-mediated diseases, are not at an increased risk for developing bladder cancer. This suggests that immune suppression alone is insufficient to promote bladder cancer development. To address whether the tumor viromes are substantially different between these two immunosuppressed populations, we performed whole genome and total RNA sequencing of 43 bladder tumors of SOTR and 15 bladder tumors from PLWH. Nearly half of tumors from SOTR contained viral sequences. The most common were from BK polyomavirus (N=9, 21%), JC polyomavirus (N=7, 16%), carcinogenic human papillomaviruses (N=3, 7%), and torque teno viruses (N=5, 12%). Viral integration analysis and immunohistochemistry against Large T antigen expression indicate that BKPyV was clonally integrated into the majority of tumors in which it was detected. In tumors from PLWH, only torque teno viruses were detected in two patients (13%). This is more similar to the general population where DNA virus sequences, especially polyomaviruses are rarely detected in bladder tumors. Together our results suggest that polyomaviruses and papillomaviruses contribute to carcinogenesis and the overall increased risk of bladder cancer in SOTR. It is unclear why these viruses are absent in PLWH, but the introduction of these viruses from the donor graft to a naïve host may be one explanation.

Presenter: Gabriel Starrett, Stadtman Investigator

CRISPR gene editing to target latent EBV Infection

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Abstract:

Epstein Barr Virus (EBV) is an oncogenic human gammaherpesvirus that establishes latency in B cells. EBV drives higher rates of cancers in people living with HIV and immunosuppressed transplant recipients. Many EBV+ lymphomas depend on the viral genome for survival and proliferation, but restricted viral gene expression during latency limits potential targets for therapy. CRISPR editing to damage or eradicate the viral genome presents a powerful strategy for treating latent infection. However, off-target editing of the host genome is an important consideration for clinical use. To address this issue, we use an obligate homodimer CRISPR construct containing the FokI nuclease fused to a catalytically dead cas9 (FokI-dCas9). This molecule requires proper positioning of two guide RNAs (gRNAs) for editing to occur, theoretically reducing the incidence of off-target edits. Preliminary screening of gRNA pairs in EBV+ HEK293 cells demonstrated successful editing of the EBV B95.8 genome at predicted loci upon delivery of each of the seven gRNA pairs tested. I also verified knockdown of the key latency EBNA1 viral protein in infected cells expressing EBNA1-specific gRNAs. We hypothesize that combinations of gRNAs targeting multiple regions of the EBV genome will more effectively reduce viral genome copy number than single gRNA pairs. I am currently developing viral vectors to more efficiently deliver the CRISPR components. I will examine the impact of combining the gRNA pairs for multiplex editing in EBV+ cells including HEK293, Burkitt lymphoma cells, and lymphoblastoid cell lines. We expect combinatorial edits that reduce viral genome copy number will correlate with loss of viability of EBV-dependent lymphomas. Alongside examining viral genome copy number and cell viability, I will assess the ability of EBV to reactivate and transform B cells. We expect combinatorial edits that target oncogenes and accrue EBV genomic damage will reduce reactivation and infectivity.

Presenter: Victoria Stepanyants, Postbaccalaureate Cancer Research Training Award Fellow, HIV and AIDS Malignancies Branch

Guanylate binding protein 5: Impairing virion infectivity by targeting envelope glycoproteins

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Abstract

Guanylate binding protein (GBP) 5 is an interferon-inducible cellular factor with a broad antiviral activity, reducing the infectivity of progeny virions by interfering with processing and incorporation of the viral glycoproteins. It is believed to inhibit the infectivity of viruses such as HIV-1, highly pathogenic influenza A, and dengue by reducing the proteolytic activity of furin. However, the exact mechanism by which GBP5 inhibits processing of viral glycoproteins and whether it only affects glycoproteins with a furin-dependent processing remains poorly understood.

HIV-1 Δ*env* luciferase reporter viruses were pseudotyped with either HIV-1 envelope (Env), SARS-CoV Spike (S) or SARS-CoV-2 S glycoprotein in 293T cells and purified by sucrose cushion; particle infectivity was determined by measuring the luciferase activity. GBP5 was found to reduce HIV-1 Env, SARS-CoV S, and SARS-CoV-2 S incorporation and virion infectivity in a concentration-dependent manner. To determine the effect of GBP5 on glycoproteins further, we analysed virus- and cell-associated proteins by an SDS-PAGE. GBP5 expression resulted in increased mobility of HIV-1 Env, SARS-CoV S, and SARS-CoV-2 S glycoproteins, suggesting an effect on glycoprotein glycosylation. To confirm that GBP5 affects glycoprotein glycosylation, we treated cell-associated lysates with PNGase endoglycosidase. PNGase treatment resulted in the same mobility shift of glycoproteins as observed in the presence of GBP5, indicating that GBP5 indeed affects protein glycosylation.

Our work provides evidence that GBP5 not only inhibits protease cleavage of viral glycoproteins but also affects protein glycosylation. Furthermore, using S protein of both SARS-CoV and SARS-CoV-2, our work also shows that GBP5 reduces glycosylation of glycoproteins regardless of whether they require a furin-dependent or furin-independent processing. These findings expand our understanding of the cytoplasmic trafficking of the viral glycoproteins. Elucidating the exact mechanism by which GBP5 modulates glycosylation of viral glycoproteins could uncover a novel target site for the development of an antiviral drug.

Presenter: Hana Veler, Postdoctoral Research Fellow (Visiting)

Rationale for the use of pacritinib in KSHV-multicentric Castleman disease

Yiquan Wu, Victoria Wang, and Robert Yarchoan

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Abstract:
The interleukin-6 (IL-6)/JAK/STAT3 pathway plays important role in primary effusion lymphoma (PEL) and Kaposi sarcoma herpesvirus (KSHV)-associated multicentric Castleman's disease (MCD), which is driven by KSHV-infected B cell plasmablasts. We explored the potential of different JAK inhibitors for use in PEL and KSHV-MCD. As shown by ATP assay, flow cytometry and trypan blue counting, 1 μM pacritinib efficiently inhibited cell growth and induced apoptosis of PEL cell lines, and it was superior to the other JAK inhibitors tested. Several cellular kinases including STAT3 were inhibited, as revealed by the kinase profile array and STAT3 ELISA. In addition to JAK2, pacritinib targets FLT3 and IRAK1; the possibility that these might contribute to the effect in PEL using small molecule inhibitors and siRNA knockdown were explored. Several inhibitors and siRNAs targeting FLT3 also inhibited the growth of PEL cells in vitro, suggesting that FLT3, which is involved in B cell development, may play a role in pacritinib's growth inhibition of PEL. mRNA sequencing and RT-PCR showed that KSHV viral genes including RTA and LANA, and some key host genes including several cyclins and IL-6 were downregulated by pacritinib. Finally, pacritinib suppressed KSHV viral-IL6(vIL-6)-induced IL-6 production by peripheral blood mononuclear cells, which may model an important step in the pathogenesis of KSHV-MCD. These results suggest that pacritinib warrants testing for the treatment of KSHV-MCD and PEL.

Presenter: Yiquan Wu, Research Fellow (Yi-quan.wu@nih.gov)

Selective expression of HPV oncogenes from a single integration site in cervical cancer tissues and cell lines with multiple viral integration sites

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Abstract

Integration of a virus genome into human chromosomal DNA is critical in papillomavirus-induced carcinogenesis. Here we provide a striking evidence that the virus-host fusion RNA transcripts primarily are derived from a single integration site in clinical cervical cancer tissues having hundreds of HPV integration sites and in three cervical cancer cell lines, CaSki, SiHa and HeLa cells having multiple HPV DNA integration sites. The host genomic elements that are near the integrated HPV DNA are critical for efficient expression of the viral oncogenes and clonal expansion of viral integrated cells. The expression of viral oncogenes E6 and E7 involves in selection of a host 3' splice site (s) and/or host RNA polyadenylation signal. We discovered that specific siRNA knockdown of the host RNA portion of the virus-host fusion transcripts could abolish the expression of viral E6 and E7 proteins, inhibit cell growth and promote cell senescence in HPV16+ CaSki and HPV18+ HeLa cells. These observation of how the expression of virus genomes integrated at specific host genomic sites is controlled provides our further understanding of HPV carcinogenesis and may provide new opportunities for precision medicine.

Presenter: Lulu Yu, Research fellow