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HIV RESERVOIR MAINTENANCE BY B CELL-MEDIATED TRANS INFECTION OF NAÏVE T CELLS
Abigail D Gerberick¹, Nicolas Sluis-Cremer², Paolo Piazza¹, Diana DeLucia¹, Charles Rinaldo¹ and Giovanna Rappocciolo¹
¹Department of Infectious Diseases and Microbiology, Graduate School of Public Health and ²School of Medicine, University of Pittsburgh

Background: The latent HIV reservoir established early in infection is a major barrier in curing HIV infection. Within the CD4T cells that form part of the reservoir, CD4T naïve (TN) cells are always found to harbor virus DNA and produce more virus upon in vitro reactivation compared to other CD4T cell subsets. Paradoxically, TN isolated from peripheral blood are resistant to direct HIV infection in vitro and do not express the HIV co-receptor CCR5, but R5 tropic HIV has been isolated from these cells ex vivo. Antigen presenting cells (APCs; B cells, dendritic cells (DCs) and macrophages) can mediate transfer of HIV to CD4T cells via a process termed trans infection and we have shown that APCs from HIV nonprogressors (NP) lack the ability to trans infect CD4T cells, unlike normal progressors (PR). Our aim was to determine susceptibility of TN to APC mediated trans infection with HIVBal, a CCR5 tropic HIV strain and the frequency of latently infected TN isolated from HIV-NP.

Methods: CD4T cells, B cells and monocytes were purified from PBMCs of seronegative donors by magnetic microbead separation. B cells were activated by CD40L/IL4 and DCs were differentiated from monocytes by GM-CSF/IL4 culture. TN were purified by negative selection and cultured in CCL19 for 2 days prior to testing. B cells and DCs were pulsed with HIV-1Bal and cultured with TN cells or CD4T cells at a 1:10 ratio. Co-culture supernatants were tested for HIV-1 p24 antigen by ELISA. Cell phenotypes were determined up to 16 days after co-culture by flow cytometry. HIV-NP and PR total CD4T cells and TN were purified by magnetic microbead separation and HIV-1 DNA was quantified via PCR.

Results: CCR5neg TN isolated form 10 seronegative donors were always found to be susceptible to B cell-mediated trans infection with HIVBal, but resistant to both cis infection and DC-mediated trans infection. Total CD4T cells were efficiently trans infected by both B cells and DCs. We detected significantly higher levels of HIV DNA in TN isolated from PRs compared to NPs, and significantly higher levels of HIV DNA in total CD4T cells compared to TN in NPs.

Conclusions: Our in vitro and ex vivo data reveal an important role for TN in the maintenance of HIV latent reservoirs and that B cell mediated trans infection of TN is the primary mechanism of HIV infection of these cells. Furthermore, we were able to detect significantly higher levels of HIV DNA in total resting CD4T cells of than in TN of HIV-NPs, suggesting that the absence of HIV reservoir in TN could lead to the ability of these individuals to control viremia in the absence of cART.
Hemoglobin variants influence *Plasmodium falciparum* sexual differentiation

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*Plasmodium falciparum* is the parasite responsible for most cases of severe malaria, a life-threatening disease that affects nearly half of the world’s population. The lifecycle of *P. falciparum* is dependent on asexual and sexual reproduction cycles in the human and Anopheles mosquito respectively. Sexually differentiated parasites arise from asexual parasites during the intraerythrocytic stage of infection in the human host and complete their development and reproduce within the Anopheles vector. Multiple epidemiological studies have shown that individuals with certain variants of hemoglobin (HbVAR), a population known to have tolerance to malaria disease, produce a greater number of sexually differentiated parasites during *P. falciparum* infection than individuals with the most common hemoglobin type, hemoglobin A (HbA). However, mechanistic studies determining the role of HbVARs on sexual differentiation have never been studied. In this study we designed a culture system that allows for infection of red blood cells expressing unique HbVARs with asexual *P. falciparum* parasites. Highly synchronized cultures of *P. falciparum* strain NF54 were established initially in HbA RBCs through multiple rounds of sorbitol synchronization and magnetic separation. Late-stage parasitized RBCs were then separated and placed in cultures of HbVAR RBCs. Parasite development and their subsequent sexual differentiation among HbVAR and HbA cultures were followed over the two-week culture period in which sexual stage parasites mature into either male or female gametocytes. Parasitemia was measured using flow cytometry, and proportions of sexual differentiation were determined. Our preliminary results show a significantly higher sexual differentiation rate among parasites grown in HbVAR RBCs compared to those differentiated in HbA RBCs (p=0.02). Our results provide the first direct evidence that the Hb type in RBCs influences sexual differentiation of *P. falciparum*. Though further studies using this *in vitro* culture system should be completed and more variants of HbVAR RBCs should be examined in order to confirm these results, our findings provide insight to a potential new mechanism of the initiation of sexual stage differentiation.
Rabies Virus: Understanding and Responding to the Public Health Problem

Rabies is a viral encephalitis that is incurable and belongs to the Rhabdoviridae family and genus Lyssavirus. Rabies virus is found all over the world, from poverty to upper-class, male to female, young to old, everyone can be affected by rabies. Lyssaviruses are all genetically related and have adapted to replicate within the central nervous system. Early on many researchers believed that only one of the species of the virus was able to cause rabies, but that has been debunked over the years to show that at least seven species of the virus can cause rabies. Rabies virus has a bulleted capsule and can range from 75nm to around 200nm with the membrane surrounded by a studded membrane. When inside the host, the lyssavirus has three tasks to complete. The first, gaining access to a host cell. Second, transcribe, translate, and replicate. Lastly, reassemble components and leave the cell. Rabies is found throughout the world, the main reservoir is rabid dogs, however, the order Carnivora have also been found to be reservoirs. Lyssaviruses are extremely fragile and cannot survive in the open environment, hence why a host is so important for the transfer of the virus. The main transfer of the virus is from a bite from an infected host; however, this is not the only way transfer occurs. Scratches, abrasions or open wounds exposed to saliva or infectious material are other possible modes of infection.
Systematic Analysis of Cross-Genus Horizontal Gene Transfer among Bacterial Pathogens in a Single Hospital

Background
Multidrug-resistant bacteria pose a serious health threat, especially in hospitals. Horizontal gene transfer (HGT) of mobile genetic elements (MGEs) facilitates the spread of antibiotic resistance, virulence, and environmental persistence genes between nosocomial pathogens. Despite recent advances in microbial genomics, studies of HGT in hospital settings remain limited in scope, predominantly as corollary analyses to genomic investigations of bacterial transmission. The objective of this study was to systematically identify and track the movement of MGEs by horizontal transfer within a single hospital system.

Methods
We screened the genomes of 2,173 bacterial isolates from healthcare-associated infections collected over 18 months and identified identical nucleotide regions in bacteria belonging to distinct genera. To further resolve the most prevalent shared sequences, we performed long-read sequencing on a subset of isolates to assemble highly contiguous genomes. Using these genomes, we resolved and tracked the presence of lineages of plasmids and chromosomally encoded MGEs throughout our genome dataset. Lastly, we cross-referenced our genomic findings with deidentified patient care data to make inferences on the epidemiology of the MGEs we resolved.

Results
Many of these genomes contained plasmids and chromosomal elements encoding one or more of the shared sequences we identified, which were often arranged in a mosaic fashion. We then tracked the appearance of ten different plasmids in all 2,173 genomes, and found evidence of plasmid transfer independent from bacterial transmission. Finally, we identified two cases of plasmid transfer between pathogens of different genera within individual patients, including one plasmid that was likely transferred to another pathogenic strain in a different patient. In this second case, analysis of patient care records supported the genomic evidence of transfer by revealing epidemiologic links between the two patients.

Conclusions
By combining whole-genome sequencing with epidemiologic data, we were able to infer cases of likely HGT within the hospital that would not have been identified by studying solely the transmission of bacterial pathogens. This work expands our understanding of HGT in healthcare settings, and demonstrates the utility of HGT-focused genomic epidemiology, which can inform efforts to limit the spread of drug-resistant pathogens in hospitals.
Seroprevalence of Spotted Fever Group Rickettsial Antibodies in Humans in Uganda using Enzyme-Linked Immunosorbent Assay

Author: Dzigbordi Kamasa-Quashie - IDM  
Mentor: William Nicholson, PhD  
Practicum Site: Centers for Disease Control and Prevention, Rickettsial Zoonoses Branch

Description of The Problem: Rickettsioses, caused by obligate intracellular bacteria, are among the earliest known vector-borne diseases. Rickettsiae are traditionally classified into two groups: spotted fever group (SFG) and typhus group. These pathogens are transmitted via arthropod vectors such as mites, lice and most notably, ticks. While studied extensively in North America and other developed countries, little is known about the presence of rickettsial diseases in Sub-Saharan Africa, partially due to symptomatic similarities between other acute febrile illnesses such as malaria and leptospirosis. These similarities, along with inadequate diagnostic capacity, contribute to the misdiagnosis and underreporting of rickettsioses and other infectious diseases in this region. In recent years, Uganda has experienced multiple infectious disease outbreaks. As a suspected cause of febrile illness in this country, the need to understand the prevalence and epidemiology of rickettsioses is imminent in order to protect and improve the health of communities. Studies based in Uganda support the presence of SFG rickettsiae in domestic animals and ticks however, very few have focused on the identification of this pathogenic group in humans. This project aims to address that gap through the use of enzyme-linked immunosorbent assay (ELISA).

Objectives/Aims: To determine the nationwide prevalence of SFG rickettsial antibodies in human sera samples collected from Ugandans using ELISA.

Methods: This project evaluated sera specimens for serological activity to SFG rickettsial antibodies, as part of the 2011 Uganda National Acute Febrile Illness Agent Detection Study. All sera specimens were collected during the 2011 Uganda AIDS Indicator Survey. In order to create a sample representative of all districts in Uganda, households were selected using a cluster-based random sampling strategy. The survey population was comprised of both males and females, ranging from 15-59 years of age. All participants were either residents of the selected households or visitors of the household the night before the survey. Each participant voluntarily agreed to provide a blood sample. A total of 4,039 samples were received for testing however, due to volume insufficiency, 3992 were analyzed. All specimens were tested at a 1/100 dilution using a SFG rickettsia enzyme-linked immunosorbent assay IgG antibody kit per instructions from the manufacturer (Fuller Laboratories, CA).

Results: Overall, 1972 (49.4%) sera samples were reactive for SFG rickettsial antibodies. Comparatively, 1725 (43.2%) were non-reactive and 295 (7.4%) were equivocal or indeterminate. Higher prevalence rates were seen in males, older age groups and the rural population. Regional prevalence ranged from 35.0% to 64.8%, with the highest being seen in the Northeast region.

Conclusions: This is the second survey to examine a representative sample of human sera in Uganda. Additionally, the large sample size provides strength to the importance of SFG rickettsiae as emerging pathogens in this country. Due to the inability to differentiate between species via serology, additional research is needed in order to determine species prevalence.

Public Health Significance: Limited information regarding SFG rickettsioses in Uganda reinforces the importance of this study. The results of this study will aid in the development of prevention programs and the improvement of disease surveillance in regions where SFG rickettsiae are most prevalent.
ABSTRACT

Title: CARE PROVIDERS PERCEPTIONS OF HIV POSITIVE HAITIAN MIGRANTS NEEDS LIVING IN THE DOMINICAN REPUBLIC

Authors & Affiliation: Inngide Osirus, B.S.¹ - IDM, Jasmine Abrams, PhD²

Description of The Problem:

Human Immunodeficiency Virus (HIV) is one of the greatest global health challenges. While HIV is incurable, it is preventable and can be managed with treatment. Important aspects of HIV prevention include raising awareness, ensuring individuals know their status, and assisting HIV positive individuals with starting and remaining in HIV treatment and care. These primary and secondary prevention strategies steps are imperative to decreasing the overall burden of HIV in a community and globally. Using the HIV treatment cascade, a framework that outlines stages of HIV care, to drive prevention efforts is integral to achieving the 90-90-90 global HIV targets: 90% of all persons living with HIV (PLHIV) are aware of their HIV status, 90% of all PLHIV who know their status are receiving antiretroviral therapy (ART), and 90% of those on ART are virally suppressed. This represents one of the key monitoring strategies for supporting expansion and linkage of HIV care, treatment, and prevention services. Additionally, using the cascade can highlight gaps and progress along the continuum of care that can help scientists and practitioners determine the best opportunities for intervention.

The Centers for Disease Control and Prevention in the Dominican Republic named their priority population migrants of Haiti and Dominicans of Haitian descent, as this demographic group is diagnosed with HIV at higher rates in comparison to the general population and there are no official HIV prevention programs targeting Haitian migrants. This qualitative and quantitative needs assessment will explore barriers that exist in HIV testing and gaps in the treatment cascade for Haitian migrants in the Dominican Republic.

Objectives/Aims:

The objective of the key informant interviews of medical doctors and community health workers were to explore barriers to HIV prevention and treatment among Haitian migrants. With that knowledge, the needs assessment allowed us to explore the needs of Haitian migrants living with HIV in the Dominican Republic to help determine appropriate intervention strategies.

Method(s) Used/Approach Taken:

Key informant interviews with directors/staff of community-based, governmental, and academic organizations (n = 13) to better understand:

- Systemic, social, and individual level barriers to HIV testing
- Factors associated with retention in care
- Factors associated with treatment interruption
- Explore future directions/interventions to decrease disease burden

Qualitative data were thematically analyzed through the following steps:

- Become familiar with data by reading and rereading interview notes
- Use NVIVO 12 to create codes from key informant interviews
- Use a thematic analysis approach to generate themes from codes
- Define and name themes

Public health records were reviewed to determine:

- Rates of HIV among different populations in the Dominican Republic
- Rates of HIV testing
- Rates of engagement along the treatment cascade among PLWH

Results:

Results of thematic analysis conducted with data generated from the key informant interviews, revealed the need for intervention with Haitian migrants at the national level. One interviewee suggested doing this through the framework of a Human Security Model for Haitian migrants living in the Dominican Republic. Collectively, interviewees stated that the program needs to address: 1) human rights (to decrease discrimination and increase access to services) 2), cultural humility and stigma reduction training for Dominican medical providers, and 3) international care coordination (between Haiti and the Dominican Republic) and 4) education for persons living with HIV/AIDS. Additionally, interviewees recommended having more educational opportunities for providers and patients, Haitian Creole translators at treatment centers, and gender-based violence interventions.

Discussion/Implications/Conclusions:

The results demonstrate that Haitian migrants in the Dominican Republic have many unmet needs and that effectively addressing HIV among this demographic group will require an intersectional approach between human rights policy, systems level trainings, social support, and increased educational and employment opportunities.
Student investigator: Julianne Sangimino, VMD

- Alumnus of the University of Pennsylvania School of Veterinary Medicine (class of 2017). Current MPH candidate at the University of Pittsburgh Graduate School of Public Health in the Department of Infectious Diseases & Microbiology (anticipated graduation: spring 2021).

Mentors:

1. Dr. Shelley C. Rankin, PhD - The University of Pennsylvania School of Veterinary Medicine, Professor of Clinical Microbiology.
2. Dr. Jeremy Martinson, DPhil - The University of Pittsburgh Graduate School of Public Health, Assistant Professor of Infectious Diseases and Microbiology.
3. Dr. Jonathan Lustgarten MS, PhD, VMD - VCA Animal Hospitals, Senior Biomedical Informatics Specialist.

Working title: A predictive model of CRE transmission dynamics and community prevalence based on geospatial analysis of canine patients who tested positive at the Ryan Veterinary Hospital at the University of Pennsylvania

Project description/abstract:

Carbapenem-resistant Enterobacteriaceae (CRE) are gram-negative, rod-shaped bacilli such as E. coli, Klebsiella, Salmonella, and Shigella that exhibit resistance to carbapenem antibiotics through a variety of chromosomal mutations and acquired resistance mechanisms. In addition to carbapenems, they are frequently resistant to other classes of antibiotics, and “pan-resistant” CRE have been reported. The mortality rate for human patients with CRE infections is high - up to 50% in some studies - and the prevalence of these infections is rising, especially in health care settings. These “superbugs” pose an urgent and increasing threat to public health, yet there are critical gaps in our understanding of their transmission dynamics and overall prevalence in non-health care settings such as neighborhoods and communities. If these gaps are not addressed, our understanding of CRE will be stunted, and the prognoses for those affected will remain grim given the current lack of effective treatment for CRE infections.

Like other MDR infections, CRE infections are not unique to the human population. Dogs that have been colonized or infected with CRE have been reported by the University of Pennsylvania’s Ryan Veterinary Hospital in Philadelphia, Pennsylvania. At this time, it is unclear how these dogs were exposed to CRE - whether from a “point source” (i.e. a shared location in their environment, such as a hospital or boarding facility), or whether they are transmitting it between one another and potentially other species. To date, the prevalence and geospatial distribution of CRE has not been studied in the canine population, nor has the potential impact of CRE-positive pets on human infection and transmission dynamics.

This study will use existing individual-level spatial information (as well as data that will be collected over the upcoming summer) to create a geospatial map of CRE-positive cases around the Ryan Veterinary Hospital using Esri ArcGIS software. Additionally, we will incorporate what is known about the genomic variation and molecular epidemiology of these individual cases. Using Kernel density estimation and Kriging interpolation, our primary aims will be to make
evidence-guided, geospatial predictions for a) the overall prevalence of these pathogens in the community, b) transmission dynamics within the canine population, and c), if applicable, the projected trajectory for the spread and dissemination of CRE throughout the community. It is our goal that ultimately such models can be merged with CRE data from the human health care perspective to help define the role of CRE-colonized canines in human CRE infection and transmission cycles.
Abstract Title
Analyzing the Barriers to Reaching the Joint United Nations Programme on AIDS (UNAIDS) “90-90-90: Treatment for All” Targets in the Caribbean

Authors & Affiliation
Laura Dytrt, MPH Candidate, University of Pittsburgh Graduate School of Public Health

Problem/Issue:
The Caribbean region has the second highest prevalence of people living with HIV/AIDS in the world, with large differences in prevalence between countries. The Caribbean faces many barriers to reaching the UNAIDS “90-90-90: Treatment for All” goals. Identifying these gaps can improve HIV care services and contribute to the UNAIDS goal of ending the AIDS epidemic by 2030.

Objectives/Specific Aims:
The objectives of this essay are to:
   I. Analyze the barriers to care at each step of the HIV care continuum in the Caribbean using the social ecological framework as a guide.
   II. Analyze best practices and policies in other nations that have been successful in achieving 90-90-90 targets and apply them to the context of the Caribbean.

Research Methods/Approaches:
To achieve these objectives issue analysis supplemented by a literature review will be used. The issue analysis will focus on identifying and analyzing standard policies and practices in the Caribbean that relate to HIV care and acceptance in the region. The literature review will be conducted to determine best policies and practices related to the HIV care continuum to identify what policies and practices can be applied to the Caribbean.

Results/Outcomes:
Barriers to achieving the UNAIDS 90-90-90 targets occur at policy, community, institutional, interpersonal, and individual levels. These barriers include polices that discriminate against sexual and gender minorities widespread stigma, gender-based violence, different approaches to testing and linkage to care, and differences in accessibility of preventative measures. Best practices that can be implemented in the Caribbean include removal of discriminatory, improvements in continuity of HIV care, and addressing the socioeconomic and structural factors that contribute to individual HIV transmission.

Discussion:
Conclusions
Understanding barriers to each step of the HIV care continuum in any setting can identify areas where improvements can be made in an effort to both reduce new HIV infections and eliminate AIDS as a public health concern.

Limitations
Limitations include an overall lack of data and research about HIV in the Caribbean, qualitative data that relied on self-report measures, and inability to access certain data sources. All of these factors could have influenced the results of this analysis.

Implications for Public Health
The implications of this project will be to improve public health policy and practice in the Caribbean in areas related to HIV care.
Marina S. Levochkina - IDM

Title: High Neutrophil-Lymphocyte-Ratios and Positive Infection Status After Traumatic Brain Injury Increases Hospital Resource Utilization and Worsens Long-term Outcomes

Authors: Marina S. Levochkina*, Leah E. Vaughan*, Shannon Trombley, Jason J. Calcagno, Amy K. Wagner

*Co-authors

Affiliations:
1. Department of Physical Medicine and Rehabilitation, University of Pittsburgh
2. Safar Center for Resuscitation Research, University of Pittsburgh
3. Clinical and Translational Science Institute, University of Pittsburgh

Abstract:

Individuals who suffer a moderate-to-severe traumatic brain injury (TBI) are at an increased risk of developing an acute infection, neutrophilia, and lymphopenia. Studies have shown that developing these conditions all independently lead to longer times on mechanical ventilation and longer hospital stays, thereby increasing their chances in acquiring complications such as organ dysfunction and sepsis which further leads to morbidity and mortality. This also puts a severe financial and psychological burden on the patient and understanding the extent that these states affect outcome will bring awareness to the secondary injuries related to TBI. We hypothesized that a high Neutrophil-Lymphocyte-Ratio (NLR), which indicates a state of lymphopenia and neutrophilia, coupled with presence of infection in the first 21 days post trauma will lead to both longer ventilation times and longer hospital stays. We also postulated that high NLR in the acute phase is associated with a lower six-month Glasgow Outcome Score (GOS) mediated by infection status. We assessed neutrophilia, lymphopenia, and infection status by using electronic medical records to retrospectively collect absolute lymphocyte and neutrophil counts along with collecting positive microbiology cultures. Other clinical variables included: worst in 24 hour post-injury Glasgow Coma Scale (GCS), Injury Severity Score (ISS), mechanism of injury, hospital days, days requiring mechanical ventilation, and GOS at six months post-injury. We utilized group-based trajectory (TRAJ) analysis to represent the dynamics of NLR values over time and to stratify individuals into low (n=192) and high(n=81) NLR subgroups. Both TRAJ groups were similar in age, race, mechanism of injury, best in 24 hour post-injury GCS, and ISS. Compared to the high TRAJ group members, those in the low TRAJ had 48% greater odds of acute infection, longer length of hospital stay (18.2±1.0 vs. 14.0±0.9; p=0.0048), more days requiring mechanical ventilation (14.86±1.08 vs. 8.59±0.90; p < 0.001), and a higher proportion of unfavorable outcomes at 6 months post-injury (56% vs 44%; p=0.0399). Kaplan-Meier estimates suggested that the time to first infection was significantly
earlier for a greater proportion of the high NLR TRAJ compared to the low NLR TRAJ (p=0.0114). ANCOVA analysis was conducted to compare high and low NLR TRAJ groups with positive and negative infection status while covarying for demographic and clinical injury factors (age, CT, injury type, and GCS). These results yielded that patients with high NLR and presence of infection had significantly longer ventilator days (t=0.70) and longer hospital length of stays (t=0.97). Binomial logistic regression analysis was done in assessing the association between NLR TRAJ membership to six-month GOS. Positive infection status accounted for 17% (mediation percentage) of the relationship between NLR TRAJ and six-month GOS (p=0.0128). It is clear that there is a coupled relationship between NLR and infection status post-TBI that is affecting the patient not only in the acute phase but also into the chronic phase. Despite recent improvement in the acute moderate-to-severe TBI mortality rates, it is evident that more needs to be done to understand the mechanisms behind the cellular response post-TBI and acquiring concomitant infections and their subsequent burdens on global outcomes.
Megan Arden, IDM

Title: Utilizing performance management data to inform infectious disease program planning at a local health department

Background: Performance management is a systematic process that helps an organization achieve its mission and strategic goals. As part of the accreditation process, the Allegheny County Health Department (ACHD) implemented a formal performance management system in 2015 to track goals and objectives across all programs and services. The performance management system tracks a list of metrics that are used to generate the overall program that helps leverage funding through the local public health administration law (ACT 315). Within the Bureau of Community Health Promotion and Disease Prevention, there are three Infectious Disease programs: Immunization, Sexually Transmitted Diseases and HIV/AIDS, and Tuberculosis.

Purpose: The goal of this project is to analyze performance management data for the three Infectious Disease programs to help set performance metrics for future years.

Methods: Performance management data was analyzed for all three programs, over the past two years, 2018 and 2019. Additionally, links to the Plan for a Healthier Allegheny (ACHD’s Community Health Improvement Plan), the ACHD Strategic Plan, Healthy People 2020, and 10 Essential Public Health Services were identified. This information was used to generate a performance management brief for each program.

Results: Overall, the Immunization and Tuberculosis programs achieved their proposed objectives and activities in 2019. The STD and HIV/AIDS program reported partial achievement on most objectives. Further analysis is needed to determine how many objectives across all three programs lined to the Plan for a Healthier Allegheny and supported Strategic Plan goals and initiatives.

Conclusion: The information from this analysis will help inform program planning activities for 2020 and beyond. Though there remains room for further improvement, the programs reported many improvements in their service delivery in 2019. In 2019, ACHD published a new strategic plan; the department is currently working on a new community health improvement plan to be released in 2020. This will provide programs with the opportunity to realign programmatic, departmental, and community priorities. Finally, performance management data is critical for quality improvement projects at ACHD; this data should be utilized to help determine quality improvement initiatives and inform progress moving forward.
NK cells: the key to successful HIV immunotherapies

Renee R. Anderko, Charles R. Rinaldo, Robbie B. Mailliard
Department of Infectious Diseases and Microbiology, University of Pittsburgh Graduate School of Public Health

The challenges associated with achieving total elimination of replication-competent HIV-1 have prompted a shift in focus to designing therapeutic strategies that effect a functional cure. Antibodies have garnered widespread interest recently for their potential importance in preventing and controlling HIV-1 infection. In particular, early administration of antibodies supported the generation of CD8+ T cell responses that mediated long-term control of viremia following discontinuation of ART, but the mechanism underlying this immune-mediated control remains unclear. To date, no study has identified natural killer (NK) cells as the definitive link between antibodies and durable T cell responses. Therefore, we explored the ‘helper’ role of NK cells in HIV-1 infection, hypothesizing that NK cells would provide dendritic cell (DC)-mediated ‘help’ in cellular immunity to HIV-1. Freshly isolated NK cells from virally suppressed HIV-1 seropositive individuals were co-cultured with monocyte-derived immature DCs in the presence or absence of innate stimuli or opsonized target cells. Responding NK cells were assessed for their ability to produce DC-modulating cytokines, and DCs harvested from the co-cultures were analyzed for their expression of maturation-associated surface markers, as well as their subsequent ability to induce type-1 immune responses. DC-derived co-stimulatory factors efficiently activated the ‘helper’ function of NK cells as evidenced by their robust production of IFNγ. Opsonizing antibodies also synergized with IFNα to promote NK ‘helper’ cell activity. Furthermore, interactions between NK cells and DCs induced the development of mature DCs with a heightened ability to produce IL-12 and to drive the development of anti-HIV-1 cellular immunity. Our data demonstrate the capacity of NK cells to enhance DC-mediated immune responses to HIV-1 and highlight the potential for harnessing the reciprocal crosstalk between NK cells and DCs in the design of novel anti-HIV-1 therapies.
Roberta S. Dos Reis¹, Shilpa Sant², Marc C.E. Wagner¹, Velpandi Ayyavoo¹*
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HIV-1-associated neurocognitive disorders (HAND) is characterized by neuroinflammation that trigger the pathogenic cascade resulting in neurodegeneration, leading to cognitive impairment. However, the molecular events underlying HIV-neuropathogenesis remain elusive, due to lack of brain-representative experimental models.

To fill this gap, we developed a 3D human brain organoid (hBORG) model containing neurons and astrocytes along with incorporation of microglia. Both infected and uninfected microglia infiltrated into hBORGs resulting in a triculture system (MG-hBORG) that mirror the multicellular network observed in in vivo. To investigate if our MG-hBORG model supports productive viral infection, we assessed viral titers and results indicate that virus release increased over time and plateaued at day 10 post-infection. These results were further supported by the assessment of expression of viral RNA and multiply spliced transcripts by RT-qPCR. Further, we measured the levels of TNF-α and IL-1β in the supernatant from MG-hBORG HIV-1+ cultures compared to mock culture. TNF-α levels were significantly elevated by 45-fold 3 days after microglia incorporation, which directly correlated with the peak of viral replication. In contrast, IL-1β secretion occurred at later time points, and is enhanced by 7-fold in HIV-infected MG-BORG compared to mock culture. Moreover, HIV-infection induced cell cytotoxicity in hBORG by 25.8% 15 days post infection. Assessment of the expression levels of the neuronal marker βIII-Tubulin demonstrated 2.3-fold decrease, indicating neuronal loss in infected MG-hBORG compared to mock.

Thus, our 3D model recapitulates many hallmarks of HIV-associated neuropathology, as inflammatory response and neurodegeneration, and offers great promise for basic understanding of how HIV-1 infection alters the CNS compartment and induces pathological changes, paving the way for the discovery of new biomarkers and therapeutic targets.
ABSTRACT FORM: Deans Day

REGULATION OF NEUROINFLAMMATORY FACTORS BY NEUROPROTECTIVE MICRONRNAS IN HIV-1 INFECTED MICROGLIA

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Description of The Problem:
Despite successful antiretroviral therapy, more than half of individuals living with HIV-1 exhibit HIV-1 Associated Neurocognitive Disorder (HAND). Individuals with HAND experience a spectrum of cognitive, motor, and/or mood dysfunctions. Currently, there is no treatment or prevention methods for HAND. HIV-1 virus enters through the blood brain barrier (BBB), and establishes infection in macrophages and microglial cells present in the central nervous system (CNS). Macrophages and microglia cells have been key target for HIV-1 in CNS. Exposure of neurons to viral proteins as well as the immune/inflammatory released by infected target cells during infection fuels the development of HAND. To combat this problem, we propose to target neuroinflammatory factors that have been identified to have a role in development of HAND using miRNAs. These miRNAs target biological pathways including homeostasis of cellular interaction, production of neurotoxic and neuroprotective factors, cell proliferation and differentiation. In this study, we explore the effects of these candidate miRNAs and their role in regulating the production of neuroinflammatory chemokines and cytokines. Through this knowledge, there will be an increased likelihood of developing novel therapeutic strategies and treatment for neurocognitive disorders.

Objectives/Aims:
The goal is to evaluate the regulation of specific miRNAs in inflammatory responses using HIV-1 infected microglia cell line.

I. Generate lentivirus expressing miRNAs and stably transduce microglia cell line (HCM3).
II. Investigate how lentivirus-expressing miRNAs regulate the expression of inflammatory factors in response to LPS and HIV-1 infection.

Method(s) Used/Approach Taken:
HIV-1 virus stock (YU2) was prepared with and without VSV-G-Env pseudo-type and tittered using TZM and U87 cells. Similarly, specific miRNAs expressing lentivirus vectors were generated for transduction of microglial cells. A titration assay was also performed on these viruses and analyzed through a fluorescence microscope. Microglia (HCM3) cell line was transduced with miRNA expressing lentiviruses and then infected with an MOI of 1.0 of HIV-1 virus. Supernatants were collected 24- and 72-hours post infection, as well as pre and post LPS treatment and amount of cytokines in supernatants was measured by ELISA.

Results:
We have observed higher titers in the lentivirus expressing miRNAs 20a* and 106b*. MiRNA-126 had a significantly low titer and was concentrated to ultimately meet similar titer ranges with other candidate miRNAs. After LPS stimulation, miR-106b showed reduced levels of TNF-α 72 hours post transduction suggesting neuroprotective roles of miR-106b in microglia cell line HCM3. All 4 candidate miRNAs presented a suppressive effect on IL-1β expression 72 hours post transduction and post LPS stimulation. Overexpression of miR-106b in microglia cell line HCM3 may have a suppressive effect on IL-1β expression as its levels are decreased after HIV-1 infection. Moreover, the overexpression of miR-106b decreased TNF-a expression, supporting the protective role of this candidate miRNA. There was an increasingly high levels of IL-6 secretion among all candidate miRNAs however from IL-6 within all samples even though they were diluted by 1:50 ratio.

Discussion/Implications/Conclusions:
HIV-1 infection dysregulates miRNA expression, thus altering the expression of host cellular factors that are either neurotoxic or neuroprotective in CNS. Understanding the therapeutic effects of these cellular miRNAs dysregulated by HIV-1 infection in microglia cells may provide information for the development of additional antiviral strategies.
Immunometabolism of Lung Granulomas in Non-human Primates with Tuberculosis

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Description of The Problem: Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), triggers the formation of granulomas in the host. Granulomas are composed of many different types of host immune cells including T and B lymphocytes, macrophages, and neutrophils. The metabolic pathways used by immune cells in granulomas are important for cell function and glycolytic metabolic pathways in granulomas may be involved limiting or promoting disease. By understanding immunometabolism in TB granulomas, we can improve diagnosis and potentially create new therapeutics to combat TB disease.

Objectives/Aims: To determine which cell subsets use glycolysis as an energy source in granulomas and identify potential drivers of glycolysis in granuloma macrophages.

Method(s) Used/Approach Taken: GLUT1 is a glucose transporter that transports glucose into the cell. To determine what cell subsets express GLUT1 in a granuloma, IHC was performed on lung granuloma from non-human primates (NHP) infected with Mtb. These slides were stained for macrophage markers including CD11c and CD163 and GLUT1 before being imaged by fluorescence microscopy. Image analysis was performed using ImageJ to determine the total area and percent area each cell marker occupied in granuloma cross sections, as well as co-localization between the macrophage markers and GLUT1. To determine a possible relationship between glucose uptake and mycobacterial antigens, we also performed a glucose uptake assay and hypoxic experiments using 2-NBDG on monocyte-derived macrophages that were stimulated with inactivated Mtb.

Results: Image analysis revealed co-localization of different cell markers and GLUT1. When looking at co-localization of CD11c and CD163 with GLUT1, we found that CD11c+CD163- epithelioid macrophages, the cells in granulomas that are most commonly infected with Mtb, expressed more GLUT1 than interstitial and alveolar macrophages. Moreover, we see evidence that macrophages increase their glucose (2-NBDG) uptake when stimulated with inactivated Mtb.

Discussion/Implications/Conclusions: We found that macrophages in TB granulomas express GLUT1 and increase glucose uptake in the presence of Mtb antigen. Through these experiments, we can better understand granuloma immunometabolism and can use this information to improve monitoring of disease progression or to develop new treatments for Mtb infection.