U.S. FOOD & DRUG ADMINISTRATION

Summary of Viral Diseases Research Programs of Laboratory of Emerging Pathogens

Scientific Site Visit Research Report
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Diagnosis and Pathogenesis of Filoviruses (FV) and Hepatitis A Virus (HAV)

Goals and Objective of this program:

Conduct mission-oriented research in Filoviruses and HAV in support of the safety of the blood supply in the US

Principal Investigator: Gerardo Kaplan, Ph.D.

Rationale for the work in FV and HAV

- Are major human pathogens that **can be transmitted by blood** and pose a significant risk to the safety of the blood supply in the US.

- Are **endemic** in Africa and the US, respectively, and have been **imported** to the US.

- Current FV outbreak in Congo and **unprecedent HAV epidemic in the US** that started in 2016 and spread to 37 states (approx. 44,000 cases, 27,000 hospitalizations, and 420 deaths).

- **FDA regulates FV and HAV products**, including vaccines, diagnostics, and blood donations.

- Kaplan Lab provide regulatory expertise, develop methods and reagents in support of products, and conducts research to inform regulatory decisions.

Projects under this research program

Filovirus Program (***plan to discontinue program in the near future***)

1. Developed Filovirus BSL-2 neutralization tests
2. Developed ultra-sensitive EBOV antigen test
3. Developed assays to determine EBOV sequence variation
4. Performed analysis of anti-EBOV T-cell immune response

HAV Program

1. Cell entry of HAV
2. HAV cellular receptor 1 (HAVCR1) as a cell entry factor for hepatitis C virus (HCV)
3. HAV infection by exosome mimicry

4-year Accomplishments

Filovirus publications

1) Konduru et al., 2018.
J Virol Methods 254:1-7

2) Zang et al., 2019.
Adv Mater 31:e1902331
(Impact Factor: 27.398)

3) Tiper et al., 2022.
PLoS One 17:e026373

4) Chabot et al., *2022.*
J Virol 96(18):e0116621

HAV publications

1) Costafreda & Kaplan 2018.
J Virol 92:e02065-17

2) Kachko et al., 2018.
J Virol 92:e01742-17

3) Costafreda & Kaplan 2019.
J Virol 93:e02040-18

4) Costafreda et al., 2020.
Nat Microbiol 5:1096-1106
(Impact Factor: 17.74)

Summary of Main Findings

- ✓ Discovery of the **exosome mimicry model of HAV infection**: cargo delivery of exosomes requires two lipid receptors, HAVCR1 and NPC1, but not a viral envelope.
- ✓ **Infectivity of exosomes from HAV-infected cells** is mediated by cargo delivery of **free viral RNA** and not intracellular uncoating of viral particles.
- ✓ This exosome mimicry pathway can be targeted for therapeutic interventions to prevent viral infection, modulate exosome-mediated treatments, and enhance mRNA vaccine delivery.

Proposed Future work

- ✓ Extend our knowledge on HAV cell entry to develop methods for pathogen reduction of non-enveloped viruses.
- ✓ Analyze clinical markers of HAV infection in plasma from serial donations obtained during the current HAV epidemic in the US.

Diagnosis and Pathogenesis of Hepatitis Viruses that Threaten the Safety of Blood and Related Products

Goals and Objectives:

1. Develop reference reagents to evaluate and standardize nucleic acid assays
2. Understand prevalence and impact of viral hepatitis in North America
3. Develop novel models for studying hepatitis virus infectivity and pathogenesis

Principal Investigator: David McGivern, Ph.D.

Development of reference reagents and standards for assays intended to detect viral nucleic acids

Background and Rationale: Well characterized Reference Panels permit evaluation of the analytical performance of different nucleic acid amplification tests intended to detect viral pathogens. Biological Standards allow for the interlaboratory standardization of tests.

Hepatitis E virus (HEV) secondary standards: HEV is an emerging pathogen that can be transmitted by transfusion.

- Developed heat-inactivated secondary standards for harmonization of Nucleic Acid Amplification Technology (NAAT) based assays for HEV detection.
- Calibrated standards against the WHO International Standard in a Collaborative Study involving 10 laboratories.[1]

Reference Panels for SARS-CoV-2:

(i) Cross-center collaboration with Center for Devices and Radiological Health to produce FDA Reference Panels for the evaluation of molecular diagnostic devices for SARS-CoV-2.

(ii) Participating Laboratory in the Collaborative Study for the Establishment of the 1st WHO International Standard for SARS-CoV-2 RNA.

[1]*Fares-Gusmao et al. J. Clin. Virol. 2022*

Understanding the prevalence and disease impact of viral hepatitis in North America

Background and Rationale: For persons infected with hepatitis B virus (HBV) and hepatitis C virus (HCV) disease outcomes are variable, but the underlying mechanisms are poorly characterized. The contribution of HEV to disease progression in persons with chronic liver disease is poorly understood.

Research Progress

- Characterized the prevalence and disease impact of acute HEV infection among persons living with chronic hepatitis B in the US and Canada.[1]
- Identified novel biomarkers of liver disease progression in viral hepatitis: In collaboration with colleagues at NIH, we have shown that the gene *Chitinase 3-like 1* (*CHI3L1*) encodes a profibrogenic factor, which is overexpressed in the aging liver and in HBV- and HCV-associated cirrhosis.[2]
- Developed a novel sandwich ELISA for detection of HEV ORF2 antigen in blood.[3]

[1]McGivern et al. Open Forum Inf Dis. 2019; [2]Nishimura et al. PNAS. 2021; [3]McGivern lab, unpublished.

Novel models for studying hepatitis virus infectivity and pathogenesis

Background and Rationale: HEV transmission and pathogenesis are poorly understood, in part due to the lack of small animal models of infection. For HBV, the lack of robust cell culture models has limited investigation of virus-host cell interactions.

Novel animal and cell culture models for hepatitis virus infectivity will facilitate studies of pathogenesis and biomarkers of infection.

Research Progress

- Established a gerbil model of acute HEV infection for transmission and pathogenesis studies.
- Established a novel model of chronic HEV using gerbils treated with the immunosuppressive drug tacrolimus.
- Established HBV and HEV cell culture infection systems based on primary human hepatocytes derived from chimeric mice with humanized livers.

McGivern lab, unpublished

Accomplishments 2018-2022

- **Selected publications from a total of 10 papers**:
  - HEV secondary standards for RNA detection: Fares-Gusmao et al. J. Clin. Virol. 2022
  - Host biomarkers of disease progression in chronic HBV and HCV: Nishimura et al. PNAS 2021.
  - Prevalence and impact of acute HEV among persons with chronic hepatitis B: McGivern et al. Open Forum Inf. Dis. 2019.

- Research Collaboration Agreements established with investigators at NIH and University of Southern California.

Future Directions

Apply next generation sequencing approaches in the gerbil model of acute and chronic HEV to define mechanisms of liver injury at the molecular level.

Develop standardized methods to evaluate the effectiveness of novel Pathogen Reduction Technologies against HBV and HEV using primary human hepatocytes from chimeric mice with humanized livers.

Evaluating Pathogenesis and Markers of Arbovirus Infections and Developing Reference Reagents to Improve Blood Safety

Goals and Objective:

1. Develop reference reagents to evaluate and harmonize nucleic acid assays
2. Studies on pathogenesis and identification of biomarkers of flaviviruses using primary isolates from asymptomatic infections
   a) Impact of genetic variability in flavivirus infectivity and pathogenesis
   b) Identification of biomarkers for differential detection of DENV, WNV and ZIKV

Principal Investigator: Maria Rios, Ph.D.

Research Project 1: Develop Reference Reagents to Evaluate and Harmonize Nucleic Acid Test (NAT) Assays

Mission Relevance: NAT assays are most sensitive and specific methods for blood screening and diagnostics. Reference reagents are regulatory research tools for evaluation, validation and harmonization of NAT assays

Project accomplished: Reference reagents for arboviruses and for blood group genotyping were produced, validated and available for stakeholders

- RNA Reference Reagents (RR) and/or International Standards (IS) for Emerging Arboviruses
  - WNV (RR), DENV-1 to 4 (IRR), CHIKV (IS) and ZIKV (RR)

- DNA Reference Reagents for Blood Group Genotyping – 18 members covering 42 blood group alleles
  - Endorsed as WHO International Reference Reagents for blood group genotyping

Project 2a: Study on DENV, WNV and ZIK using primary isolates: Impact of genetic variation in flavivirus infectivity and pathogenesis

Mission Relevance: Flaviviruses are pathogenic to humans, can be fatal and are transmitted by transfusion. There is no treatment or effective vaccine. WNV is endemic while imported cases of CHIKV, DENV and ZIKV are reported in the U.S.

Rationale: Laboratory adapted isolates MAY NOT represent the virus in nature. Primary isolates from asymptomatic infections should provide insights on events driving infection outcome

Major Findings on ZIKV:

- Comparative ZIKV study using cell lines and monocyte derived macrophage cultures showed augmented viral isolation using macrophage despite presence of antibodies. The study also revealed variability in isolation and growth patterns among 42 samples tested. *Sippert, Viruses 2019*
- Sequencing of 12 ZIKV isolates revealed 2 clades exhibiting genetic variability compared to reported clinical isolates (range: 8 to 54 nucleotide mutations and 2 to 11 amino acid substitutions per isolate) indicating a role of genetic variation in infection outcome. *Assis, Am J Trop Med Hyg 2020*
- Two stable full-length ZIKV-reporter-virus (ZIKV-nano Luciferase and ZIKV-GFP) were developed to study impact of genetic variability on infectivity and pathogenesis. *Volkova, Viruses 2020*

Project 2b: Identification of differential biomarkers for DENV, WNV and ZIKV infections

Mission Relevance: High antibody cross-reactivity among DENV, WNV and ZIKV impairs differential diagnostic, delaying proper treatment options and overall public health. NAT are highly specific but reliant to time-of-infection and viral-loads, and offer limited feasibility

Rationale: At an early stage, DENV, WNV and ZIKV infections are indistinguishable which could be asymptomatic or milder in nature, but can quickly progress to distinct severe disease with neurological or hemorrhagic manifestations, requiring differential diagnostic for proper medical interventions

Major Findings:

- A total of 45 immune biomarkers (cytokines, chemokines and growth factors) for discrimination of DENV, WNV and ZIKV were analyzed and found to be upregulated in asymptomatic-infections as compared to non-infected subjects: DENV (n=25/45); WNV (n=5/45) and ZIKV (n=7/45)
- TIM-1, IL-1ra, IL-4 and C3a were identified as potential biomarkers to differentiate among DENV, WNV and ZIKV infections. *Fares-Gusmao et al, Sci Rep 2019*

Ongoing study:

- miRNAs are under investigation as biomarkers for discrimination of DENV, WNV and ZIKV. Profiling ZIKV asymptomatic-infection identified of 21 up-regulated and 16 down-regulated miRNA compared to non-infected subjects. *Konduru, unpublished*

Accomplishments (2018-2022)

Selected articles (from a total of 17): 5 reviews, 2 chapters and 10 peer-review articles

- **Reference Reagents to Evaluate and Harmonize Nucleic Acid Test (NAT) Assays**

  Arboviruses: *Genome Announc, 2018; Transfusion, 2018; J. Mol. Diag., 2019*

  Blood Group Genotyping*: J. Mol. Diag., 2019; J. Mol. Diag., 2020; Annals of Blood. 2022*

- **Study on flaviviruses (DENV, WNV and ZIKV) infections using primary isolates: Genetics, infectivity, and pathogenesis** *Viruses 2019; Am J Trop Med Hyg 2020; Viruses 2020*
- **Identification of differential biomarkers for DENV, WNV and ZIKV infections** *Sci Rep 2019*

Research Collaborative Agreement: NIH/NHGRI

Major Collaborations: NIH/DTM; FDA/NCTR; FDA/CDRH; FDA/CBER/OTAT
FioCruz MG-Brazil; Several blood establishments

Outside OBRR Funding: FDA-MCMi and FDA-OMHHE total $500,000.00

Future Directions

- ZIKV-reporter-viruses will be used to investigate the impact of mutations on infectivity, replication and pathogenesis; use ZIKV-n-Luc to investigate the role of Ig in pregnancy in collaboration with Dr. Struble from OCGT
- Evaluate TIM-1, IL-1ra, IL-4 and C3a as differential markers in archived samples, and in vitro after infection of PBMCs and MDM with each viruses
- Evaluate identified miRNAs as discriminatory tool for DENV and ZIKV infection using repository samples.