LABORATORY OF RETROVIRUSES
DIVISION OF VIRAL PRODUCTS

VRBPAC March 6 2019

Hana Golding

LAB OVERVIEW

LR UNITS

Hana Golding, Ph.D. (PI and Lab Chief)

Unit of Viral Immunology and Pathogenesis

FTEs:

Marina Zaitseva Ph.D. (Staff Scientist)

Surender Khurana Ph.D. (Staff Scientist)

Jody Manischewitz, M.S., Tatiana Romantseva, MS, Lisa King, B.A.

ORISE Fellows: 6-7 postdoc and post-bacc / year

Arifa Khan, Ph.D. (PI)

Unit of Molecular Retrovirology

FTEs:

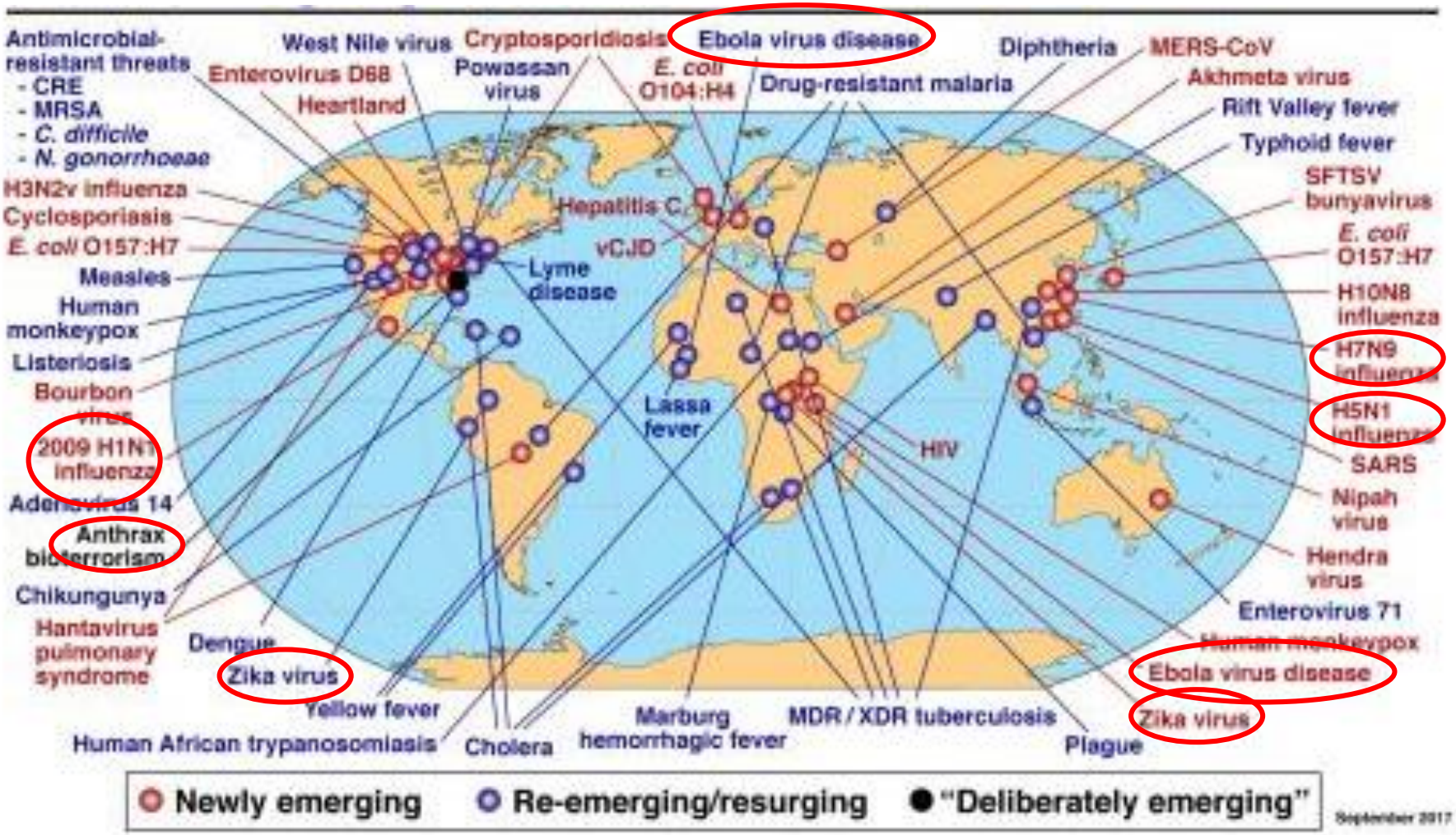
Hailun Ma, Ph.D. (Staff Fellow)

Belete Teferedegne Ph.D. (Staff Fellow. Moved to DVRPA in Jan. 2018)

Sandra Fuentes Ph.D.

ORISE Fellows: 3-5 postdoc and post-bacc / year

EVOLVING LANDSCAPE OF INFECTIOUS DISEASES



During the last decade CBER had to respond to multiple emerging and re-emerging diseases including Avian influenza with pandemic potential, Ebola, and Zika. Rapid response included shift in efforts

LR Response: facilitate rapid deployment of vaccines against emerging diseases

- **Identify regulatory and scientific gaps in knowledge, methods for vaccine release, and correlates of protection**
  - **LR researcher-regulators provide expertise for review, and re-orient their scientific programs to address the challenges of new vaccines, including the use of new cell lines and manufacturing platforms, novel immunogen/adjuvant design, and new endpoints for clinical trials.**

- **Developed advanced technologies for improved analyses of:**
  - **Safety of novel cell substrates**
  - **Humoral immune responses post-infection and vaccination**
  - **Adjuvant safety and mode of action**
  - **Vaccine potency assays**
  - **Animal models for preclinical evaluation of vaccines including safety and effectiveness**

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Regulatory Responsibilities

- **Vaccines against human pathogens**
  - **HIV, Influenza, Zika, Ebola, RSV, Adjuvanted vaccines (multiple pathogens)**
  - Non replicating and replicating viral vectors:
  - Nucleic acid Vaccines (DNA; mRNA)
  - Live attenuated vaccines
  - Recombinant proteins and peptide-based vaccines

- **Novel Adjuvants and vaccine delivery systems**

- **Universal Influenza Vaccines (new)**

- **Novel cell substrates and detection of adventitious agents using next generation sequencing technologies (new)**
  - Mammalian tumorigenic and tumor-derived cell lines
  - Insect cell lines for baculovirus expression vectors
  - Avian cell lines

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Approval of Vaccine BLA

BLAs that were approved between 2013-2018:

- **Q-PAN-H5N1**: AS03-adjuvanted H5N1 (A/Indonesia) *(Khurana/Golding)*
- **Fluad:** MF59-adjuvanted seasonal influenza vaccine for the elderly (>65 yr) *(Zaitseva)*
- **Shingrix:** AS01-adjuvanted VZV (gE) vaccine for the elderly (>50 yr) *(Zaitseva)*
- **Flublok**: Baculovirus-expressed recombinant trivalent HA proteins produced in Sf9 insect cells (for persons > 18 yrs) *(Khan)*

Development of New Immunological Assays and Animal Models to Evaluate Vaccine Safety and Efficacy

Hana Golding, Ph.D.

Unit of Viral Immunology and Pathogenesis

Golding Lab:
Scientific projects led by Zaitseva and Khurana

Dr. Zaitseva is leading several research projects:

- **Adjuvant safety**: Mechanisms of production of pro-inflammatory mediators (cytokines and PGE2) in human cell-based assays predictive of *in vivo* reactogenicity.
- **Bioluminescence imaging of live mice:** mechanism of protection against vaccinia challenge

Dr. Khurana is leading several research projects:

- **In-depth analyses of the humoral immune responses generated by different vaccine candidates vs. infections** (*Influenza, RSV, Ebola, Zika*):
  - Whole genome phage display libraries (GFPDL)
  - SPR technologies for antibody affinity measurements of polyclonal antibodies from humans and NHP

  Immunogen design/expression (*RSV, Influenza*)

  Animal models for preclinical evaluation of vaccine candidates with emphasis on safety and effectiveness **(***Influenza, RSV***).**
- New potency assay for rapid release of influenza vaccines (antibody–independent)
- New reporter-based neutralization assay (*RSV*)
- **Universal influenza vaccines: safety and efficacy**

Adjuvant safety (Marina Zaitseva, Staff Scientist)

Goal: Development of *in vitro* assays using Human cell targets predictive of adjuvant toxicity *in vivo*

- **Adjuvants are included in vaccine formulations to activate antigen presenting cells (APC)**
- **Often, strong activation of APC by adjuvants may induce excessive release of pyrogenic and inflammatory substances causing adverse reactions in vaccine recipients. Animal models may not be predictive of human responses !**
- **Muramyl dipeptide (MDP) adjuvant was associated with fever and reactogenicity in rabbits and humans: used as a prototype of reactogenic adjuvant.**
- **We investigated the mechanism of production of prostaglandin E2 (PGE_2), a proximal mediator of fever, and of pyrogenic cytokines IL-1β, IL-6, and IL-8 in human monocytes activated with MDP adjuvant**

Results: T cell derived GPIbα augment MDP induced pyrogenic response and reactogenicity

- Partially activated T cells (purified by CD3 beads) shed Glycoprotein Ib alpha protein (GP1bα) that binds to Mac-1 integrin on monocytes
- T cell derived GPIbα dramatically increased production of PGE_2 and pro-inflammatory cytokines in human monocytes activated with MDP
- Blocking of Mac-1 by antibodies in monocytes *in vitro* and experiments in Mac-1 KO mice *in vivo* confirmed the role of Mac-1 in inflammatory response to MDP
- Novelty: We describe for the first-time a contribution of GPIbα/Mac-1 signaling to production of pro-inflammatory substances in monocytes in response to MDP adjuvant
- Outcome: Further study of T cell-monocyte nexus might help in the assessment of inflammatory potential of novel adjuvants. The *in vitro* based assays are valuable for downs-election of novel adjuvants.

NEW TECHNOLOGIES FOR UNDERSTANDING ANTIBODY RESPONSES FOLLOWING VACCINATION & INFECTION

(Surender Khurana)

- **TRADITIONAL ASSAYS USED FOR VACCINE RESPONSES:**
  - **Plaque Reduction Neutralization Test (PRNT) (Many viruses)**
  - **Hemagglutination Inhibition assay (influenza)**
  - **Virus Neutralization (Many viruses)**

METHODS DEVELOPED

WHOLE GENOME FRAGMENT PHAGE DISPLAY LIBRARIES (GFPDL)

- **COMPLETE ANTIBODY EPITOPE REPERTOIRE**

SURFACE PLASMON RESONANCE (SPR)

- **ANTIBODY KINETICS (AFFINITY MATURATION)**
  - **ANTIBODY ISOTYPE**

ANIMAL MODELS FOR EVALAUTION OF SAFETY & EFFICACY OF VACCINES/THERAPEUTICS

THESE TOOLS WERE DEVELOPED AND APPLIED IN STUDIES OF HUMAN SAMPLES: INFLUENZA, RSV, EBOLA, AND ZIKA INFECTION & VACCINATION

DEVELOPMENT AND EVALUATION OF SAFETY, POTENCY AND EFFECTIVENESS OF VIRAL VACCINES: RSV & INFLUENZA: Key Accomplishments

RSV

- Antigenic fingerprinting of RSV following primary human infection in Children identified importance of anti-G antibodies.

- Bacterially produced non-glycosylated G protein was shown to be safe and effective vaccine against RSV: Mice and Cotton rat challenge studies

INFLUENZA

- Evidence for anti-PA-X antibodies following infection with highly pathogenic H7N7 avian influenza in humans.

- A high-throughput potency assay for rapid release of influenza vaccines

- Adjuvants improve vaccine induced antibody responses in humans:
  - Expanded Ab repertoire against protective targets (*epitope spreading*)
  - Increased Ab affinity maturation
  - Broader cross-protection against diverse avian influenza strains
  - Similar finding in several prime-boost protocols

- UNIVERSAL INFLUENZA VACCINES: Development of *in vitro* assays and animal models to evaluate potency, safety and effectiveness (including VAERD).

RESPONSE TO EMERGING VIRAL DISEASES: ZIKA & EBOLA

ZIKA VIRUS: Acute infection (plasma; urine)

Whole genome immune profiling revealed differential human IgG and IgM Ab repertoires in serum and urine following Zika virus infection.

- **Antibody affinity to ZIKV-E inversely correlated with the disease severity**
- **ZIKV Serodiagnostic test based on NS peptides identified by GFPDL**

EBOLA VACCINATION AND INFECTION (Humans, NHP)

- **Human antibody repertoire following VSV-Ebola, DNA and protein vaccination identified novel protective targets and revealed importance of IgM antibodies in Ebola virus neutralization.**
- **Strong correlation between anti-GP antibody affinity and protection in EBOV animal challenge studies**

IMPACT:

Antibody affinity & Durability are key parameters to be followed in EBOV vaccine studies.

Development of Sensitive Virus Detection Assays for Safety of Vaccines and Related Biologics and Evaluation of Viruses with Potential Concerns for Human Infections

Arifa S. Khan, Ph.D.

Unit of Molecular Retrovirology

KHAN LAB: *Scientific projects*

Project I.

- **DEVELOPMENT OF NEW TECHNOLOGIES FOR INVESTIGATING ADVENTITIOUS AND ENDOGENOUS VIRUSES**

A. Evaluation of next generation sequencing (NGS) platforms for virus detection

I. Method Standardization

II. Bioinformatics pipelines

III. Development of reference materials

B. Investigations of endogenous and occult viruses in vaccine cell substrates: (Hailun Ma: Lead)

I. Sf9 cells: Rhabdovirus; RT activity; Whole genome sequencing

II. Vero cells: ERVs

Project II.

- **DEVELOPMENT OF *IN VITRO* AND *IN VIVO* MODELS FOR SIMIAN FOAMY VIRUS INFECTIONS IN HUMANS**

A. In vitro models for latent and active SFV infections

I. Characterization of SFV-K3T A549 cell clones

II. Identification of biomarkers for SFV replication

III. Identifying determinants of SFV fitness

B. Analysis of SFV infection in naïve and SIV-infected rhesus macaques to predict clinical outcome of humans infected with SFV or coinfected with SFV and HIV

KHAN LAB PROJECT I A: DEVELOPMENT OF NEW TECHNOLOGIES FOR INVESTIGATING ADVENTITIOUS AND ENDOGENOUS VIRUSES: Evaluation of Next Generation Sequencing Platforms for Virus Detection

Goals

- **NGS Standardization for detection of known and novel adventitious viruses** for evaluating safety of cell substrates, vaccines and related biologics.

Accomplishments

- NGS potential for sensitive detection of adventitious viruses in complex biological samples was demonstrated by similar detection of 4 model viruses by 3 laboratories using independent sample preparation methods, different sequencing platforms, and bioinformatics pipelines. (*Khan et al., mSphere, 2017)*.
- Five, well-characterized, large-scale reference virus stocks were developed for NGS standardization and are currently being used by some vaccine manufacturers.
- A new Reference Virus Database (RVDB) was developed and is publicly available at the GWU HIVE and used by some vaccine sponsors. (*Goodacre et al., mSphere, 2018).*

Regulatory Impact

- Availability of viral stocks for NGS standardization can facilitate its use for broad virus detection to evaluate safety of biologics
- NGS can enhance product safety by supplementing or replacing some current assays that have limitations for virus detection
- NGS laboratory efforts is directly facilitating review of regulatory submissions with NGS and development of regulatory guidance for using NGS for adventitious virus detection

KHAN LAB PROJECT I B: DEVELOPMENT OF NEW TECHNOLOGIES FOR INVESTIGATING ADVENTITIOUS AND ENDOGENOUS VIRUSES: Investigations of Endogenous and Occult Viruses in Vaccine Cell Substrates (Hailun Ma: Lead)

Goals

- **Investigating NGS for characterization of new cell substrates: Sf9 insect cells** are used for baculovirus-expressed vaccines

Accomplishments

- A novel rhabdovirus was detected using degenerate PCR and NGS (*Ma et al., JVirol, 2014*)
- Virus-negative and virus-positive cell clones were isolated from the ATCC Sf9 cell line.
- Infectivity assay for rhabdovirus was developed with the virus-negative cell line
- Cell clones with rhabdovirus variants in the X-gene were obtained and found to be infectious
- NGS analysis identified different families of endogenous retroelements that are being investigated to characterize the novel RT activity, which is constitutively produced from Sf9 cells

Regulatory Impact

- Rhabdovirus discovery resulted in establishment of PCR assays and viral clearance steps by manufacturers of baculovirus-expressed vaccines
- Sf-rhabdovirus negative cell clone provides an important reagent for developing a sensitive assay for infectious virus detection
- A "clean" Sf9 cell line may be obtained for manufacturing and research purposes
- Ongoing work to characterize endogenous retroviruses in the Sf9 genome will identify viruses with potential function to assess if they can pose a safety concern

KHAN LAB PROJECT II A: DEVELOPMENT OF *IN VITRO* AND *IN VIVO* MODELS FOR SIMIAN FOAMY VIRUS (SFV) INFECTIONS IN HUMANS:
In Vitro Models for Latent and Active Simian foamy Virus Infections

Goals

- Develop relevant *in vitro* models to **identify viral/host determinants of SFV replication** for assessing the potential of latent virus activation and clinical outcome humans infected due to cross-species transmission from non-human primates

Accomplishments

- Stable SFV-infected cell clones were obtained from infection of human A549 cells with a naturally-occurring rhesus macaque SFV isolate
- Clones were characterized for virus expression and particle production: latent, persistent, and chronic phenotypes were identified. Copy number was determined by ddPCR.
- Virus rescue experiments indicated SFV latent infection was due to lack of early expression of the transactivator Tas gene
- RNA-Seq differential gene expression analysis suggests immune signaling pathways may be involved in SFV chronic infection

Public Health Impact

- SFV-A549 cell clones are a relevant model for natural virus infection in monkeys (and possibly humans). Identification of markers for virus replication could help investigate latent virus activation and potential clinical outcome in human infections
- SFV-A549 cell clones provide useful research reagents to study the outcomes of virus coinfections in humans that are exposed to different nonhuman primates species infected with different virus strains in natural or research settings