Biomarkers of Malaria and *Babesia* Detection, Immunity and Pathogenesis

Scientific Site Visit Report Summary

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BPAC, November 4, 2021

Research Project 1: Identify Malaria Reservoirs and Candidate Transmission Blocking Vaccines

Mission Relevance: Malaria presents a serious clinical burden and remain a blood safety risk in United States and globally. There is no FDA-licensed donor screening assay or vaccine against malaria.

Major Findings:

- Identified more than **200 novel *Plasmodium falciparum* gametocyte-specific biomarkers** by transcriptome analyses. Of these, novel **Pfg-17 gametocyte gene** allowed highly sensitive detection of the asymptomatic *P. falciparum* infectious reservoirs in endemic areas (JID, 2018)
- Antibodies against two novel *P. falciparum* gametocyte antigens – **Pf77 and PfMDV-1** reduce oocyst development in the mosquito midgut by **93% and 84%**, respectively. Pf77 and PMDV-1 are pan-developmentally expressed, relatively genetically conserved and naturally immunogenic in endemic areas (Sci Trans Med 2021)
- Preclinical evaluation of Pf77 and PfMDV-1 as a **multistage mRNA malaria vaccine** is ongoing in collaborative studies.

Research Project 2: Identify Novel Immunodominant *Babesia microti* Antigens to Develop Superior Detection Assays and Vaccine Candidates (1)

Mission Relevance: Human babesiosis is a major public health risk in United States. Assays of superior sensitivity for detection of early low-grade infections and persistent chronic infections are needed.

Major findings:

- By genome-wide immunoscreening, we identified **56 novel immunodominant *Babesia microti*** antigens. Our studied have clarified the domain architectures, potential biological functions, and evolutionary relationships of the *B. microti* antigens including the BMN-family antigens
- A combination of three novel antigens provided **96% sensitivity and 100% specificity** in identifying *B. microti* patient sera in an ELISA (Sci Rep 2020)
- A multiplex antibody bead assay under development is **comparable to IFA** in detecting *B. microti* exposed blood donors and **superior to IFA** in detecting window period cases.

Research Project 2: Molecular Basis of *B. microti* pathogenesis (2)

Mission Relevance: A better understanding of host biomarkers and molecular mechanisms underlying asymptomatic infections and severe babesiosis would help reduce clinical burden and improve blood safety.

Major Findings:

- We have shown expression of **BAHCS1, MCFRP1, PiβS and SERA** on the surface of *B. microti*-iRBC indicating a possible role in cytoadherence
- Exploring the role of parasite sequestration in persistent chronic infections and in organ damage experienced in severe babesiosis in mice and hamsters
- Cytoadherence assays being performed to identify parasite ligand(s) and host receptor(s) using *B. microti* iRBC isolated from animals and humans
- Identification of host biomarkers of severe and asymptomatic infections in animal models and in human babesiosis by flow cytometry and genomics studies are ongoing.

Accomplishments (2016-2020)

- **Selected Publications from a total of 21 papers**
  - **I. Biomarkers of malaria detection and pathogenesis**
    e.g., PLoS One 2017; J Inf Dis 2017; Inf Immun 2018; Sci Rep 2018; Malaria J 2019; *PNAS 2020*
  - **II. Malaria vaccines and vaccine-induced immune mechanisms**
    e.g., Clin Transl Immunol 2018; Inf Immun 2019. Sci Transl Med 2021
  - **III. *Babesia microti* antigen discovery, detection, vaccines and epidemiology**
    e.g., Transfusion 2018; Sci Rep 2020; Open Forum Inf Dis 2021; Cell Host Microbe 2021
- **CRADA:** PATH-MVI (2016-2020)
- **US patent:** 1 patent filed (*B. microti* antigens, 2018)
- **Research Collaborative Agreement:** Griffith University, Australia
- **Major Collaborations:** NIAID, Johns Hopkins Malaria Research Institute, Yale School of Public Health and Univ of Penn. Medical School

Improving blood safety by reducing the risk of transfusion-transmission of *Leishmania* through vaccination

Objective: to develop methods and animal models to evaluate biomarkers of safety and efficacy of live attenuated *Leishmania* parasite vaccines

PI: Hira L Nakhasi, PhD, FASTMH

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Leishmaniasis and its Relevance to U.S. Public Health

- *Leishmania* infection causes several forms of diseases:
  - Visceral leishmaniasis (VL) could be fatal if untreated
  - Cutaneous leishmaniasis (CL) causes disfiguring skin lesions that could be chronic
- *Leishmania* parasite is a blood borne pathogen and can be transmitted by transfusion and travels to endemic areas
- Leishmaniasis is endemic in the US
- Currently donor screening is not an option because it does not meet the incidence threshold and/or prevalence in the US donor population
- However, vaccination of US travelers and military personnel stationed in endemic areas can be an alternative to enhance blood safety

Rationale for using live-attenuated *Leishmania* parasites as vaccines against Leishmaniasis

- No licensed vaccine
- Previous attempts to develop vaccines have not resulted in a successful vaccine
- Inoculation with a low dose of wild-type *Leishmania* parasites, commonly known as Leishmanization provides protection

  -*discontinued due to safety concerns*

- Most successful licensed vaccines against other pathogens are live attenuated
- Genetically modified live-attenuated *Leishmania* parasites that are safe, and provide protection is safe alternative to Leishmanization

Selva et. et. 2019 PLoS NTD

Preclinical evaluation of genetically modified live- attenuated vaccine candidate

Candidate Vaccine : Centrin gene deleted *Leishmania major* parasites (*LmCen-/-*)

- Centrin gene was deleted using the state-of-the-art CRISPR/Cas9 technology
- Immunization in preclinical animal models induced robust immune response
- **Safe and efficacious** against CL infection
- **Safe and efficacious** against VL infection
- **Safe alternative** to leishmanization which can cause lesions

Selva et. al. JI. 2009; Gannavaram et. al. Sci. Rep. 2017; Dey et. al. Cell Host Microbe. 2018; Zhang et. al. Nature. Commun. 2020; Karmakar et. al. Communication. Biol. 2021

Accomplishments (2016-2020)

- **23** Publications in peer-reviewed journals
- **1** US patent issued, and **1** US Patent application filed by FDA
- **3** Cooperative Research And Development Agreements (CRADA)
- Established consortium of national and international collaborations
- Extramural funding (>$7 million) for the consortium from
  - Wellcome Trust, UK,
  - GHIT Fund, Japan, and
  - NIH/NIAID, USA

Studies to Reduce the Risk of Transmission of Chagas Disease from Blood and Blood Products

Major objectives of this program are to develop novel biomarker detection assays for Chagas disease and study disease pathogenesis and transmission

PI: Alain Debrabant, PhD

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Chagas Disease in the United States and Mission Relevance

- Caused by vector-borne parasite ***Trypanosoma cruzi and transfusion-transmission***
- About **300,000** infected persons including **76 autochthonous** cases in the Southern States
- Vertical transmission rate **1-5%**
- Risk of transmission by **blood transfusion** reduced since implementation of donor testing for antibody in 2007
- Need for alternative, **biomarker detection assays** to further improve donor testing and rapid diagnosis
- Need to better understand **chronic Chagas disease**, parasite persistence, and vertical transmission

Blood biomarkers of Chagas disease and assay development

Major Findings

- Identified and characterized ***T. cruzi* secreted proteins** as novel **biomarkers** of CD and generated high affinity and specificity detection reagents (aptamers and monoclonal antibodies)
- Developed **new assays** in ELISA and Lateral Flow (LF) formats that detect the *T. cruzi* biomarkers in blood of infected mice, and in Chagas disease patients, either asymptomatic or with cardiac symptoms
- The **Tc-5171-ELISA** detected a reduction in biomarker levels post drug treatment in mice and in patients suggesting its utility to assess efficacy of drugs in pre-clinical and clinical stages of development
- Collaborative studies are on-going to further evaluate these assays to measure **treatment efficacy and cure** in clinical trials and for **rapid diagnosis**

Nagarkatti et al. Scientific Reports 2020 - PMID: 33177582

Chagas disease pathogenesis and transmission

Major Findings

- Using proteomic studies and live fluorescence, we demonstrated the presence of *T. cruzi* proteins (GRP78, HSP70 and Mucin) at the **surface of infected cells** and in extracellular vesicles suggesting their role in pathogenesis
- Using transgenic *T. cruzi* parasites, we identified the **gut as a major parasite reservoir** in a mice model of chronic Chagas disease
- Developed an in vitro **3D culture system of human trophoblasts** and showed that 3D- grown JEG-3 cells were **refractory** to *T. cruzi* infection and released paracrine **factors of resistance,** suggesting the role of trophoblasts in limiting parasite vertical transmission
- Future studies involve identification of mechanisms of **immune evasion** and **parasite persistence** in the gut during a chronic infection

Silberstein et al. 2018 PLoS One – doi: 10.1371
Silberstein et al. 2021 Front. Microbiol. – doi: 10.3389

Accomplishments (2016-2020)

Blood biomarkers of Chagas disease and assay development

- *Nagarkatti et al. Scientific Reports 2020*
- *Fortes et al. Antimicrob. Agents Chem. 2021 (under revision)*

Chagas disease pathogenesis and transmission

- *Silberstein et al. PLoS One 2018*
- *Silberstein et al. Front. Microbiol. 2021*

Evaluation of a novel pathogen reduction technology for *T. cruzi*

- *Jankowska et al. Front. Med. 2020*

Major collaborations and other accomplishments

- Research Collaborative Agreement (RCA) with Kephera Diagnostics, Framingham, MA to develop a Chagas POC test
- RCA with University of Texas at El Paso on the NIH Funded Clinical Study to evaluate novel biomarkers to assess Chagas disease treatment
- Employee Discovery and Invention Reports filed with FDA for monoclonal antibodies against Chagas Disease biomarkers

Advanced Technology for Reducing the Risk of Transfusion Transmission of Infectious Agents

Scientific Site Visit Report Summary

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BPAC, November 4, 2021

The Ebolavirus RMA Project

- A resequencing microarray able to sequence the Ebolavirus genome in an efficient and computationally simple process
- Full-genome length virus RNA samples sequenced
  - Ebola virus (Zaire), Bundibugyo virus, Sudan virus and Taï Forest virus
  - Confirmed by comparison of Ebolavirus-RMA results to NGS sequencing of the same samples
- Escape mutant variants sequenced
  - Recombinant VSV-Ebola-GP virus stocks passaged in the presence of neutralizing antibodies were sequenced with the Ebolavirus-RMA.
  - By the 3rd passage, GP sequence substituting a D for N amino acid in the epitope of the neutralizing antibody revealed the mechanism of escape
- These studies indicate that Ebolavirus-RMA can detect genomic drift and is suited for further evaluation as a tool to sequence clinical specimens in epidemiological and surveillance studies

The Laser-based Detection Project

- The Laser-based device for multiplex pathogen detection in blood represents a novel technology for diagnosis and blood screening.
- Minimal sample preparation, rapid readout and flexibility make this a promising technology
- Collaborating with Creative LIBS Solutions (CLS), evaluation has optimized the instrument and detected five pathogen types
  - Two parasite species, Gram-negative and Gram-positive bacteria, HIV spiked in whole blood.
- To evaluate the device for clinical specimens, the instrument was recalibrated for plasma.
  - Repository blood donor specimens positive with licensed tests for HIV (n=8) or HCV (n=8) or negative (n=12) were correctly identified with the Laser-based device.
- Such a device has potential in a hospital setting to test blood collected for transfusion without sending samples out to a central testing facility.

The Blood Borne Pathogen Resequencing Microarray Project

- BBP-RMAv.1 demonstrated sensitive multiplex detection of HCV, HBV, HIV, *B. microti, L. donovani, T. cruzi, P. falciparum, S. aureus, S. epidermidis, E. coli and Y. enterocolitica* spiked into human plasma or whole blood.
- BBP-RMAv.2 expanded to 55 blood borne virus, 5 bacterial and 16 protozoan species, strains and genotypes, yet retained the sensitive detection and rapid analysis by a custom bioinformatic pipeline.
- As an alternative to the microarray, we are testing a nanopore sequencing device to compare the workflows and outcomes of these two devices.
- The high specificity and potential to discover nucleotide changes in the target pathogen suggest this platform is best suited in a confirmatory role.

Accomplishments (2016-2020)

- Ebolavirus Resequencing Microarray Project
  - Genome sequence determined in 24 hours
  - Genomic drift in Ebola GlycoProtein demonstrated
  - Tiper et al. in review *PLoS One*

- Laser-Based, Rapid, Multiplex Detection of Blood Borne Pathogens
  - RCA with Creative LIBS Solutions
  - Multari et al. 2019 *J. Appl. Micro.*
  - Multari et al. in review *J. Appl. Micro*

- Blood Borne Pathogen Resequencing Microarray-NGS comparison
  - Kourout et al. 2016 *Transfusion*
  - Ongoing research