Malaria Research Program at CBER

Studies to improve Blood Safety from the risk of transfusion-transmitted malaria, and malaria pathogenesis and control

Scientific Site Visit Review Summary
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CBER, FDA
CBER Mission-Oriented obligations and research projects

- Blood safety from transfusion-transmitted malaria
  - Development of DNA and antibody based tests for blood donor screening

- Provide expertise for the review of malaria vaccine INDs
  - Studies on malaria biology, pathogenesis, vaccination and biomarkers of virulence and immunity

Only malaria research program at CBER

Malaria Program Members:
Sanjai Kumar, Ph.D., Supervisory Biologist (PI)
Hong Zheng, M.S., Biologist
Victoria Majam, M.S., Biologist
Babita Mahajan, Ph.D., Post doctoral Fellow
Transfusion transmitted malaria in the US

1963-1998
• 3 cases per year. All four species of Plasmodium implicated
  - Approximately 50% of cases were caused by malaria naïve travelers

1995 to 2005
• 0.5 case per year. *P. falciparum* is the predominant species
  - Caused by donors born in a malaria endemic country

• There is no laboratory test to screen blood donors for malaria infections

• Blood safety is maintained by risk based deferral policy

• Loss of approximately 150,000 donors each year

US travelers: 28 million each year

US immigrants: asymptomatic carriers
Laboratory tests to detect malaria parasite infections in blood donors

DNA Test

- 2.4 parasites/ml of blood
- Could leave up to 1200 parasites in a unit of blood
- For *P. vivax* infectious dose is 10 infected RBC or 0.02 parasites/ml in a unit of blood

Nested PCR - QIAmp kit

ELISA

Reactivity with recombinant *PfCSP*, *PfAMA-1*, and *PfMSP1*$_{42}$

<table>
<thead>
<tr>
<th>Patient Sera</th>
<th>No. of Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em></td>
<td>4/4</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>8/8</td>
</tr>
<tr>
<td><em>P. malariae</em></td>
<td>2/2</td>
</tr>
<tr>
<td><em>P. ovale</em></td>
<td>7/8</td>
</tr>
<tr>
<td>Normal Sera</td>
<td>0/2</td>
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</tbody>
</table>

- Three *P. falciparum* proteins can be used to detect antibodies to all four *Plasmodium spp*
Live Attenuated Malaria Vaccines and Biomarkers of Virulence and Efficacy

• R,TSS, the most successful recombinant malaria vaccine induced only 30% protection for a short duration
• Data from natural immunity, irradiated sporozoites and genetically attenuated sporozoites suggest that whole parasites are the most effective vaccines
• Current research trend suggests that several INDs based on live attenuated malaria vaccines will be submitted to FDA in the coming years
• A program to identify the molecules associated with parasite growth, and survival for targeted gene deletion to generate live attenuated vaccines
• To identify the biomarkers that could predict the virulence and efficacy of live attenuated vaccines
Genetically altered live attenuated malaria vaccines

**Gene KO strategy for Centrin**

- **P. falciparum**
  - Single crossover
  - Target locus
  - Blood Stage Schizonts
  - Electroporation & Transformation
  - In vitro drug selection
  - Genetic analysis and microarray

- **P. berghei**
  - Double crossover
  - Target locus
  - In vivo selection of resistant parasites
  - Attenuated Sporozoite
  - Genetic analysis and microarray
Genome-Wide Expression Profiling in Malaria infection Reveals Transcriptional Changes Associated with Lethal and Nonlethal Outcomes

- Identify a set of host biomarkers that distinguishes between lethal and non-lethal malaria infections

- Metabolic acidosis resulting from the accumulation of lactate is an important prognostic indicator for the severity of malaria. Increased activity in the glycolysis pathway is associated with pyruvate conversion to lactate

- In 17XL, all genes in the glycolysis pathway are upregulated during the entire course of infection
Summary

- A nested PCR that detects 2 *P. falciparum* parasites/ ml of blood and a pan-*Plasmodium* ELISA that recognizes all four species
- Identified *P. falciparum* parasite molecular factors and associated biological pathways induced in response to febrile temperatures
- Identified both host and parasite biomarkers that distinguish between virulent and non-virulent strains of malarias

Future Directions

- DNA and antibody based tests for the detection of malaria parasites in blood donors
- Suitable molecules for targeted gene deletion to create growth deficient malaria parasites
- Genetically attenuated live malaria vaccines and their safety, efficacy and virulence profiling
- Biomarkers that could predict the safety and efficacy of attenuated malaria vaccines
Collaborations

- FDA
  Hira Nakhasi, Anangmuthu Selvapandian
- NIH
  Tom McCutchan
- WRAIR
  Chris Ockenhouse
- Virginia Tech
  Dharmendar Rathore
- NCBI
  Aravind Iyer