Uncovering Novel Antigens Expressed in Various Sporozoite Compartments

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Naval Medical Research Center (NMRC)
Malaria Vaccine Program

Malaria Vaccines for the World Meeting
22-24 April, 2013
Lausanne, Switzerland
Objectives - ICE

1. Identify (I) - Screen and prioritize sporozoite / liver stage antigens as vaccine candidates using sera and T cells from RAS-immunized, protected volunteers.

2. Characterize (C) - Determine sub-cellular localization (compartmental distribution) of down-selected antigens using polyclonal sera and immuno EM.

3. Evaluate (E) - Design vaccine reagents concordant with sporozoite localization and evaluate for protection in animal models.

- Likely processed by hepatocytes to induce T cell responses
  - Vaccine: DNA + Adeno5

- Likely to induce functional antibody responses
  - Vaccine: recombinant protein in adjuvant
Overall Strategy to Identify PE Antigens Inducing Antibody and T-cells in the RAS Protective Model

Expression Libraries: Proteome, Transcriptome, dCDA

Pre-Erythrocytic Gene Selection: 1,000

Gateway Cloning

Wheat Germ Cell-free System

Cloning & Protein Expression: 300

RAS Human sera: 150

RAS T-cell Screening

ELISPOT

Ag Characterization Polyclonal AB

IFN-γ Long-Term ELISPOT Response to PF93 Antigen

DNA Vaccine, Adeno 5 & Rec. Protein

Py Protection Studies

Ag Characterization Polyclonal AB

IFA, ILSDA, Immuno EM,
Whole Sporozoite Vaccine Induces Sterile Protection
Plasma & PBMC Used for Antigen Screening

Immunizations via mosquito bites

4 weeks

Spz Challenge

Leukapheresis

Plasma
PBMC

Rec. Protein by Wheat Germ

Western Blot & ELISA

HLA & overlapping peptides

Cultured & Ex-vivo ELISpot
# Overall T cell Reactivity of 21 *P. falciparum* Antigens to RAS Volunteers Protected vs. Non-Protected

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<td>N/D</td>
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</table>

16 out 21 (76%) tested showed positive recall in IFN-γ ELISPot assay
Overall Breadth of the T cell Reactivity of RAS Volunteers to *P. falciparum* Pre-erythrocytic Antigens

Mann-Whitney: Not Significant

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<th>Fraction of Antigens Positive by IFN-γ ELISpot (# recognized/# tested)</th>
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<tr>
<td>0.2</td>
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<tr>
<td>0.0</td>
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</table>

**Protected**
- 5 subjects
  - 4 tested against 21 antigens
  - 1 tested against 10 antigens

**Non-Protected**
- 5 subjects
  - 1 tested against 3 antigens (not shown)
  - 4 tested against 21 antigens
  - 1 tested against 10 antigens
Identification of Novel *P. falciparum* Antigens Expressed in Sporozoites
Immuno EM Results – Seven Examples

- CSP – **surface expressed** (traditional antigen)
- CelTOS – **micronemes / cytoplasmic** (traditional)
- PF02 + PF56 – **surface expressed** (novel)
- PF78 – **membrane associated** (novel)
- PF106 – **rhoptry / surface expressed** (novel)
- PF144 – **microneme expressed** (novel)
- PF61 – **cytoplasmic expressed** (novel)
- PF119 – **nuclear** (novel)
Localization of CSP Antigen in *P. falciparum* Sporozoites
Surface + Internal + Shed
Schematic Localization of CSP in Sporozoites by Immuno EM

Surface-Expressed & Shed Protein

CSP

Micronemes

CSP

Rhoptries

Shed CSP Antigen
Localization of CelTOS Associated to Micronemes

*P. falciparum* Sporozoites
Schematic Localization of CeITOS in *P. falciparum* Sporozoites by Immuno EM

Microneme & Cytoplasmic Protein
Localization of PF02 Antigen in the Surface of *P. falciparum* Sporozoites
Schematic Localization of **PF02** in the Surface of Sporozoites by Immuno EM

**Surface Expressed Protein**

- **PF02**
- **Micronemes**
- **Rhoptries**
Expression of PF02 Antigen in *P. falciparum* Liver Stages

5-day liver stage

7-day liver stage
Localization of PF56 Antigen in the Surface and Cytoplasm of *P. falciparum* Sporozoites
Schematic Localization of PF56 on the Surface of Sporozoites by Immuno EM

Surface-expressed and secreted protein

PF56

PF56

Shed PF56 Antigen
Expression of PF56 Antigen in *P. falciparum* Liver Stage
Localization of **PF78 Antigen** Associated to the Membrane of *P. falciparum* Sporozoites
Localization of PF78 Antigen in the Membrane Complex of *P. falciparum* Sporozoites
Schematic Localization of PF78 on the Membranes of Sporozoites by Immuno EM

Membrane-bound or Associated Protein
Expression of PF78 Antigen in *P. falciparum* Liver Stages

5-day Liver Stage

7-day Liver Stage
Localization of PF106 Antigen in Rhoptries of *P. falciparum* Sporozoites
Localization of PF106 Antigen in the Surface of *P. falciparum* Sporozoites
Schematic Localization of PF106 in the Surface, Cytoplasm and Rhoptries of Sporozoites by Immuno EM
Expression of PF106 Antigen in *P. falciparum* Liver Stages
Efficacy of *P. yoelii* PF106 Ortholog (PY03424) Antigen (DNA-Pox Prime-boost)

Identification of two new protective pre-erythrocytic malaria vaccine antigen candidates

Keith Limbach, João Aguiar, Kalpana Gowda, Noelle Patterson, Esteban Abot, Martha Sedegah, John Sacci and Thomas Riche

% protection

PyUIS3 (dose=1X)  Py3424 (dose=1X)  Py3661 (dose=1X)  PyUIS3 (dose=2X)  Py3424 (dose=2X)  Py3661 (dose=2X)  PyUIS3 (dose=3X)  PyCSP (dose=1X)  Neg con (dose=1X)  Neg con (dose=2X)  Neg con (dose=3X)

7%  7%  0%  43%  21%  43%  14%  0%  0%  7%

(PF93) (PF106) (PF08)
Localization of PF144 Antigen to Micronemes of *P. falciparum* Sporozoites
Schematic Localization of **PF144** in Micronemes of Sporozoites by Immuno EM
PF144 Antigen Expression in *P. falciparum* Liver Stages

24-hour Liver Stage

5-day Liver Stage
Localization of PF61 Antigen in the Cytoplasm of *P. falciparum* Sporozoites

- **Cytoplasm Expression**
- **No Surface Expression**
Localization of PF61 Antigen in the Cytoplasm of *P. falciparum* Sporozoites
Schematic Localization of **PF61** in the Cytoplasm and Micronemes of Sporozoites by Immuno EM

Microneme and Cytoplasmic Protein
PF61 Antigen Expression in *P. falciparum* Liver Stage

5-day Liver Stage

6-day Liver Stage
Localization of PF119 Antigen on the Nucleus of *P. falciparum* Sporozoites
Schematic Localization of **PF119** on the Nucleus of Sporozoites by Immuno EM

Nuclear Protein

Nucleus

PF119
Expression of PF119 Antigen in *P. falciparum* Liver Stage

5-day Liver Stage

Nuclear Expression

10 µm
## Spatial Distribution of 13 Novel Antigens in *P. falciparum* Sporozoites

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<th>Antigen</th>
<th>Surface</th>
<th>Membrane bound</th>
<th>Rhoptry</th>
<th>Microneme</th>
<th>Undefined Cytoplasmic</th>
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Vaccine reagents (DNA plasmids, HuAd5 vaccine constructs and recombinant protein) have been generated for *P. yoelii* ortholog antigens.

Protection studies are underway guided by antigen localization on the sporozoite:
- Internal antigens: **DNA vaccine + Adeno 5** – prime-boost immunization regimen.
- Protection against *Py* sporozoite challenge by liver burden (qPCR) and sterile blood parasitaemia.
- Antigens alone and in combination with *PyCSP*.

Candidate list will be expanded using antigens identified additional screening projects and on the surface of infected hepatocytes as MHC-peptide complexes by mass spectrometry (Vince Gerbasi, section 4).

Attempts are being made to localize these antigens on the liver stage parasites by immuno EM.
Thank You

NMRC Malaria Vaccine Program

Tom Richie
Eileen Villasante
Emily Smith
Jessica Bolton
Noelle Patterson
Keith Limbach
Andrea Strein
Sharvari Sonawane
Joao Aguiar

mvi PATH

MALARIA VACCINE INITIATIVE

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Ashley Birkett

Joao Aguiar

CAMRIS INTERNATIONAL

UNIVERSITY OF MARYLAND

John B. Sacci
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Source of Support: The work of authors affiliated with the Naval Medical Research Center was supported by work unit number 0602278A.870.S.A1209.

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