Discovery of *S. haematobium* diagnostic antigens using an immunomics approach

Alex Loukas
Australian Institute of Tropical Health & Medicine
James Cook University, Australia
Diagnosis of *S. haematobium* infection

- Gold standard is microscopic detection of eggs in urine.
  - low sensitivity – light infections misdiagnosed
  - won’t detect early or non-patent infections
- Circulating antigen (eg: CCA and CAA) detection is more sensitive
  - sensitivity/specificity issues in *Sh* infection (Sanneh 2017) and *Sm* infection in Brazil (Bezerra 2018)
- Urine PCR diagnostics
  - sensitivity/specificity is high
  - expensive (DNA needs to be extracted) and not easily field deployable
- ELISA detection of *Sh* SEA.
  - mostly performed with serum
  - Complex mixture (including glycans) so specificity reduced
  - SEA is not a renewable reagent (QC/QA issues)
- Requirement for sensitive and specific antibody PoC test using **renewable** reagents to complement antigen detection test.
Protein microarrays allow high throughput antigen discovery from helminth proteomes

An Immunomics Approach to Schistosome Antigen Discovery: Antibody Signatures of Naturally Resistant and Chronically Infected Individuals from Endemic Areas

Soraya Gaze1,2, Patrick Driguez1, Mark S. Pearson1, Tiago Mendes4, Denise L. Doolan3, Angela Trieu3, Donald P. McManus2, Geoffrey N. Gobert, Maria Victoria Periago, Rodrigo Correa Oliveira, Fernanda C. Cardoso3,5, Guilherme Oliveira, Rie Nakajima5, Al Jasinskas6, Chris Hung5, Li Liang5, Jozelyn Pablo6, Jeffrey M. Bethony5, Philip L. Felgner5, Alex Loukas1,2

Immunology & Cell Biology

Original Article

Specific humoral response of hosts with variable schistosomiasis susceptibility

Patrick Driguez, Hamish EG McWilliam, Soraya Gaze, David Pedrafita, Mark S Pearson, Rie Nakajima, Mary Duke, Angela Trieu, Denise L Doolan, Fernanda C Cardoso, Algis Jasinskas, Geoffrey N Gobert, Philip L Felgner, Alex Loukas, Elis Meeusen, Donald P McManus, ... See fewer authors

SaccinCUS

A next-generation proteome array for Schistosoma mansoni

Rafael Remiro de Asis, Fernanda Ludolff, Rie Nakajima, Al Jasinskas, Guilherme C Oliveira, Philip L Felgner, Soraya T Gaze, Alex Loukas, Philip T LoVerde, Jeffrey M Bethony, Carlos E Calzavara-Silva

Of monkeys and men: immunomic profiling of sera from humans and non-human primates resistant to schistosomiasis reveals novel potential vaccine candidates

Mark S. Pearson1, Luke Becker1, Patrick Driguez2, Neil D. Young1, Soraya Gaze4, Tiago Mendes5, Xiao-Hong Li4, Denise L. Doolan3, Nicholas Moti4, Takafumi Miduya5, Donald P. McManus2, R. Alan Wilson1, Jeffrey M. Bethony2, Norman Nauch4, Francisca Mutapi11, Philip L. Felgner5 and Alex Loukas4

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Genome of the human hookworm Necator americanus

Yat T Tang1,10, Xin Gao1,10, Bruce A Ross1,10, Sahar Abubucker2, Kymbrie Hallsworth-Pepin3, John Martin3, Rahul Tyagi4, Esley Heizer1, Xu Zhang1, Veena Bhonagiri-Palsikar, Patrick Minx5, Wesley C Warren2,3, Qi Wang9, Bin Zhang8,4, Peter J Hotez8,3, Paul W Sternberg2,3, Annette Dougall1, Soraya Torres Gaze, Jason Mulvanna7, Javier Sotillo7, Shoba Ranganathan3,6, Elida M Rabelo1,8, Richard K Wilson1,8, Philip L Felgner5, Jeffrey Bethony3, John M Hawdon1, Robin B Gasser1, Alex Loukas2 and Makadonka Mitreval1,3,15
Goals

- Identify antigens to diagnose *S. haematobium* infections by antibody profiling of urine and serum of infected individuals using protein microarrays.

- Use this information to select antigens for downstream development of an antibody-based PoC diagnostic test for *S. haematobium*, ideally using urine as the diagnostic fluid.

Expression - Quality Control

N-terminus 6 Histidine (HIS) tag

C-terminus Hemaglutinin (HA) tag

NO DNA

EM PTY

BLANK

IgG mix

PBS

IgE

Ebna dye

BBK19

Schisto Ptn

anti-6His tag antibody
Selection of proteins for inclusion on the array

- Using a mixture of proteome datasets and bioinformatic analyses, 1,053 proteins were selected
- Parasite extracts (including SEA) incorporated into the array as +ve controls

- 650 *S. haematobium* proteins + 403 proteins (*Sh* homologues of *S. mansoni* proteins)
- >90% success in antigen production
### Characteristics of study cohorts

| Urine                                      | Sera                                           |
|--------------------------------------------|                                               |
| 124 urine samples from endemic (Sh only) areas of Zimbabwe and Ghana | 259 serum samples from endemic (Sh only) areas of Zimbabwe and Ghana |
| **n=36** high intensity infections (>50 eggs/10ml urine) | **n=76** high intensity infections (>50 eggs/10ml urine) |
| **n=35** medium intensity infections (11-49 eggs/10ml urine) | **n=67** medium intensity infections (11-49 eggs/10ml urine) |
| **n=36** low intensity infections (0.3-10 eggs/10ml urine) | **n=98** low intensity infections (0.3-10 eggs/10ml urine) |
| **n=7** egg negative/CAA positive infections | **n=4** egg negative/CAA positive infections |
| **n=10** egg negative/CAA negative infections (endemic negative controls) | **n=14** egg negative/CAA negative infections (endemic negative controls) |
| **n=13** non-endemic negative controls | **n=15** non-endemic negative controls |
Aim 1

Identification of antibodies from serum/urine that are detectable in all infected cohorts
IgG reactivity of subject urine (n=124 - stratified by urine egg burden)

- High (>50 eggs)
- Medium (11-50 eggs)
- Low (0.3-10 eggs)

CAA pos
CAA neg
non-end. neg
Correlation between egg burden and antibody reactivity in serum and urine

For serum:
- Mean SI by sample
- Log₂ egg burden
- p-value = 0.1782, R² = 0.087384

For urine:
- Mean SI by antigen
- All positive samples
- R² = 0.6505
- P < 0.0001

Graphs show scatter plots with regression lines for both serum and urine samples.
Array reactivity plots and ROC curves of top three antigens (urine)

(A) AUC = 0.887

(B) AUC = 0.879

(C) AUC = 0.878
ELISA validation of recombinant antigens B and C (urine)

- Cell-free antigens ideal for high-throughput discovery but not scale-up
- Lead antigens expressed in cell-based recombinant systems (yeast and E. coli)

AUC = 0.8858

AUC = 0.9396
Antigen combinations increase FoR and predictive value of infection (urine)

- High combination: 80.7% FoR
- Medium combination: 75.4% FoR
- Low combination: 68.4% FoR
- Egg-ve: 93.9% FoR

Fluorescence:
- Positive (114)
- Non-end. -ve (13)

Sensitivity: 100%
Specificity: 100%
AUC = 0.980
Aim 2

Identify a minimal antibody signature from serum and/or urine which could discriminate between individuals with very low intensity infection (CAA positive, egg negative group) and no infection (CAA negative, egg negative group).
Urine and serum antibody signature verification

- Predictive performance of each signature was examined by averaging ROC characteristics obtained from a 15-fold leave-one-out cross-validation.

- SEA accuracy (urine) = 0.85 and SEA accuracy (serum) = 0.77
Summary

- *S. haematobium* protein array (993 antigens) probed with urine (n=137) and sera (n=259) from infected and non-infected individuals.

- In both urine and serum probes, numerous antigens significantly reactive between infected and non-infected populations.

- Diagnostic “signatures” comprised of top-ranked discriminatory antigens have predictive values of infection that exceed SEA, the current ELISA gold standard.

- These antigen signatures can be produced in the lab (more straightforward and rigorous manufacturing process than SEA) and used as the basis for a PoC antibody detection test.
What’s next?

• Antigens are being produced in yeast and *E. coli* to validate proteome array results by ELISA
• Kinetics of antibody responses after treatment
• Given the robust association of *S. haematobium* infection with urogenital cancer, can we use arrays to find an immune signature that predicts progression to malignancy?
• Carefully consider a(with GSA consultation) the TPP of an antibody-based diagnostic test for use in elimination setting

• Need access to more samples (*Sh* endemic area) with matching urine/serum samples and circulating antigen test data
People

Mark Pearson
Luke Becker
Javier Sotillo
Matt Field
Denise Doolan
Carla Proietti

Phil Felgner
Rie Nakajima
Al Jasinskas

Phil Felgner
Beatrice Greco

UC Irvine

Maria Elena Bottazzi
Bin Zhan
Zhu Yun,
Peter Hotez

Baylor College of Medicine

Francisca Mutapi
Janice Murray
Taka Mduluza

NATIONAL SCHOOL OF TROPICAL MEDICINE

Maria Yazdanbakhsh
Abena Amoah

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Mike Hsieh

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Array reactivity plots and ROC curves of top three antigens (serum)

(A) AUC = 0.911

(B) AUC = 0.930

(C) AUC = 0.945
Hierarchical clustering (significantly reactive antigens)

Urine