Singapore Immunology Network
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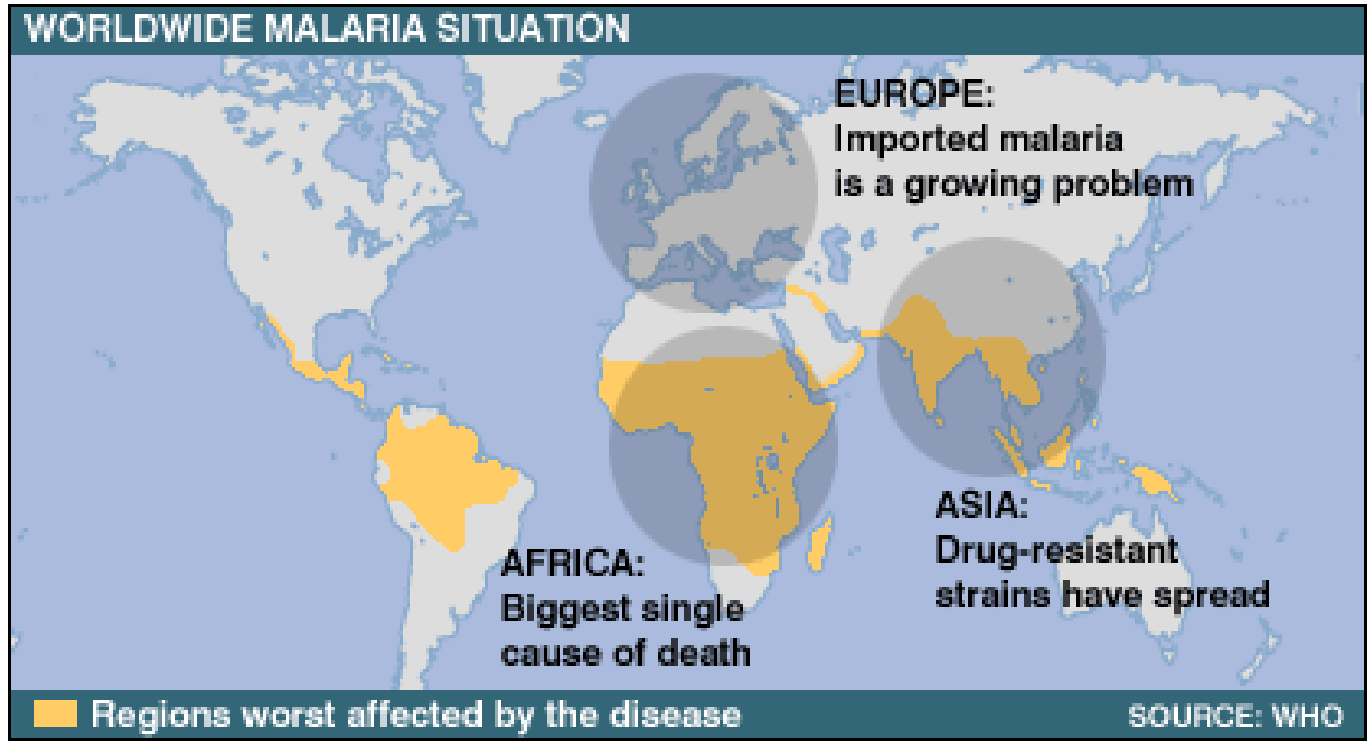
Defining targets and correlates associated with sterile protection in experimentally vaccinated humans

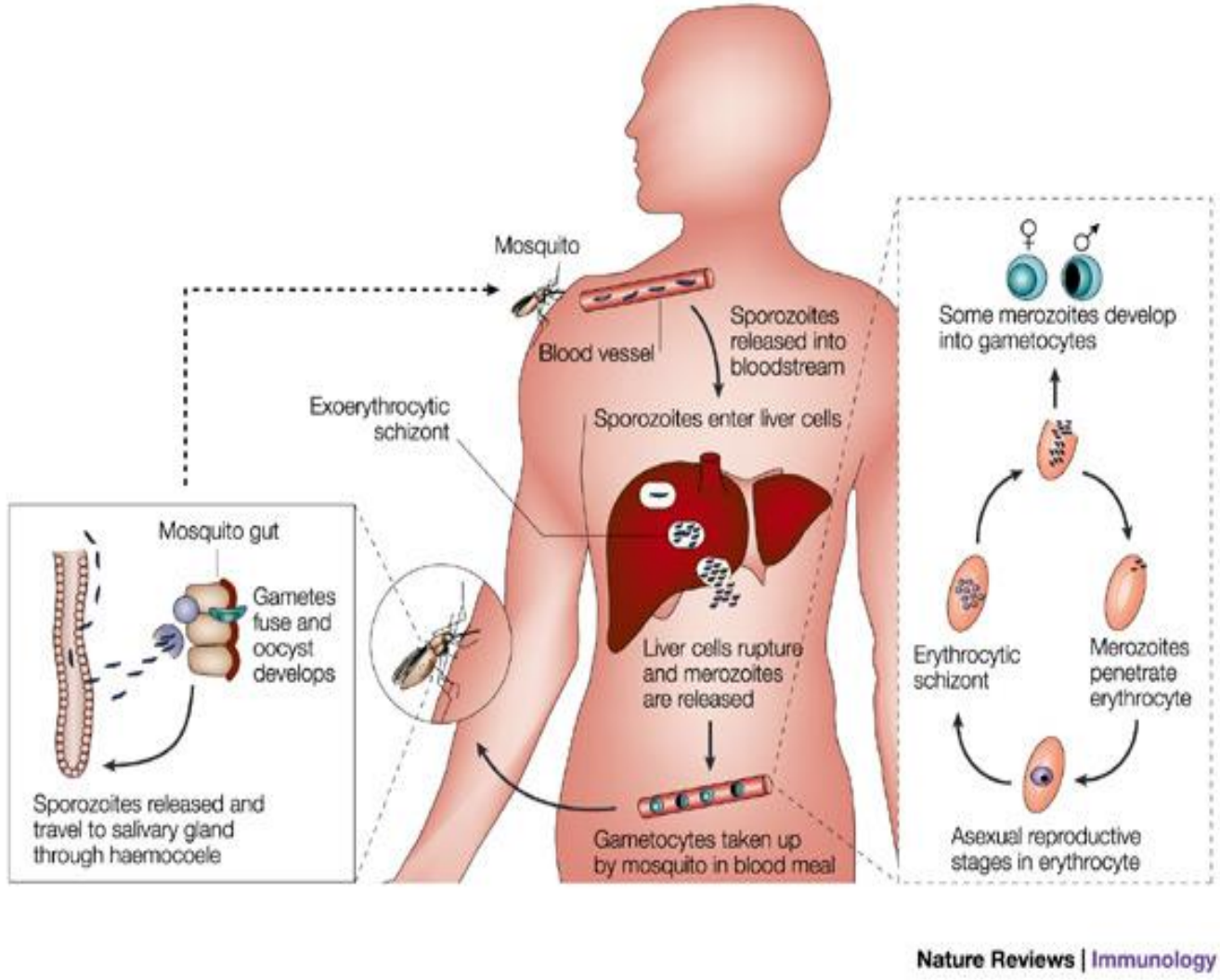
Asian Pacific Vaccinology Meeting

Laurent Rénia
Pathogen Immunobiology laboratory

Malaria: the present situation

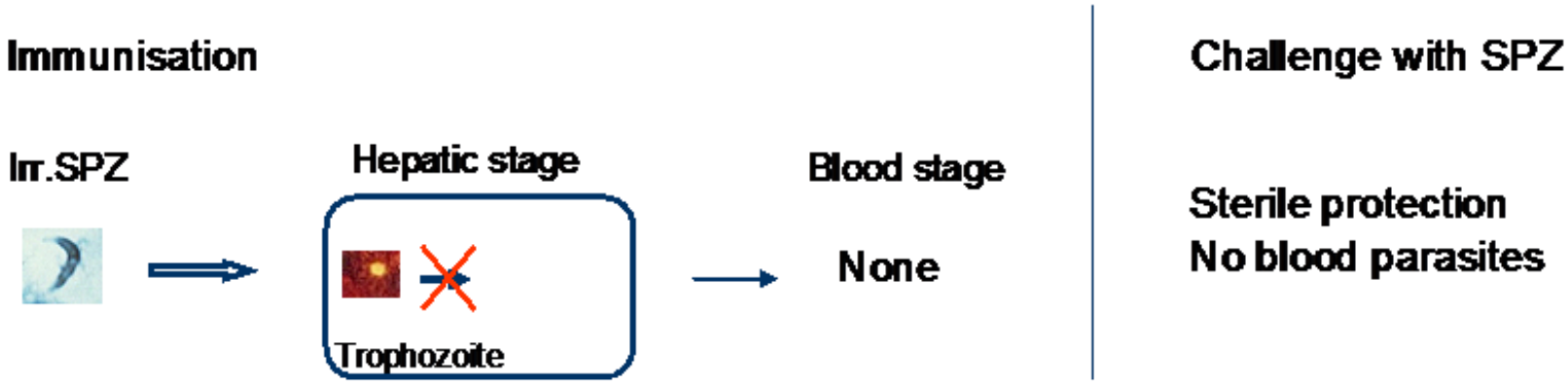


Plasmodium life cycle



4 + 1 species infecting humans: *P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi*

Gold Standard Vaccine: the irradiated Sporozoite

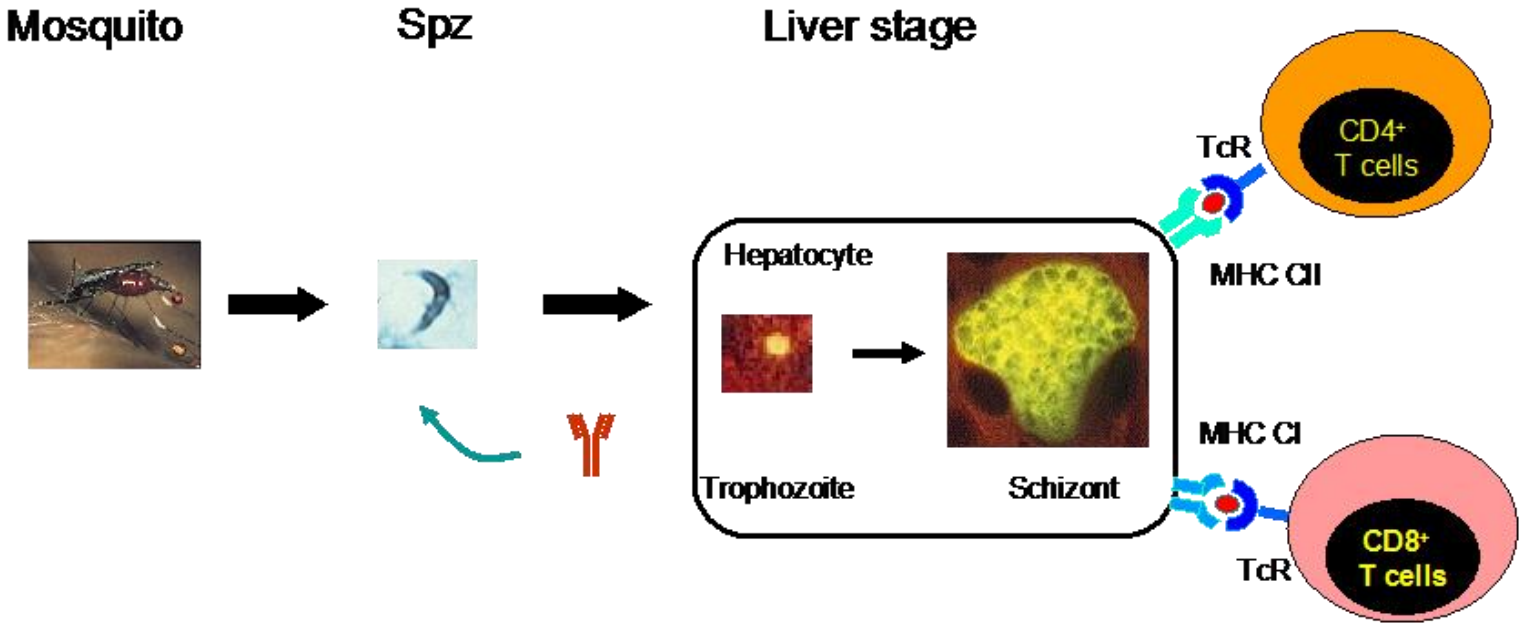


- Birds, mice, rats, monkeys, Humans

UV irradiation : Russell and Mulligan , J Mal Inst India, 1942
Richards, Nature, 1966
X-ray irradiation : Nussenzweig et al, Nature, 1967
Clyde et al, Am J Med Sci, 1973; Rieckmann et al, Trans R Soc Hyg, 1974

- Stage specific protection: protection only against pre-erythrocytic parasites (Sporozoites + Liver stage)

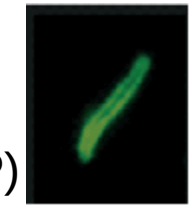
Immune responses and pre-erythrocytic stage immunity in mice



. Antibodies

. Protective effector CD8+ T and/or CD4+ T cells

. Major antigen recognized : the circumsporozoite (CSP)

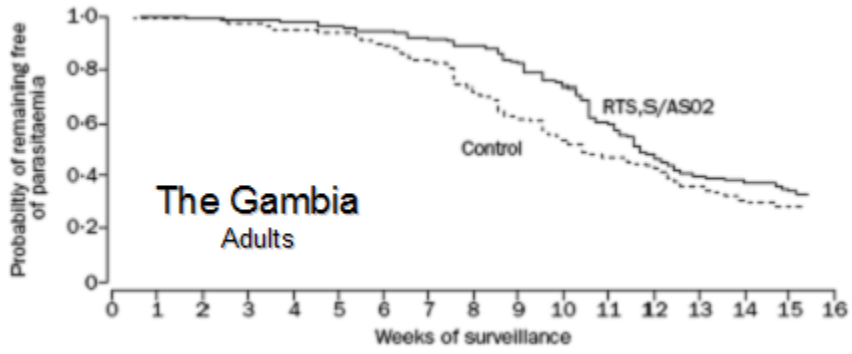


The RTS,S Vaccine: a CSP-based vaccine

CSP HBV sAg
AS03

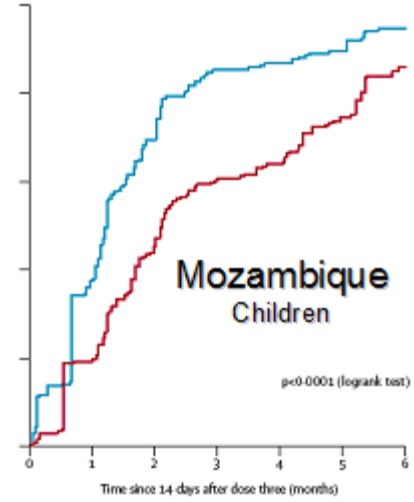
Naïve Volunteers
Adjuvant-Dependent Efficacy
48% Sterile Protection

Bojang et al. 2001 Lancet



Field Trials
Protection
At best 30% Sterile Protection
Short-lived

Alonso et al. 2004 Lancet

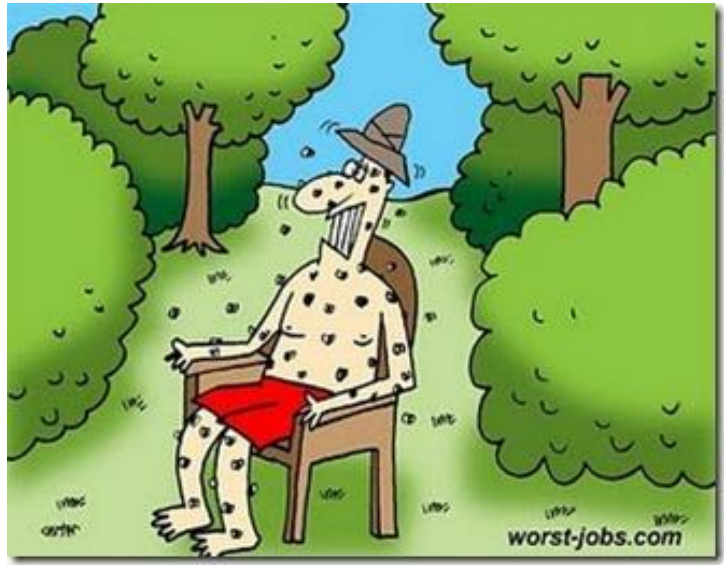


An evolving concept: Protection against disease : 28-55%

2015: Protection is strain-specific : *Neafsey et al., New Engl J Med, in press*

Malaria Vaccine

Whole Parasite Vaccines → Subunit Vaccines



Irradiated sporozoites through mosquito bites requires 1000 infective mosquito bites over a 6 month period

No subunit vaccines have reached Efficacy > 50%

Why Is there no efficient sub-unit malaria vaccine?

- Escape mechanisms developed by parasites
- Ag polymorphism, immunosuppression, enhancing antibodies…

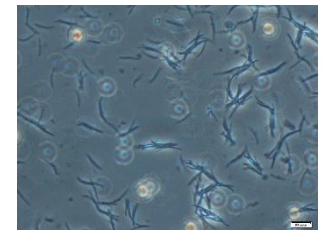
- Do we have identified the right antigen (s)?
  P. falciparum genome : 5000 malaria genes

- What immune mechanisms to target ⟷ Absence of correlates of protection *in vitro* and *in vivo*

The irradiated Sporozoite vaccine in humans

Sterile frozen /thawed irradiated sporozoites




- **Intramuscular injections: 4 x 135,000:** **Protection 0%**

Live Attenuated Malaria Vaccine Designed to Protect Through Hepatic CD8⁺ T Cell Immunity

J. E. Epstein,[1*] K. Tewari,[2*] K. E. Lyke,[3*] B. K. L. Sim,[4,5] P. F. Billingsley,[4] M. B. Laurens,[3,6] A. Gunasekera,[4] S. Chakravarty,[4] E. R. James,[4] M. Sedegah,[1] A. Richman,[4] S. Velmurugan,[4] S. Reyes,[1] M. Li,[5] K. Tucker,[7] A. Ahumada,[4,5] A. J. Ruben,[4] T. Li,[4] R. Stafford,[4,5] A. G. Eappen,[4] C. Tamminga,[1] J. W. Bennett,[8] C. F. Ockenhouse,[8] J. R. Murphy,[8] J. Komisar,[8] N. Thomas,[1] M. Loyevsky,[4] A. Birkett,[9] C. V. Plowe,[3,6] C. Loucq,[9] R. Edelman,[3] T. L. Richie,[1] R. A. Seder,[2]†‡ S. L. Hoffman[4,5]†‡

Science, 2011

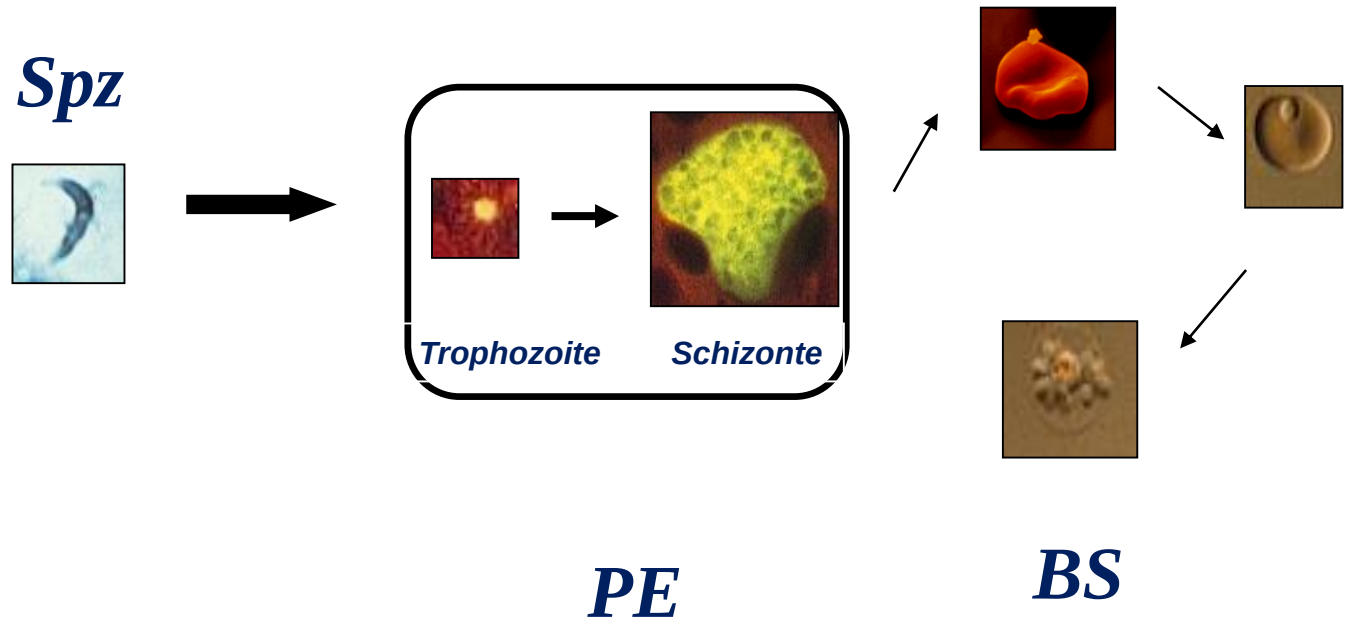
- **Intravenous injections: 5 x 135,000** **Protection: 100%**

Protection Against Malaria by Intravenous Immunization with a Nonreplicating Sporozoite Vaccine

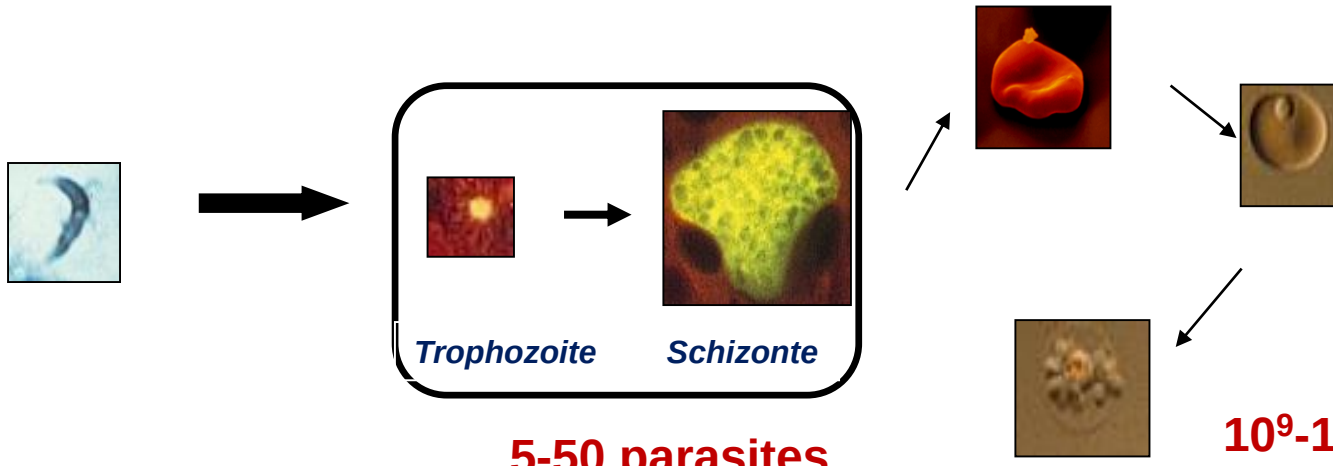
Robert A. Seder,[1*†] Lee-Jah Chang,[1*] Mary E. Enama,[1] Kathryn L. Zephir,[1] Uzma N. Sarwar,[1] Ingelise J. Gordon,[1] LaSonji A. Holman,[1] Eric R. James,[2] Peter F. Billingsley,[2] Anusha Gunasekera,[2] Adam Richman,[2] Sumana Chakravarty,[2] Anita Manoj,[2] Soundarapandian Velmurugan,[2] MingLin Li,[3] Adam J. Ruben,[2] Tao Li,[2] Abraham G. Eappen,[2] Richard E. Stafford,[2,3] Sarah H. Plummer,[1] Cynthia S. Hendel,[1] Laura Novik,[1] Pamela J.M. Costner,[1] Floreliz H. Mendoza,[1] Jamie G. Saunders,[1] Martha C. Nason,[4] Jason H. Richardson,[5] Jittawadee Murphy,[5] Silas A. Davidson,[5] Thomas L. Richie,[6] Martha Sedegah,[6] Awalludin Sutamihardja,[6] Gary A. Fahle,[7] Kirsten E. Lyke,[8] Matthew B. Laurens,[8,9] Mario Roederer,[1] Kavita Tewari,[1] Judith E. Epstein,[6] B. Kim Lee Sim,[2,3] Julie E. Ledgerwood,[1] Barney S. Graham,[1‡] and Stephen L. Hoffman,[2,3‡] the VRC 312 Study Team§

Science, 2013,

Immunisation with live sporozoites
Targeting the whole pre-erythrocytic stage



Immunity during natural infection
focus of blood stage

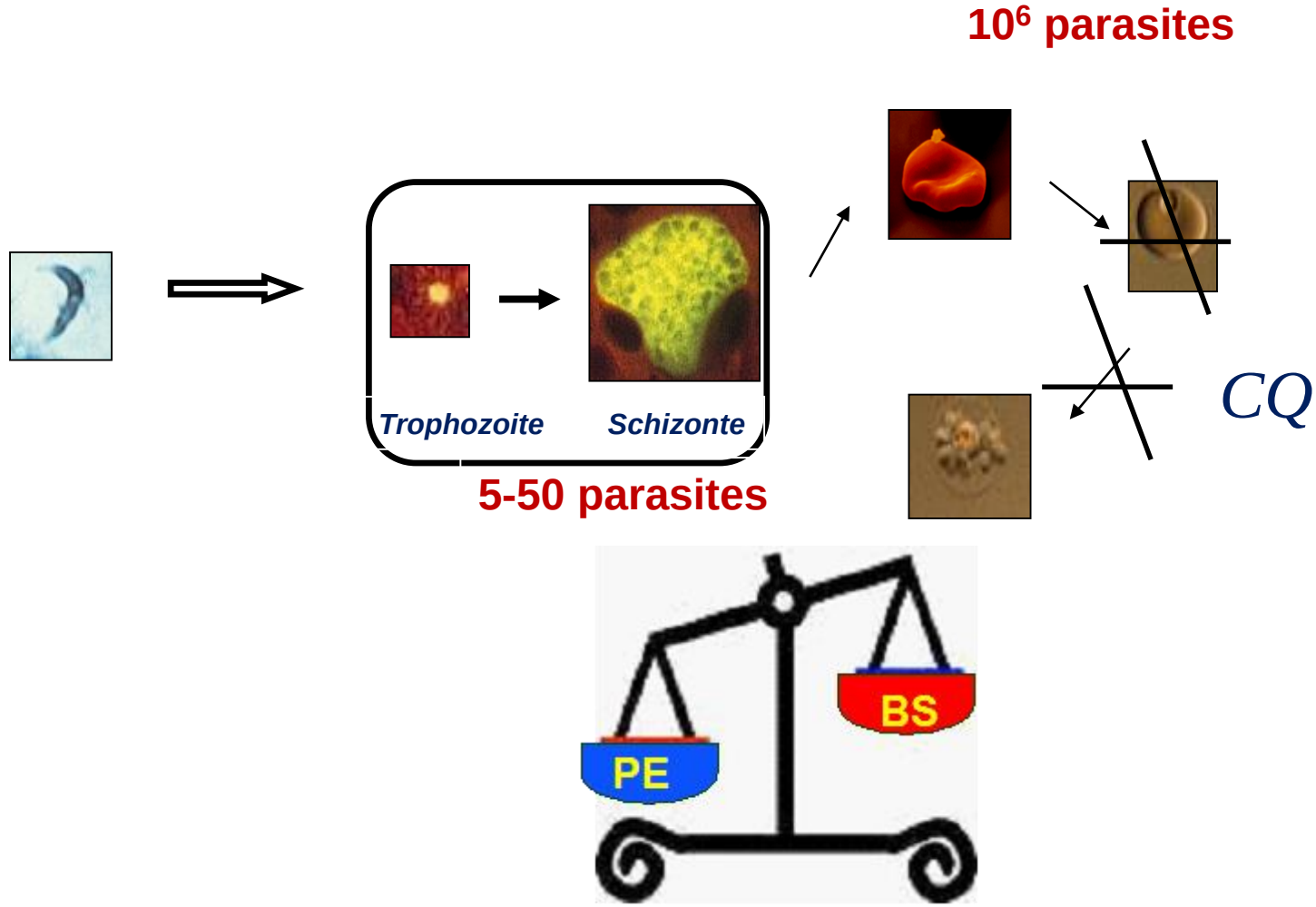


5-50 parasites

10^9-10^{10} parasites

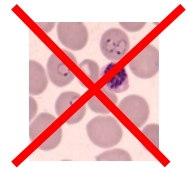
Blood stage infection induce immune diversion immunosuppression

Chloroquine has no effect on liver stage and allows expression of liver and early stage antigen repertoires

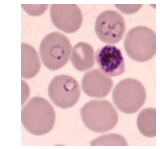


Immunization protocol with live sporozoites

CQ



Blood smears -ve



Blood smears
d3-d10
post-challenge

Days

0

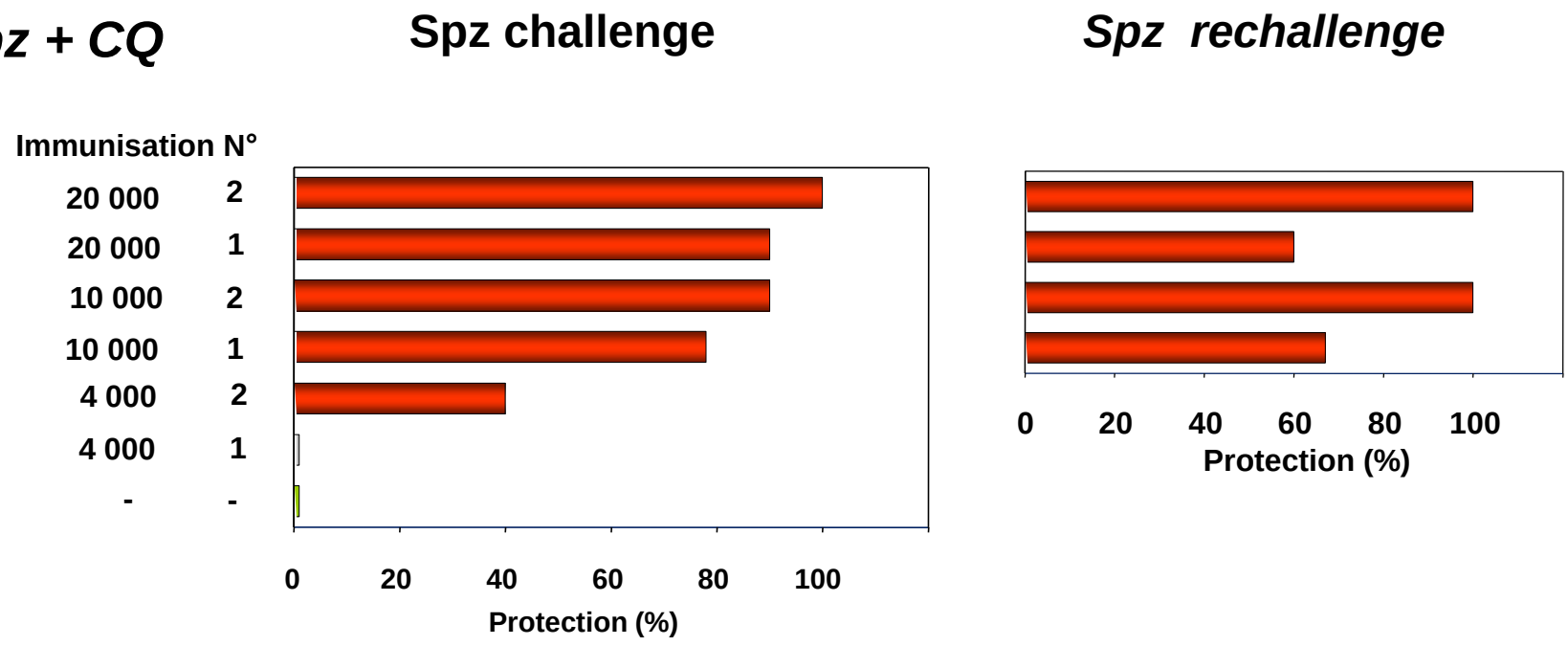
10

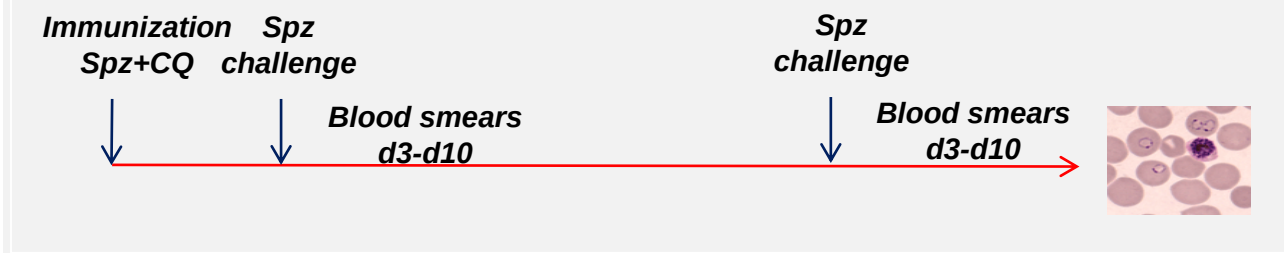
25

Normal Spz
Immunization

SPZ
Challenge
i.v. or mosquito bites)

Induction of long lasting sterile protection





The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Protection against a Malaria Challenge by Sporozoite Inoculation

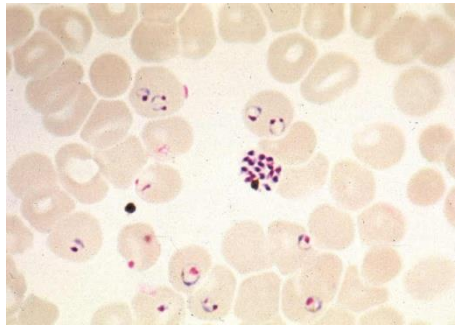
Meta Roestenberg, M.D., Matthew McCall, M.D., Joost Hopman, M.D., Jorien Wiersma, Adrian J.F. Luty, Ph.D., Geert Jan van Gemert, B.Sc., Marga van de Vegte-Bolmer, B.Sc., Ben van Schaijk, M.Sc., Karina Teelen, Theo Arens, Lopke Spaarman, B.Sc., Quirijn de Mast, M.D., Will Roeffen, Ph.D., Georges Snounou, Ph.D., Laurent Rénia, Ph.D., Andre van der Ven, M.D., Cornelus C. Hermsen, Ph.D., and Robert Sauerwein, M.D.

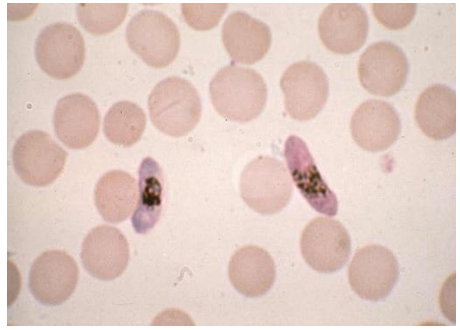
2009

Pr Robert Sauerwein
Department of Medical Microbiology
Radboud University Nijmegen
Medical Centre Nijmegen
The Netherlands

Experimental Human Infection

Production of *P falciparum* NF54 gametocytes





Infect volunteers

Exposure *to 5 infected mosquitoes*

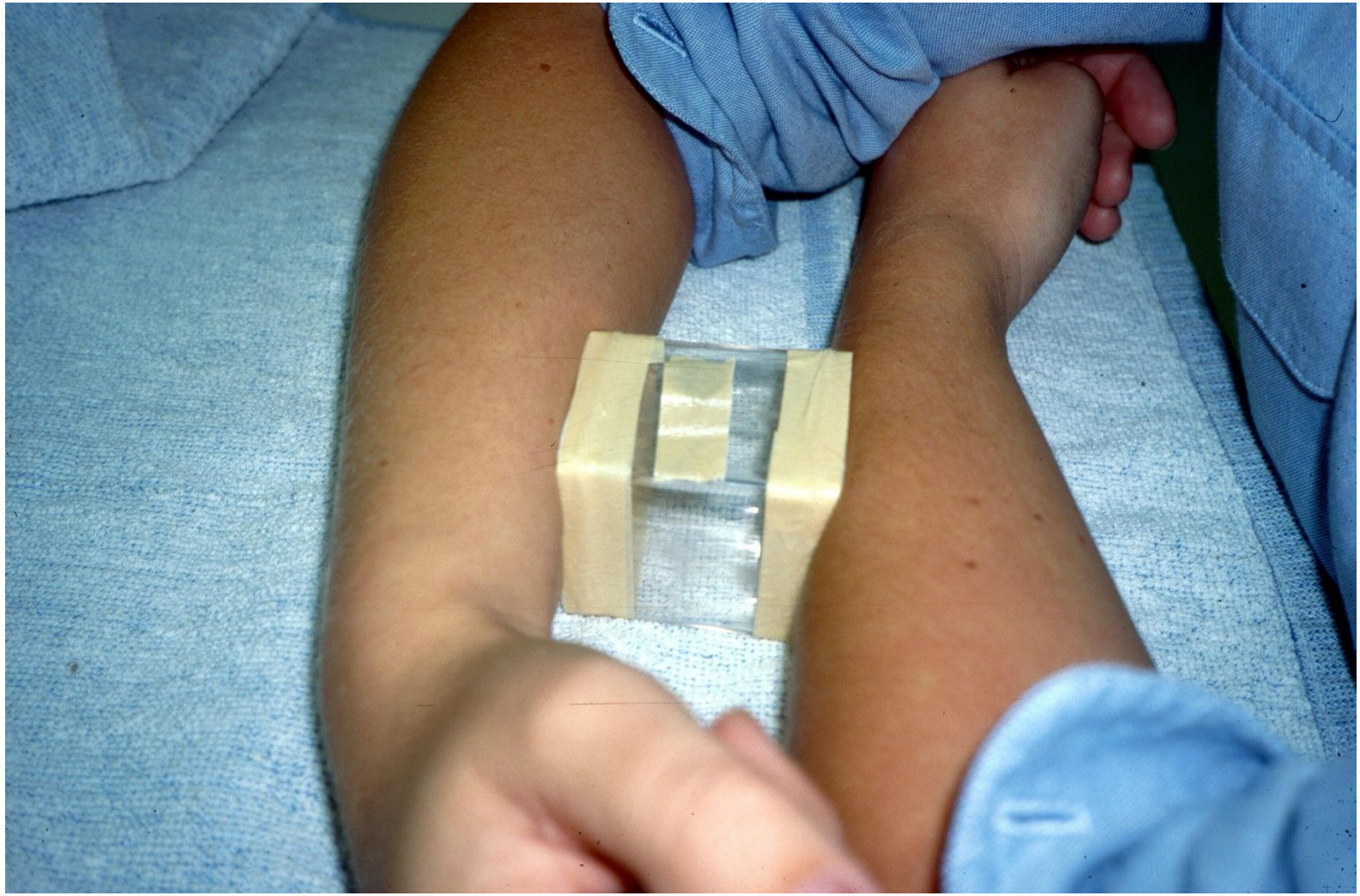
Follow-up *from day 4 -21*

- *parasitaemia : 2 times/ day*
- *clinical symptoms*
- *routine clinical lab*

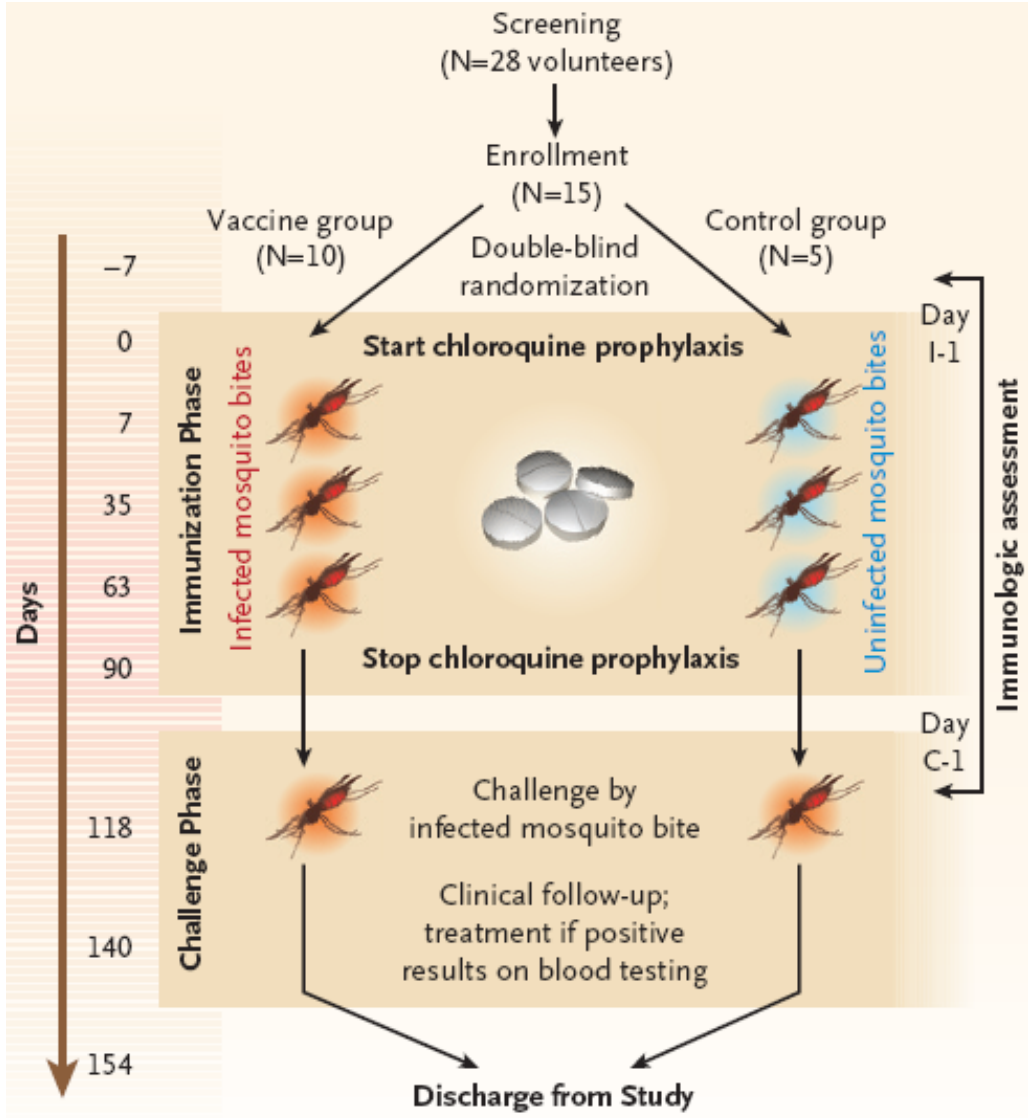
Treatment with antimalarial drug

The happy volunteers

Artificial infection

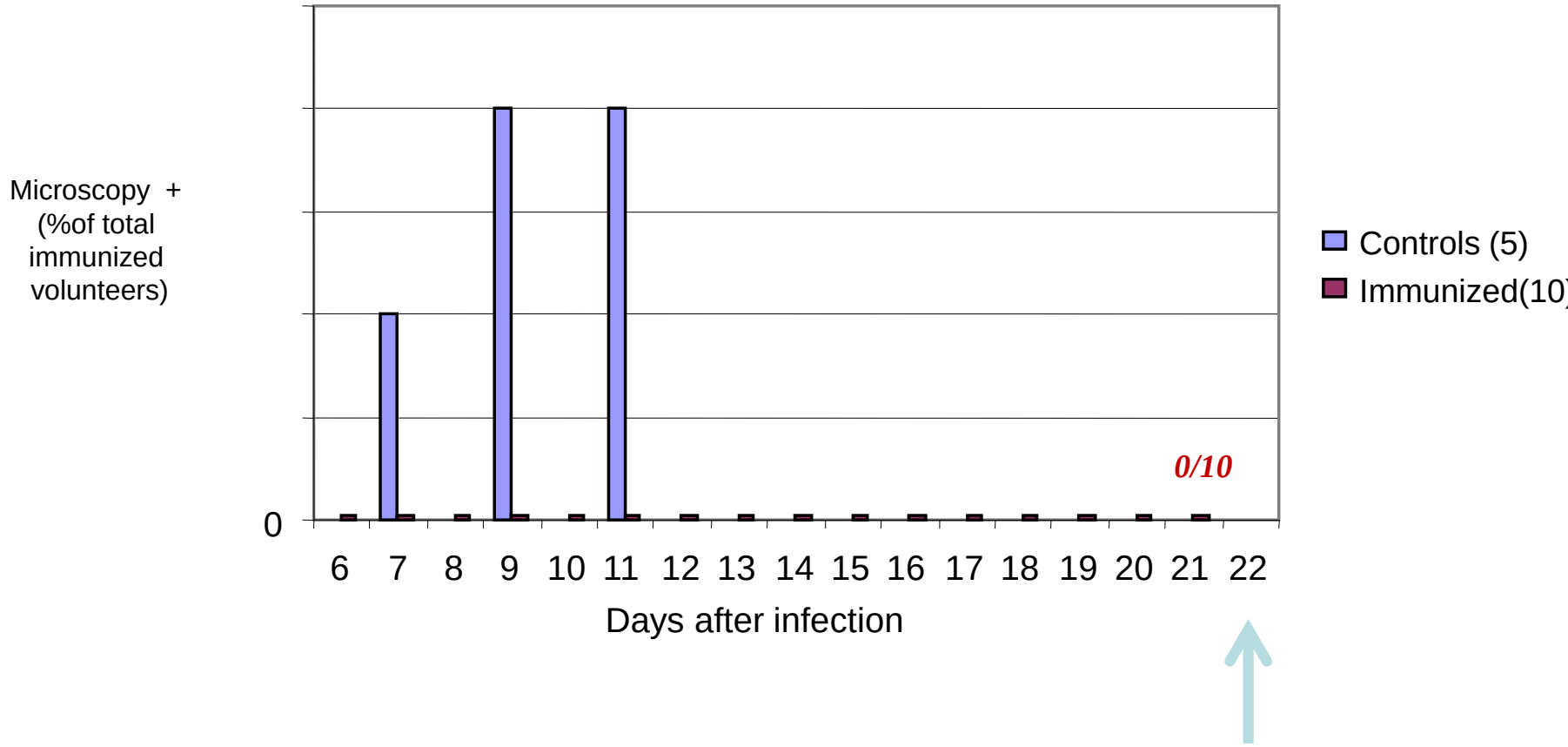


Study design and enrollment

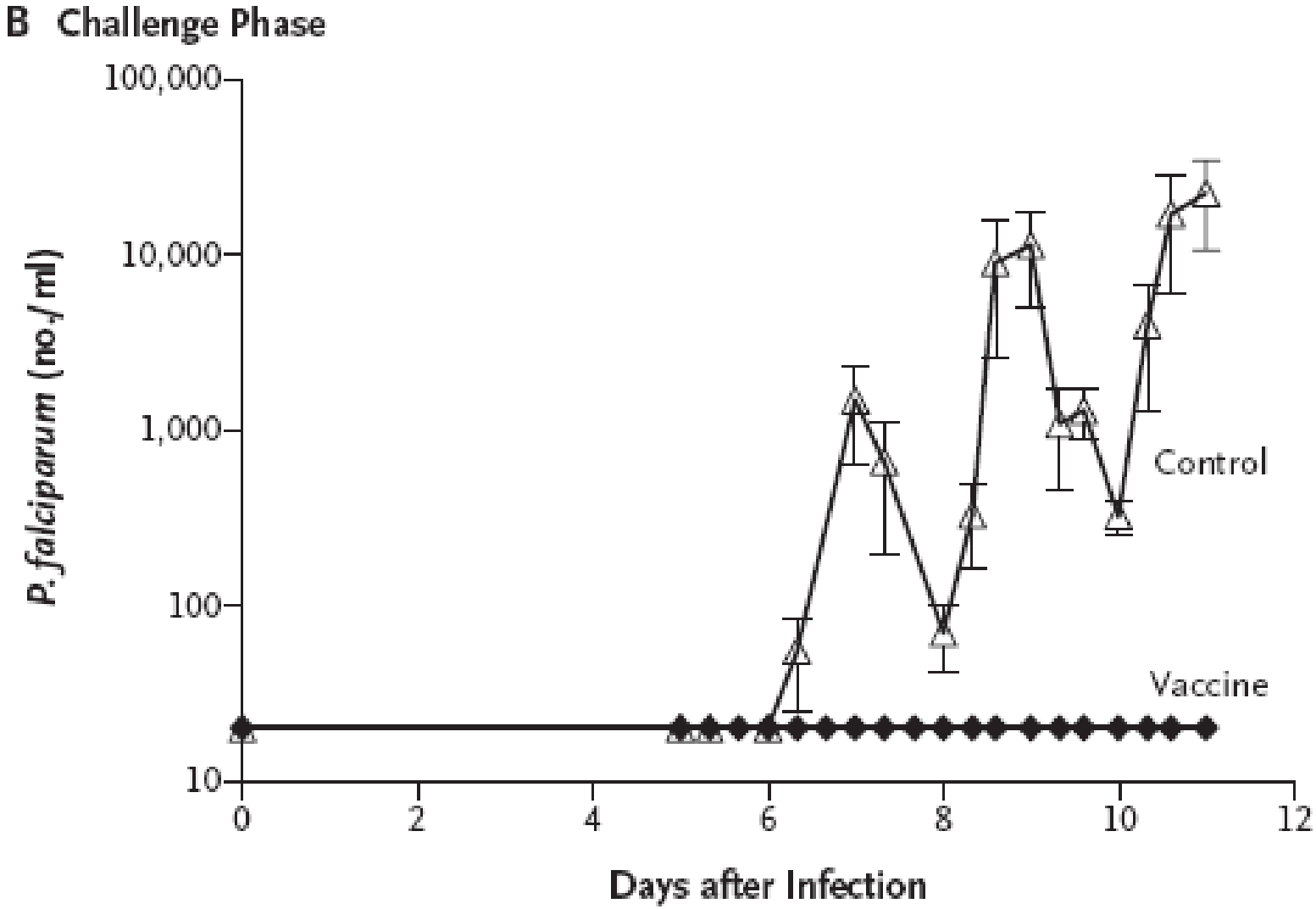


Roestenberg et al., New Eng J Med , 2009

Volunteers with positive microscopy after challenge



Parasitaemia (qPCR) after challenge



Discussion

- **Immunization with total of 45 infected mosquitoes (450-2250 sporozoites) induces 100% protection against parasitemia after a homologous challenge.**

- **Immunization shows progressive reduction of parasitemia with each infection suggestive of induction of immunity.**

- **Rechallenge after 29 months (~2 years and half): 4/6 vaccinees still completely protected (Roestenberg et al., Lancet , 2011)**

. **Immunity mainly against liver stage parasite (Bijker et al., PNAS, 2013)**

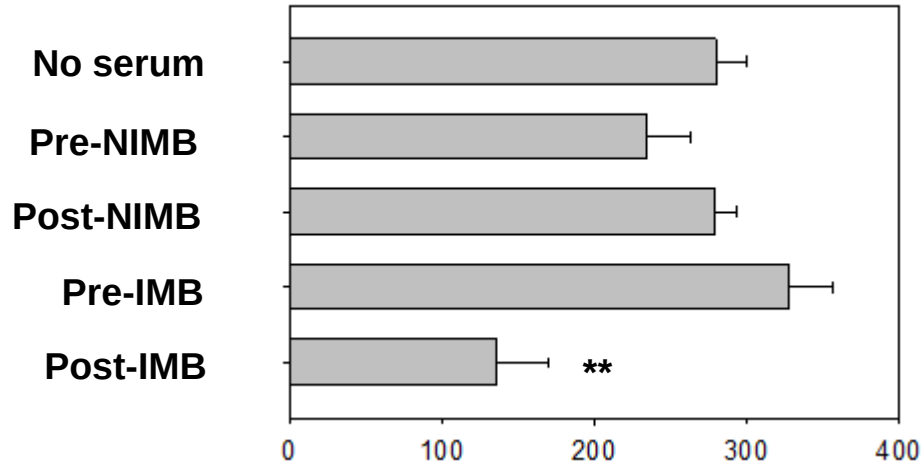
- **IMMUNE RESPONSES**

  Associated with polyfunctional effector memory CD4+ T cells expressing IFN-g, TNF-a. IL-2 specific for blood stage antigens

- **Role of antibodies ?**
- **Antigens?**

Sera from protected volunteers inhibit *Plasmodium* sporozoite invasion of primary human hepatocytes

- **"Immunized" sera from IMB individuals have antibodies against all stages**



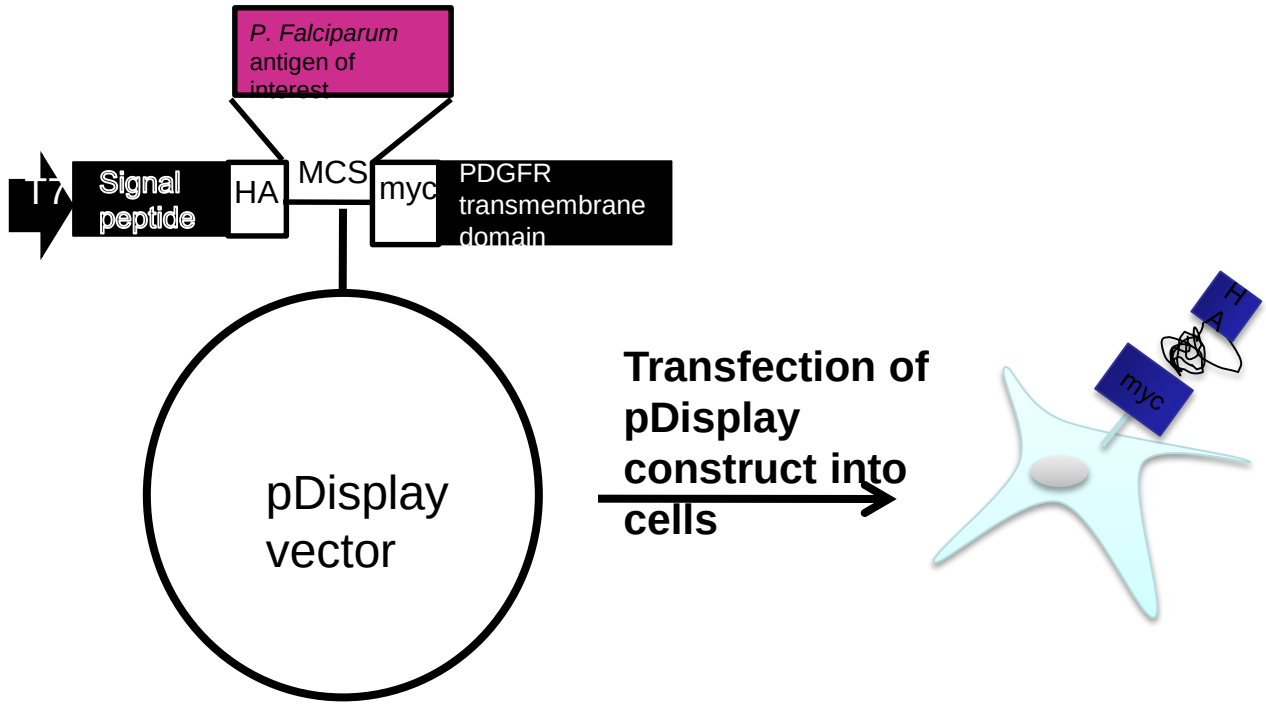
NIMB: Non-infected mosquito bites

IMB: Infected mosquito bites

Number of parasites in hepatocytes (mean±SD)

- **Immunized sera did not inhibit blood stage parasite invasion of naïve erythrocytes**

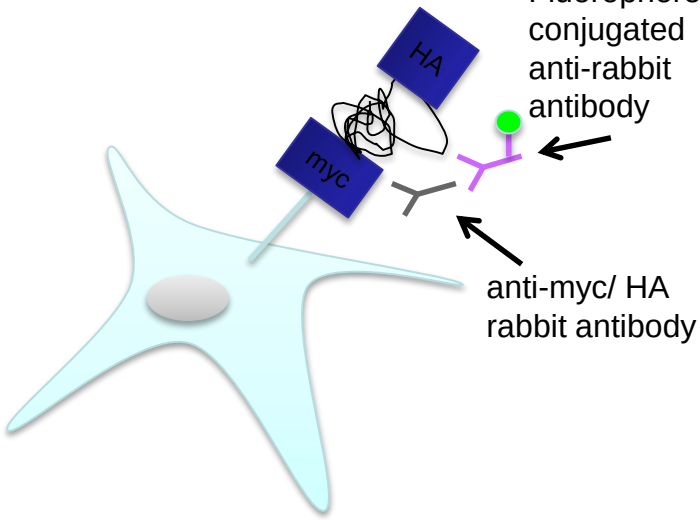
P. falciparum pDisplay antigen library
A novel system for screening serum antibodies

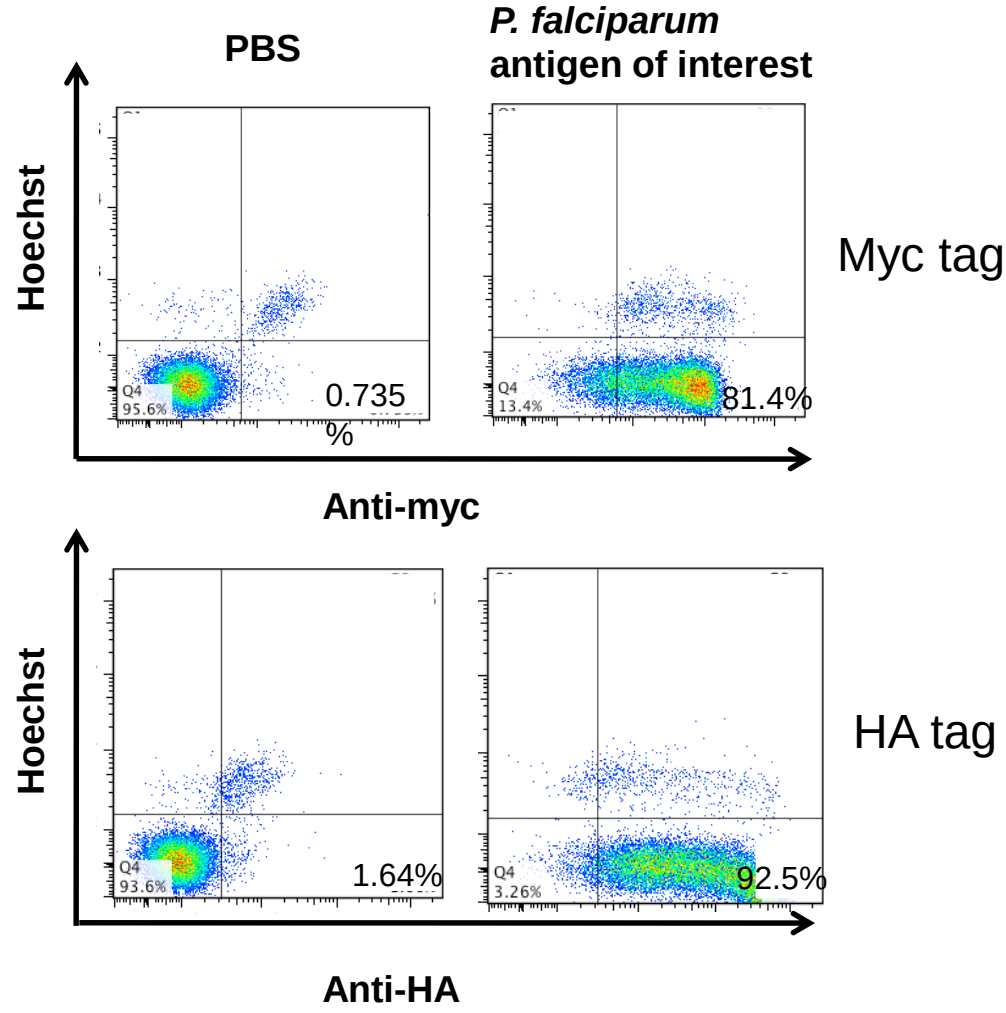


• Selected a panel of more 50 selected *P. falciparum* proteins that were expressed during the sporozoite stage to be cloned into pDisplay vector

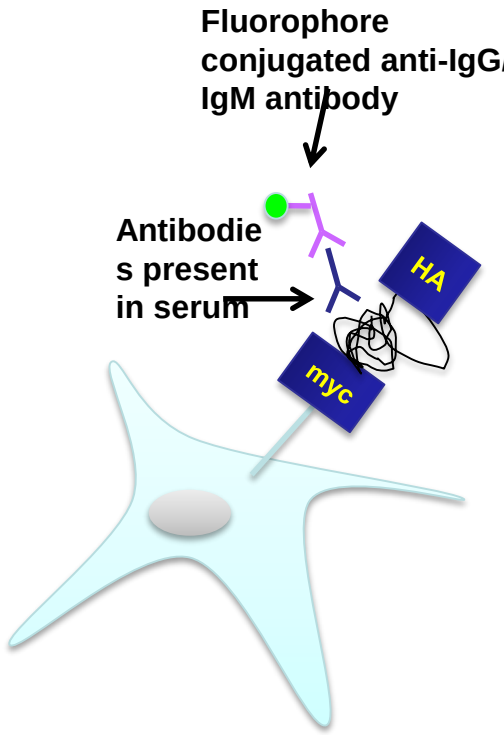
• Created a library of transfected cell lines that each express a different *P. falciparum* antigen of interest

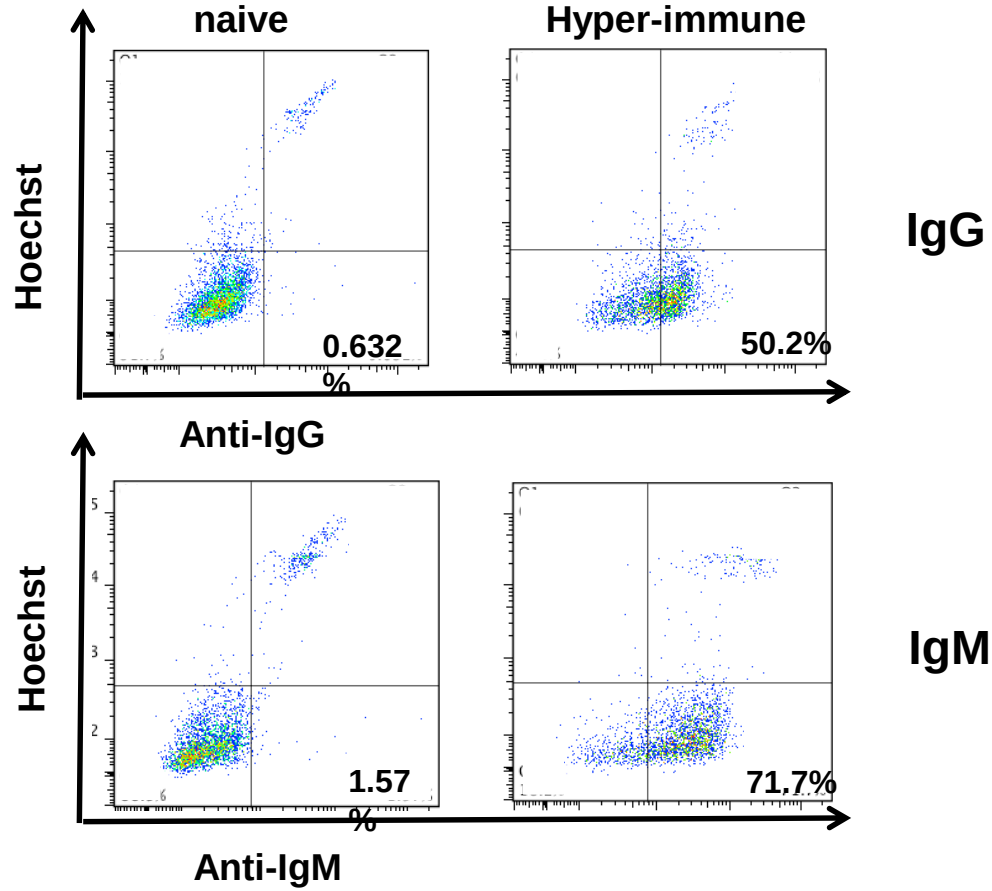
Cells that express *Plasmodium* antigen can be detected via the myc or HA tag





Utilization of cell surface expressed *P. falciparum* antigen library towards detection of sera IgG/ IgM antibodies that recognize the antigen of interest





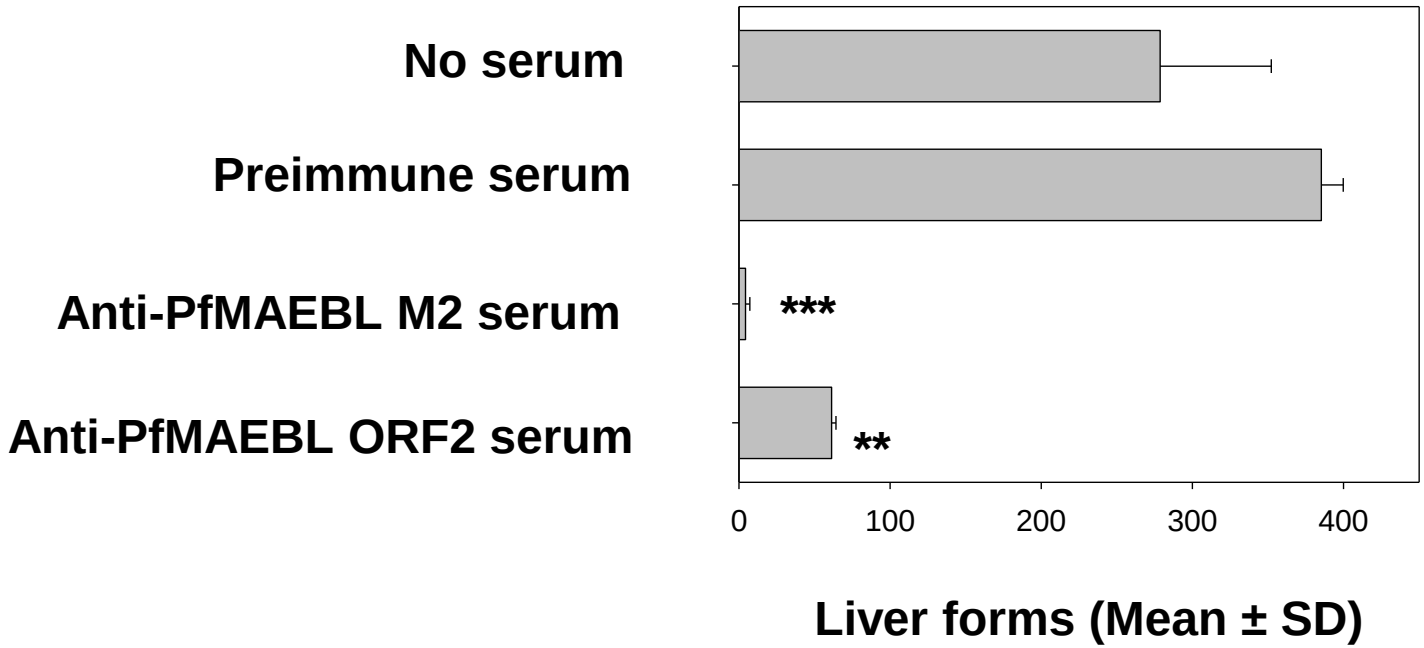
$$\text{Serum response} = \frac{\text{Cell population with bound antibodies}}{\text{Cell population that is positively transfected}} \times 100\%$$

Immunized individuals exhibit a varied and broad antibody response

Gene name	plasmodb	Amino acid position	Infectious bites									Non-infectious bites				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
EXP1	PF11_0224	28-77														
EXP1	PF11_0224	102-162														
HPTM1	PFI1590c	383-802														
HPTM3	PFF1315w	1137-1713														
LSA1	PF10_0356	650-1162														
LSA3	PFB0915w	84-590														
LSA3	PFB0915w	559-1040														
LSA3	PFB0915w	1001-1429														
maebl	PF11_0486	653-1005														
maebl	PF11_0486	958-1249														
MSP2	PFB0300c	114-272														
MSP3	PF10_0345	29-354														
MSP7	MAL13P1.174	28-281														
MTRAP	Pf10_0281	292-424														
PALPF3	MAL13P1.206	311-652														
PF38	PFE0395c	34-320														
PF92	PF13_0338	34-484														
RHOPH3	PFI0265c	16-505														
TRAP	PF13_0201	28-183														
TRAP	PF13_0201	204-499														
SEA	PF3D7_1021800	2431-3249														

Serum response >10%

Antibodies against *P. falciparum* MAEBL block sporozoite development in primary human hepatocytes



Conclusions

- Humoral immunity has a functional role in protection during live sporozoite inoculation under drug prophylaxis

- Developed a novel cell surface expressed *P. falciparum* antigen library that can be applied towards serum screening

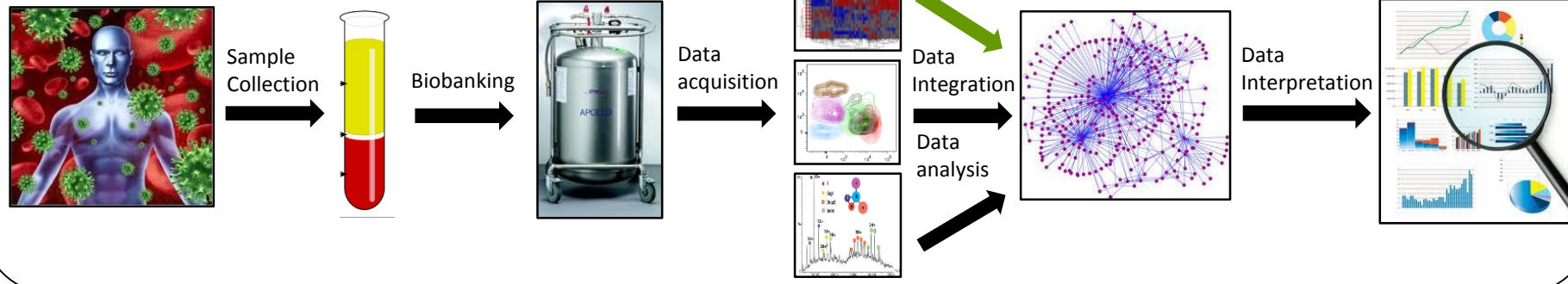
- A broad and diverse antibody repertoire was induced in protected individuals → may be a pre-requisite for achieving significant protection

- Three antigens (PfMAEBL, TRAP, PfSEA-1) were highly recognized

- Demonstrated that anti-*Pf*MAEBL antibodies play a role in antibody neutralization of pathogen → potential vaccine targets

- Whole parasite vaccines or subunit vaccines that utilize a broader antigenic repertoire may be the way to go in future

Clinical Immunomonitoring Platform

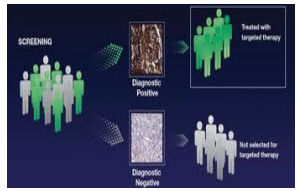


The Immunomonitoring Platform can be engaged at all phases of research:

EARLY DISCOVERY → PRECLINICAL STUDIES (In rodents and NHPs) → CLINICAL TRIAL ASSESSMENT

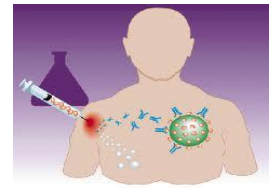
Potential Applications of the Immunomonitoring Platform:

Biomarker Discovery



Target ID

Vaccine Assessment



Drug-induced Immune Profiling

Acknowledgements

SIgN
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Anne Charlotte Gruner
Wu Ying Ying
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+ members of LR group

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Radboud University, Netherlands
Robert Sauerwein

INSERM U945, France
Georges Snounou
Dominique Mazier
Jean-Francois Franetich

Singapore Immunology Network

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Thank you for your attention!

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