Estimation of inhibitory antibodies of mice immunized with *Plasmodium vivax* Duffy Binding Protein region II (DBPII)

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Background

- Malaria is a disease caused by protozoan parasites from the genus *Plasmodium.*
- An estimated 8.55 million cases of malaria were caused by *Plasmodium vivax* in 2016, primarily affecting countries that have poor access to healthcare[1] .
- *P. vivax* preferentially invades reticulocytes via binding between the parasite's Duffy binding protein (DBP) and the Duffy antigen receptor for chemokines (DARC).
- *P. vivax* is largely absent in regions with high Fy^{a-b-} DARC negativity. (Fig 1a)
- The *Pv*DBP region II (DBP II) contains critical residues for DARC recognition. (Fig 1c)
- The necessity of this interaction makes DBP II an ideal vaccine candidate, as it elicits broadly neutralizing inhibitory antibodies.
- A persistent, long-lasting, broadly neutralizing, strain transcending inhibitory antibody is essential for a successful blood-stage *P. vivax* vaccine candidate.

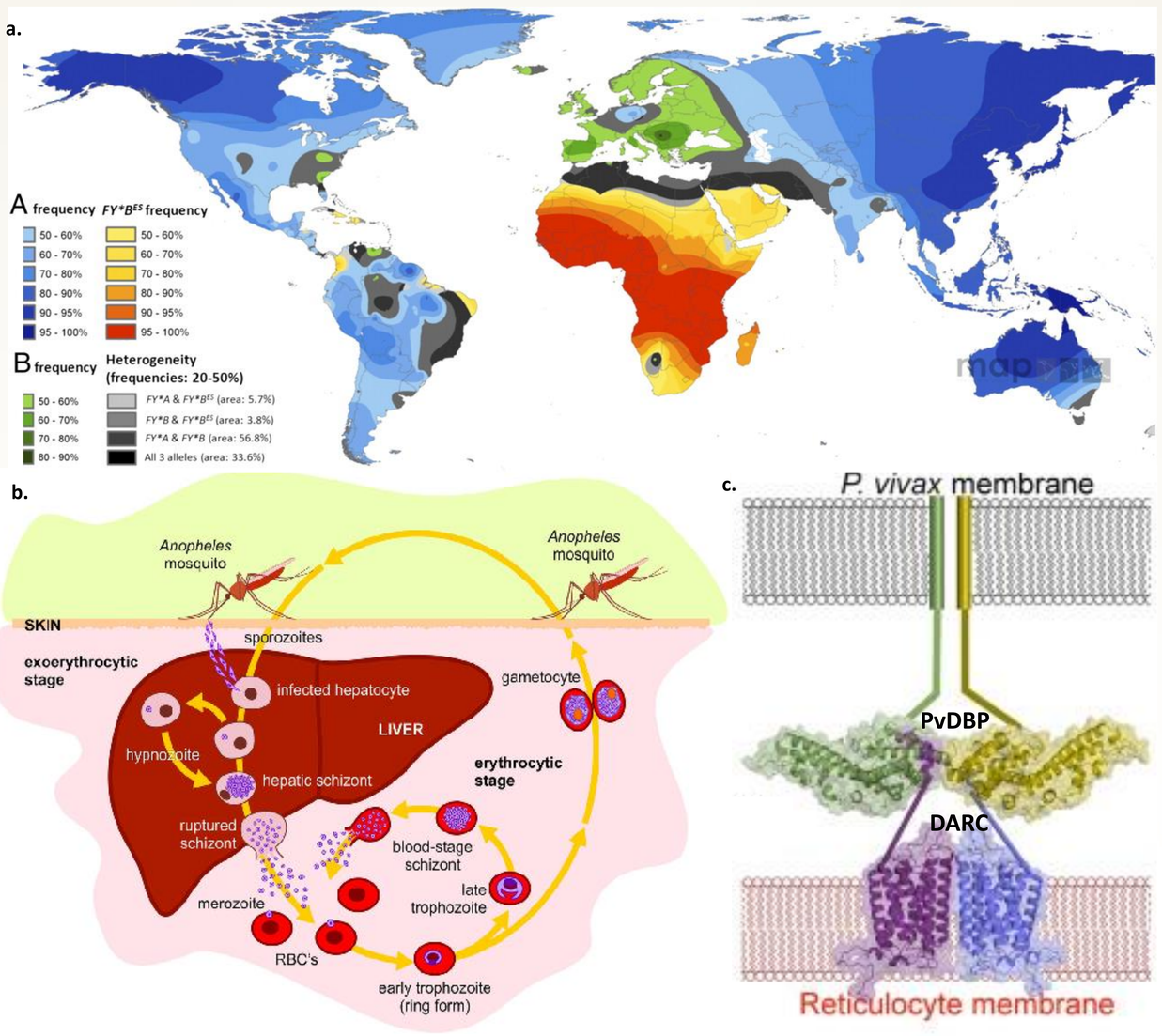

Figure 1: (a). Global map showing the frequency of the Duffy polymorphism (Source: King, C.L. *et.al.*,)[2] **(b).** *Plasmodium vivax* life cycle, which includes vector transmission, then liver and blood stage infection once in humans (Source: Lima-Junior, J. *et. al.,*)[3] **(b).** PvDBP-DARC heterotetramer (Source: Batchelor, J.D. *et. al.,*)[4]

Aims

1. To study the transfection efficiency of cos 7 cells with different DBP II antigen alleles (Sal I, 7.18, AH, P).
2. To analyze the functional inhibition of normal mouse and anti-DBP II sera.
3. To estimate the percent of functional inhibitory antibodies in mice immunized with DBP II, CpG and alum.

Methodology

- There is extreme difficulty in culturing *P. vivax in vitro*, therefore inhibition of anti-DBP II mouse antibodies was determined through transient transfection of Cos7 cells, a line of rhesus monkey fibroblasts.
- Transient transfection of Cos7 cells using DBPII_GFP were performed (figure 2a).
- Standard Cos7 cell binding assay was used to determine the functional inhibition of antibodies (figure 2b).

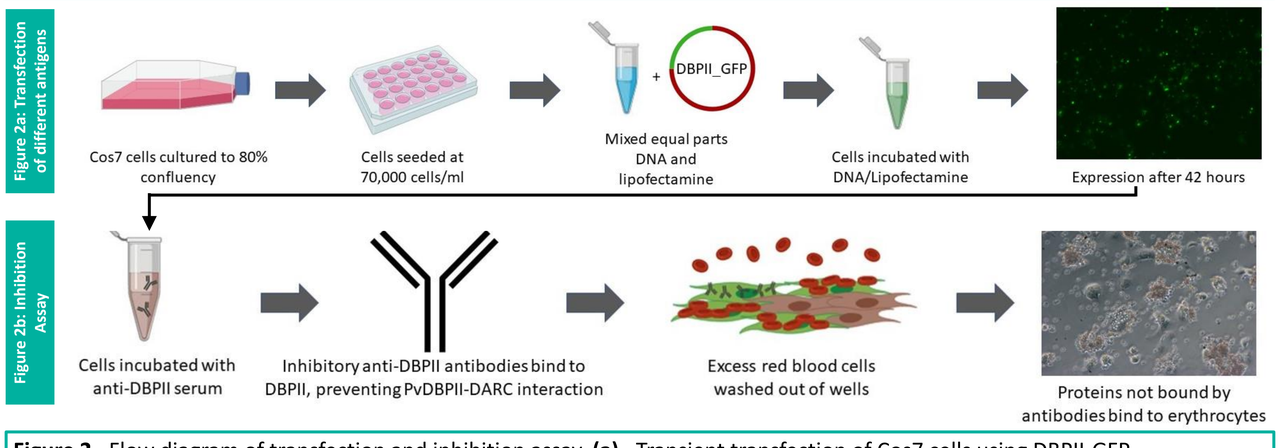

Figure 2. Flow diagram of transfection and inhibition assay. **(a).** Transient transfection of Cos7 cells using DBPII-GFP. **(b).** Inhibition assay using serum collected from immunized mice.

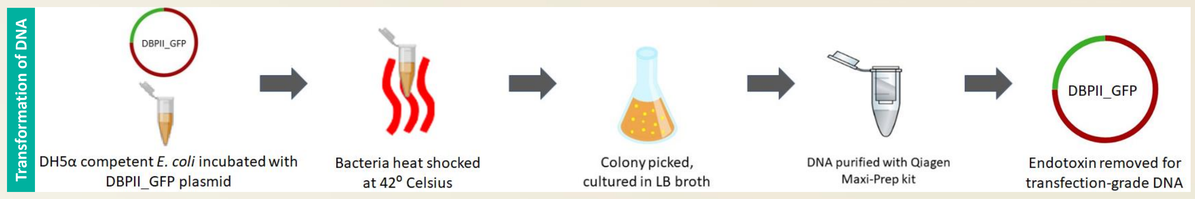

Figure 3. Heat shock transformation of optimal plasmid DNA into DH5α competent *E. coli* for further transfection.

Preliminary Results

- Transfection efficiency of antigens shown, along with binding efficiency of erythrocytes to transfected Cos7 cells. (Fig 4)
- 7.18 exhibited highest transfection efficiency, while the P allele exhibited poor expression.
- The inhibitory function of sera was determined using the following formula:

  Percent inhibition = [1 – (number of rosettes in the presence of antisera/Number of rosettes in the presence of positive control)] x 100

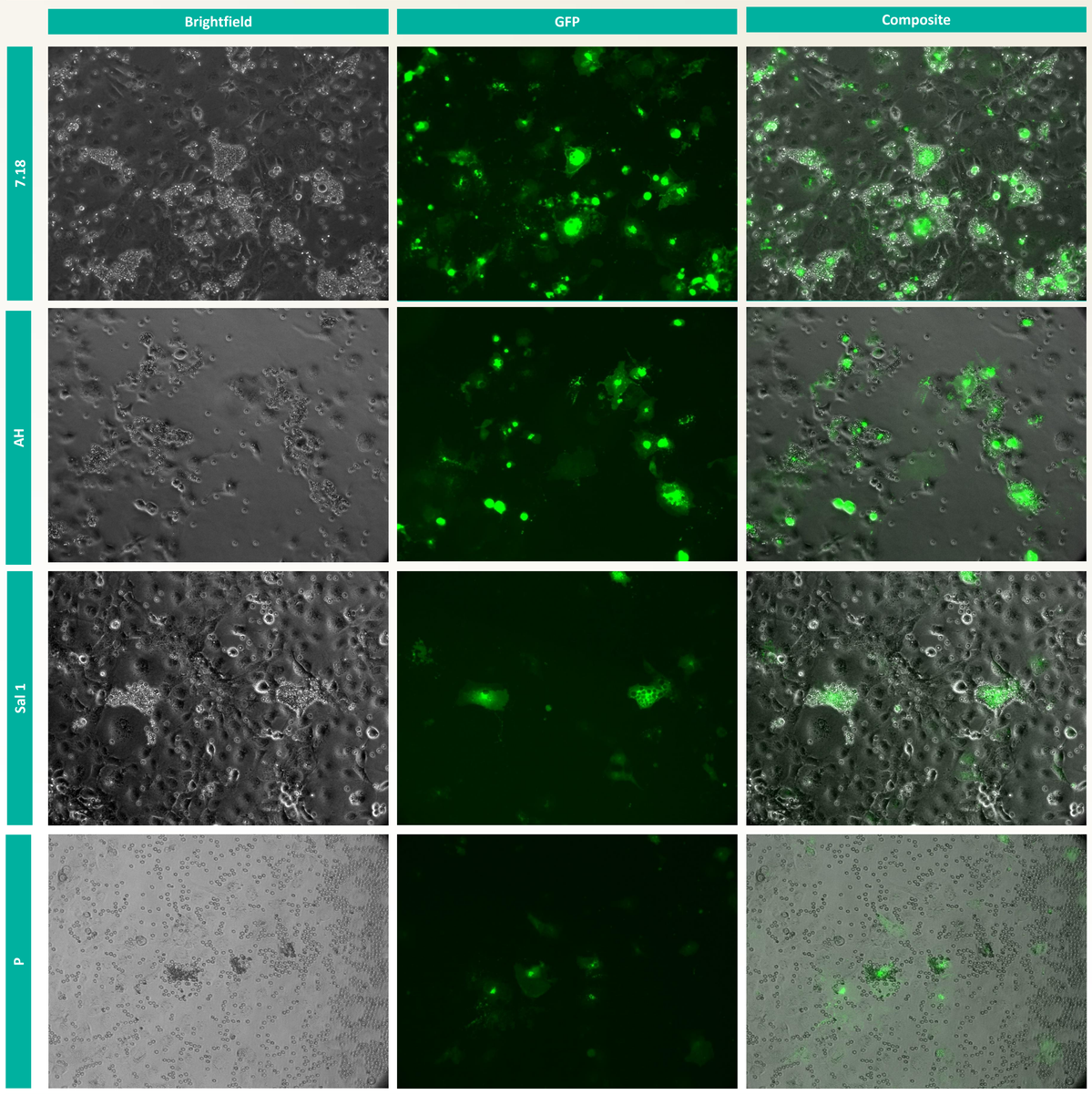

Figure 4. Erythrocyte binding was quantified by counting rosettes over 30 fields of view at 20x magnification. **Column 1** exhibits rosettes formed due to DARC-DBPII interaction. **Column 2** shows GFP fluorescence of DBPII-GFP plasmid DNA expression in transfected Cos7 cells after 42 hours. **Column 3** shows composite images of protein expression and rosette formation. Rows indicate different DBP II antigen alleles.

Upcoming Experiments

- Evaluate the percent inhibition of neutralizing antibodies of mice immunized with DBP II, CpG, and alum, using the optimal antigen DNA that is to be determined.
- Determine the differences in percent inhibition of functional antibodies in inbred, outbred, male, and female mice.

Future Directions

- We hope to test collected sera against different DBP II antigen strains, for functional inhibition their possible cross-reactivity.
- Test functional inhibition of serum in mice immunized with DEK-null2, a synthetic variant of DBP II which lacks the native immunodominant polymorphic residues.

References

1. World malaria report 2017. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.
2. King, C.L., Adams, J.H., Xianli, J., Grimberg, B.T., et al., (2011) Fy^a/Fy^b antigen polymorphism in human erythrocyte Duffy antigen affects susceptibility to *Plasmodium vivax* malaria. Proceedings of the National Academy of Sciences, 108 (50) 20113-20118; DOI:10.1073/pnas.1109621108
3. Lima-Junior, J & Lilian, P. (2016). Major Histocompatibility Complex and Malaria: Focus on Plasmodium vivax Infection. Frontiers in Immunology. 7. 10.3389/fimmu.2016.00013.
4. Batchelor, J.D., Malpede, B.M., Omattage, N.S., DeKoster, G.T., Henzler-Wildman, K.A., Tolia, N.H. Red blood cell invasion by Plasmodium vivax: structural basis for DBP engagement of DARC. *PLoS Pathog*. 2014;10(1):e1003869.