A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Identifying Antibody Responses Associated with Protection from Malaria in Pregnant Women

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Background

- In the human placenta, erythrocytes infected with *P. falciparum* (iRBCs) express on the surface VAR2CSA proteins, which mediates their adhesion to chondroitin sulfate A (CSA, a glycosaminoglycan expressed on the placental syncytiotrophoblast).

- VAR2CSA, a member of the *P. falciparum* erythrocyte membrane protein family (PfEMP1), is a transmembrane protein; its extracellular region contains cysteine-rich Duffy binding like (DBL) domains, and inter-domain (CIDR) regions rich in cysteine (CIDR)

- Over pregnancies, women develop protective immunity to placental infection in areas with high transmission of malaria. VAR2CSA protein has become the leading vaccine candidate.

- However, due to its complexity, VAR2CSA is problematic to manufacture thus hindering the development of a vaccine.

- Furthermore, for several pathogens, correlates of protection are largely based on titers of neutralizing antibodies; but this approach fails to measure protective immunity against *Plasmodium falciparum* infection

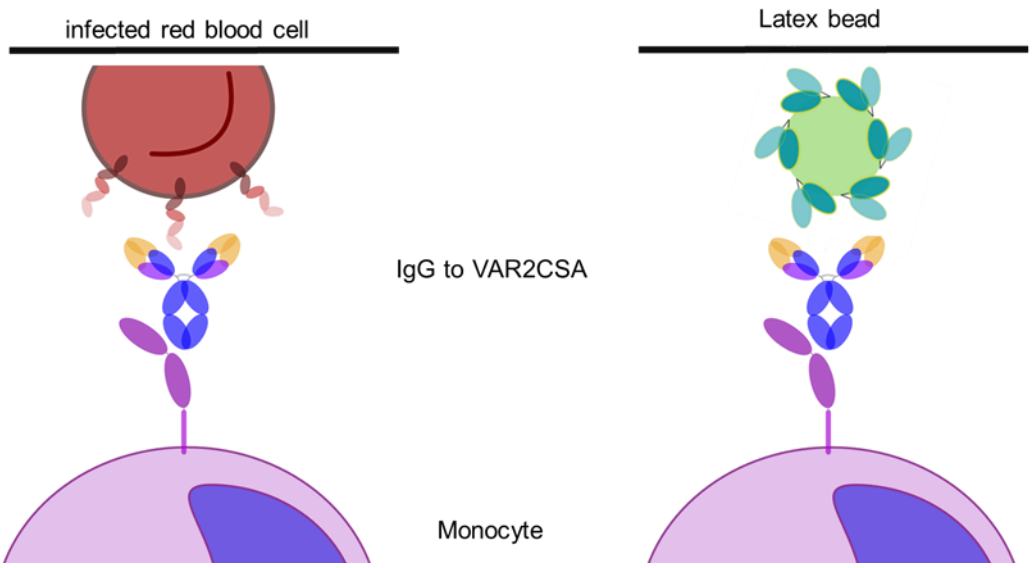
- Alternative antibody mechanisms are key to pathogen elimination, like ADCP (antibody-dependent cellular phagocytosis)

- Placental malaria is frequently accompanied by accumulation of monocytes, and studies using promonocytic THP-1 cells demonstrate the importance of functional antibodies that promote phagocytosis of infected erythrocytes in placental malaria.

Research Question

1. Does antibody mediated monocyte phagocytosis of VAR2CSA expressing iRBCs correlate with protection from placental malaria?

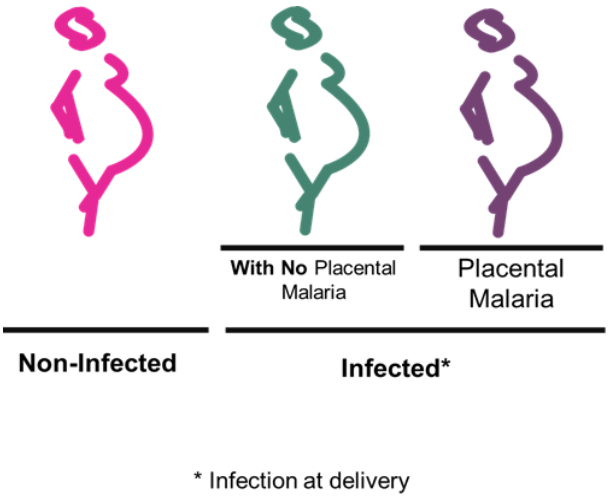
2. If so, can we identify the domain(s) of VAR2CSA that 'protective' antibodies bind?



The Fab region of the antibodies recognizes the parasite derived VAR2CSA, protein expressed on the membrane of infected RBCs. The Fc region determines the ability of the antibody to bind Fc receptors expressed on different immune cells (here monocytes), thus modulating the functional immune response.

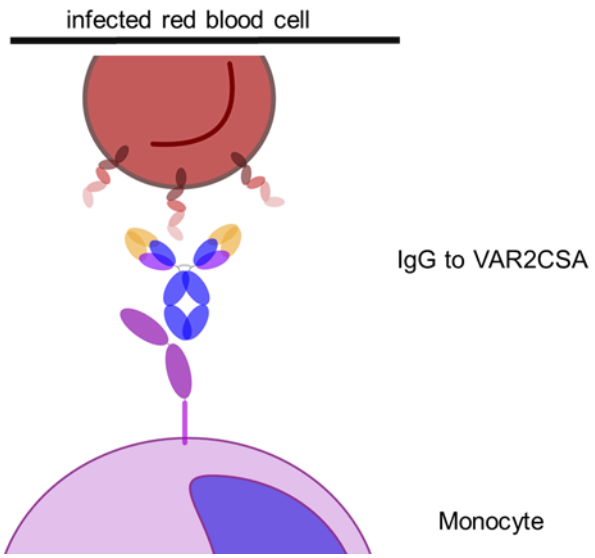
Methods

A) Placental infection in an endemic setting: The study Cohort



A cohort of pregnant women from PNG (127) categorized as follows: non-infected with *P. falciparum* (50), placental malaria (50) or infected with *P. falciparum* but with no placental malaria (27). Women were categorized based on placental pathology at delivery and samples used were taken at 14-26 gestational weeks

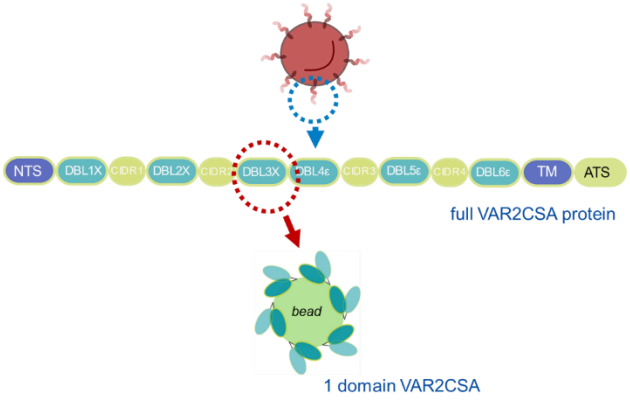
B) Antibody-dependent cellular phagocytosis (ADCP)



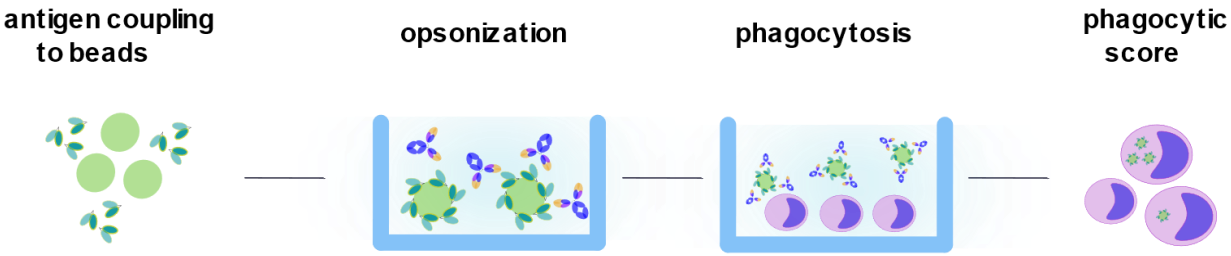
For the phagocytosis assay, we first opsonized RBCs (infected with the *P. falciparum* CS2 strain -that express VAR2CSA) with the sera samples from the PNG cohort. Positive control came from pooled positive plasma and negative controls from non-malaria exposed pooled plasma Then, *in vitro* phagocytosis assays were run using blood monocytes isolated from Australian donors (3). Then, we compared categories in pairs using the non-parametric Mann-Whitney U test at significance level p value < 0.05.

Methods

C) Which domains of VAR2CSA do antibodies bind?



We used recombinant DBL2, DBL3 and DBL5 proteins. These proteins were biotinylated and coupled separately to latex beads (FluoSpheres™ NeutrAvidin™, Thermo Fisher).



Antigen coupled beads were opsonized with antibodies. Human monocytes were isolated from buffy coats or whole blood, plated and incubated at 37◦C .Opsonized beads were incubated with the monocytes for phagocytosis. Monocyte phagocytosis was assessed by flow cytometry and presented as the phagocytic score ((% of events positive for beads) *(the geometric mean of only the % of events positive for beads)/100). For phagocytosis of *P. falciparum* infected red blood cells (iRBCs) (instead of beads) the steps followed for the protocol were identical.

Results (*)

1. IgGs induce monocyte phagocytosis of *P. falciparum* iRBCs expressing VAR2CSA

2. Monocyte phagocytosis correlates with protection from placental malaria

3. We did not find correlation between IgGs to VAR2CSA-DBL domains and protection from placental malaria

4. Fresh monocytes can replace THP-1 cells in latex beads assays

Conclusion

Here, we have explored *in vitro* the ability of malaria specific IgGs to stimulate human monocytes to phagocytose *Plasmodium falciparum* infected erythrocytes (iRBCs) and beads coated with VAR2CSA domains

We have also optimized a novel phagocytosis assay using primary monocytes that allows for the *in vitro* identification of candidate antigens for malaria vaccine development; using primary human monocytes and latex beads (coated with recombinant *Plasmodium* antigens).

In conclusion, hopefully the phagocytic assay we have optimized will broaden the understanding of acquired immunity to *P. falciparum* in malaria high transmission areas and will contribute for future malaria vaccine development

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