Identifying Antibody Responses Associated with Protection from Severe Malaria in Children

by Isobel S. Walker
University of Melbourne

Identifying Antibody Responses Associated with Protection from Severe Malaria in Children

presented by

Isobel Sylvia Walker

ORCID iD: 0000-0002-3071-1212

submitted in total fulfillment of the requirements for the degree of

Doctor of Philosophy

August 2023

Department of Medicine
Faculty of Medicine, Dentistry and Health Sciences
The Peter Doherty Institute for Infection and Immunity

The University of Melbourne

Primary Supervisor

Professor Stephen Rogerson

Dept. of Infectious Diseases, The Peter Doherty Institute, University of Melbourne, Australia

Co-supervisors

Doctor Elizabeth Aitken

Dept. of Infectious Diseases, The Peter Doherty Institute, University of Melbourne, Australia

Associate Professor Amy Chung

Dept. of Microbiology and Immunology, The Peter Doherty Institute, University of Melbourne, Australia

DECLARATION

This is to certify that:

1. The thesis comprises only my original work towards the PhD except where indicated in the statement of contribution.
2. Due acknowledgement has been made in the text to all other materials used.
3. The thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

....

Isobel Sylvia Walker
Department of Medicine, The University of Melbourne
The Peter Doherty Institute for Infection and Immunity

STATEMENT OF CONTRIBUTION

I duly acknowledge the work and assistance provided by others who contributed to the completion of this thesis:

Dr. Saber Dini and Dr Ali Haghiri: who assisted with the logistic regression and machine learning statistical analysis of Part IV and V.

Ms. Wina Hasang: who assisted with parasite culture, cell-based *in-vitro* phagocytosis assays and muliplex immunoassays.

Dr Amaya Ortega-Pajares: who assisted with performing the muliplex immunoassays.

Dr Dilini Rathnayke and Dr Agersew Mengist: who assisted with performing the flow cytometry experiments.

Dr Vanta Jameson, Sarvenaz Taghavi and Josh Kie: who assisted with the fluorescent cell sorting.

All other co-authors in the manuscript under submission (Part IV) and in the publications produced from this thesis.

Taken together, this thesis constitutes more than 90% of my own work.

ACKNOWLEDGEMENTS I

O germ! O fount! O word of love! O thought at random cast!
Ye were but little at the first, but mighty at the last.

Charles MacKay
Small Beginnings

I would like to thank:

Stephen Rogerson for his consistent support and guidance, and for supervising me and all his students with kindness and generosity.
Elizabeth Aitken for her valuable insights into everything from experimental design to big picture interpretation and for always having an open door to discuss any question.
Amy Chung for welcoming me to her lab and for the clarity and infectious enthusiasm for science that she brings to all of our meetings.
Wina Hasang for her endless patience for a thousand questions, for helping me complete experiments at all hours and on weekends, and for her friendship and positivity.
Amaya-Ortega Pajares and *Dilini Rathnayake*, for their friendship, laughter and unwavering support everyday.
All past and current members of Rogerson Lab Group and colleagues at the Doherty Institute, especially: *Meseret Workineh, Janavi Rambhatla, Siddhartha Mahanty, Juan Pablo Villanueva-Cabezas, Graham Brown, Agersew Mengist, Putri Warta, David Grimson, Abdul Mohammed A Alammar, Ricardo Ataíde, Louise Randall, Lianne Lansink* and *Zac Doan*. For being incredibly generous and inspiring people to be with in the lab and outside the lab over coffees and 'family dinners'.
The Chung lab members for all the technical and emotional support with the temperamental multiplex machine, especially *Timon Damelang, Ebene Haycroft, Milla Mclean, Kevin Selva and Pradhipa Ramanathan.*
My beautiful friends: *Isobel Leggett, Eleanor Donlon, Charlotte Georgiou, Lizzie DeAithpurua, William Troiani, Nathan Coote, Nadia Wissel, Anna Birjak*. I am so grateful for their love and support everyday.
Michael Troy, my partner for staying with me through all the wild rides, always making me smile and keeping me sane.
My family; mum, dad, Scott, Harriet, Ruben, my grandparents and angel Babi. For believing in me, for their love and encouragement, and for countless meals on wheels in tough times.

ACKNOWLEDGEMENTS II

Most importantly, I would like to thank the children and their families in Malawi and Papua New Guinea, and individuals in Australia, who donated their blood, time and energy for malaria research.

I am extremely grateful for their contributions that made this work possible. I hope that this work may contribute in some way to health interventions that serve them and others affected by malaria.

ACKNOWLEDGEMENTS III

Thanks to our collaborators:

Melbourne School of Population and Global Health:
Saber Dini, Ali Haghiri, and Julie Simpson.

Recombinant proteins and parasite lines:
Michael Duffy, Janavi Rambhatla, Eizo Takashima, Takafumi Tsuboi, Anja Jensen, Thomas Lavstsen, Louise Turner, Herbert Opi, James Beeson, Ricardo Ataíde, Jack Richards, Bruce Wines and Mark Hogarth.

Melbourne Cytometry Platform:
Vanta Jameson and Sarvenaz Taghavi and Josh Kie.

PNG cohort collection:
Moses Laman, Elvin Lufele, Laurens Manning and Timothy Davis.

Malawi cohort collection:
Karl Seydel, Terrie Taylor, Wilson Mandala and Visopo Harawa.

Institutions:
The Peter Doherty Institute for Infection and Immunity, University of Copenhagen, Ehime Institute, Malawi-Liverpool-Welcome Trust Clinical Research Programm, Malawi University of Science and Technology, Blantyre Malaria Project, Michigan State University, Papua New Guinea Institute of Medical Research, Bio21 Institute, Burnet Institute.

Funding:
My PhD was funded by the University of Melbourne Research Training Program Scholarship, Rowden White Scholarship and Nick-Christopher Top-Up Scholarship. This research project was also supported by the National Health and Medical Research Council of Australia (NHMRC).

ABBREVIATIONS

au	arbitrary units
ACT	artemisinin-based combination therapy
ADCP	antibody dependant cell phagocytosis
ADNP	antibody dependant neutrophil phagocytosis
ADCC	antibody dependant cell cytotoxicity
AMA1	Apical Membrane Protein 1
APC	Activated protein C
BSA	bovine serum albumin
CD	Cluster of Differentiation
CHMI	controlled human malaria infection trial
CM	cerebral malaria
C1s	complement component 1s
CHO cells	Chinese Hamster Ovary cells
CIDR	Cysteine Rich Interdomain Region
CSA	chondritin sulphate A
CSP	Circumsporozoite Protein
DBL	duffy binding-like domain
DC	Domain Cassette
DHE	dihydroethidium
EBA	Eryhtrocyte Binding Antigen
E.coli	Escherichia coli
EPCR	Endothelial protein C
ELISA	enzyme linked immunosorbent assay
FBS	foetal bovine serum
FSC	forward scatter
GLUPR	Glutamate Rich Protein
GPI	Glycosylphosphatidylinositol
HBECs	Human brain endothelial cells

HEPES	N-2- hydroxyethylpiperazine- N-2-ethane sulfonic acid
HIHS	heat inactivated human sera
ICAM-1	intercellular adhesion molecule
IE	infected erythrocyte
IFN	interferon
Ig	immunoglobulin
IL	Interleukin
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motif
KAHRP	Knob Associated Histidine Rich Protein
MHC	Major Histocompatibility Complex
MFI	median fluorescence intensity
mRNA	messenger ribonucleic acid
MSP	Merozoite Surface Protein
MTS	malaria thawing solution
NK	natural killer cell
PAMVAC	placental malaria vaccine candidate (PlacMalVac)
PAMPs	pathogen associated molecular patterns
PBS	phosphate-buffered saline
PBMCs	peripheral blood mononuclear cell
PECAM-1	Platelet endothelial cell adhesion molecule-1
PFA	paraformaldehyde
PfEMP1	*Plasmodium falciparum* erythrocyte membrane protein 1
PfPx-1	*Plasmodium falciparum* thioredoxin peroxidase I
PNG	Papua New Guinea
PRRs	pattern recognition reports receptors
RESA	Ring Infected Erythrocyte Surface Antigen
RH	Reticulocyte binding Homolog
RIFIN	Repetitive interspersed families of polypeptides
ROS	Reactive Oxygen Species

rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute - cell culture medium
SD	standard deviation
SSC	side scatter
STEVOR	Subtelomeric variant open reading frames
SURFIN	Surface-associated interspersed gene family proteins
TLRs	toll-*like* receptors
TNF	Tumor Necrosis Factor
VCAM-1	Vascular cell adhesion molecule-1
VSA	variant surface antigen
WGCFS	Wheat Germ Cell-Free System
WHO	World Health Organization

Contents

Part I

Abstract & Thesis Overview

1 Summary

1.1 Abstract

Malaria is a major global public health burden that affects individuals in 87 countries and causes 600,000 deaths annually. The majority of cases of severe malaria and malaria related deaths occur in children under the age of five, who lack protective immunity that is acquired by adulthood in endemic regions. Antibodies are a key component of protective immunity, however, the target antigens of antibodies and the functions of antibodies that confer protection are unclear. *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on the surface of parasite infected erythrocytes is a highly variant, multi-domain protein that mediates adhesion to a range of vascular endothelial cell receptors including Intercellular Adhesion Molecule-1 (ICAM-1) and Endothelial Protein C Receptor (EPCR). PfEMP1 is an important virulence factor and is a strong candidate target of protective antibodies against severe malaria. Therefore, the overall objective of this thesis was to understand the antibody response to PfEMP1 antigens that may confer protection from severe malaria in children.

This thesis aimed to: 1) characterize the antibody response to PfEMP1 antigens that are associated with protection from cerebral malaria (the most severe form of malaria) in a cohort of Malawian children; 2) characterize the antibody response to PfEMP1 antigens that are associated with protection from severe malaria in a cohort of Papua New Guinean children; 3) assess the association of disease severity with antibody dependent neutrophil and monocyte phagocytosis of infected erythrocytes expressing important PfEMP1 variants. For aims 1 and 2 we applied systems serology, which involves measuring multiple features of antibodies targeting multiple recombinant antigens and using machine learning with statistical methods to identify the best correlates of protection. For aim 3, we selected parasite-infected erythrocytes expressing two PfEMP1 variants (that bind to ICAM-1) and measured antibody dependent neutrophil phagocytosis (ADNP) and antibody dependent cell phagocytosis (ADCP) of opsonized infected erythrocytes by flow cytometry.

The important findings of this thesis were: 1) in both Malawian children and Papua New Guinean children, a combination of a small subset of PfEMP1 specific antibody responses could be used to differentiate between children with severe and uncomplicated malaria with high accuracy; 2) in both cohorts, DBLβ3 domains that bind to ICAM-1 receptor were targets of antibodies associated with protection from severe malaria. We also affirmed that 3) in addition to measuring the quantity of antigen specific antibodies, the biophysical properties and Fc interactions (or 'Fc features') of PfEMP1 specific antibodies are useful correlates of protection from severe malaria. A range of antibody Fc features were associated with protection, suggesting there are multiple pathways to protection from severe malaria. The antibody responses associated with protection included FcγRIIIb binding to antibodies targeting DBLβ3 domains that bind to ICAM-1, suggesting that antibodies to ICAM-1 binding PfEMP1 may induce killing mechanisms by neutrophils. In the final chapter, we found that 4) ADNP of ICAM-1+EPCR binding infected erythrocytes was associated with protection from cerebral malaria in Malawian children but was not associated with protection from severe malaria in Papua New Guinean children. Additionally, 5) ADCP of ICAM-1 binding infected erythrocytes was not associated with protection from severe malaria in either cohort of children. In considering the different methodological approaches used in this thesis, we concluded that systems serology is an effective approach to identify correlates of protective immunity to PfEMP1 but currently cannot replace more complex cell-based assays like ANDP and ADCP. Overall, this thesis highlights the important role of functional antibodies to PfEMP1 in protection from severe malaria in children from diverse geographic regions and supports the development of a DBLβ domain based therapeutic.

1.2 Thesis overview

Part II, *"Literature review"*, first describes the overall burden and clinical manifestations of malaria. Secondly, the parasite lifecycle and key pathogenic molecular mechanisms of malaria are summarized. Third, an overview of the immune response to malaria is given, including the antibody mediated response at the various stages of the parasite lifecycle. The next section focuses on PfEMP1, including structure, function and variants that are associated with severe disease. Lastly, the review details current knowledge of antibody responses to PfEMP1 and associations with protective immunity.

Part III, *"Materials & Methods"*, details the experimental procedures used to characterize antibody responses to recombinant PfEMP1 antigens and infected erythrocytes, including details of the clinical samples used throughout this thesis.

Part IV, *"Characterizing the PfEMP1 targets and Fc features of antibodies associated with cerebral and uncomplicated malaria in Malawian children"*, is currently under submission to Cell Reports Medicine and is therefore presented in manuscript format, with associated bibliography and supplementary files. The aim was to characterize the antibody response to PfEMP1 antigens in a case-control cohort of Malawian children with acute cerebral and uncomplicated malaria and identify responses associated with protection. We used a series of multiplex immunoassays to measure 11 features of antibodies targeting 39 proteins and used machine learning with logistic regression to identify key correlates of protective immunity.

Part V, *"Characterizing the PfEMP1 targets and Fc features of antibodies associated with severe and uncomplicated malaria in Papua New Guinean children"*, aimed to characterize the antibody response to recombinant PfEMP1 antigens in a case-control cohort of Papua New Guinean children with severe and uncomplicated malaria, during acute infection and in convalescence. We used a series of multiplex immunoassays to characterize nine features of antibodies targeting 29 proteins and used machine learning with logistic regression to identify key correlates of protective immunity.

Part VI, *"Antibody dependant monocyte and neutrophil phagocytosis of ICAM-1 binding IE and protection from severe malaria"*, aimed to assess the association of disease severity with antibodies that induce neutrophil or monocyte phagocytosis of infected erythrocytes expressing ICAM-1 binding variants of PfEMP1. We assessed the ability of antibodies from Malawian and Papua New Guinean children to induce ADCP and ADNP by flow cytometry.

Part VII, *"Final Discussion"*, brings together findings across all research chapters and discusses new insights into protective immunity to severe malaria and translational potential of our research findings. The strengths and limitations of the research chapters are summarized and future directions for the understanding of antibody mediated immunity against severe malaria are established.

Two published works were produced during this thesis – a book chapter describing the multiplex immunoassay used to measure antibodies to PfEMP1 antigens (Walker et al. 2022a) and a peer-reviewed literature review on the pathogenesis of malaria (Walker et al. 2022b).

Part II

Literature Review

2 Introduction to malaria and clinical manifestations

2.1 Overview of the burden of malaria

Worldwide, an estimated 229 million cases of malaria occur per year. Malaria is caused by the *Plasmodium* parasite of which there are five species that can infect humans – *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi*. The parasites are transmitted by female Anopheles mosquitos taking a blood feed (Fujioka et al. 1999). The majority of cases occur in Africa (200.5 million, 92%), followed by South-East Asia (11.3 million, 5%) and the Eastern Mediterranean (4.4 million 2%) and are predominantly caused by *P. falciparum* (99.7% of African cases). Malaria can be deadly, leading to an estimated 600,000 deaths per year – around 1000 people per day (World Health Organization 2021c).

The burden of malaria is disproportionally carried by children under five years, who account for approximately 40% of cases and at least two-thirds of malaria related deaths (61%) (Weiss et al. 2019; World Health Organization 2021c). In low transmission settings, older children are also vulnerable (Kapesa et al. 2018). The risk of infection and severe symptoms increase in pregnancy and malaria during pregnancy can lead to low birth weight - a significant risk factor for infant morbidity and mortality - as well as stillbirth and miscarriage (Guyatt et al. 2004; Rogerson et al. 2018). Malaria is treatable and artemisinin-based combination therapy (ACT) is the most effective and the most commonly used treatment for malaria in Africa. However, progression from mild to severe malaria can occur rapidly in children (Molyneux et al. 1989) and prompt seeking of and access to treatment is a limiting factor for survival rates. A median of half of children with febrile malaria see a medical provider and 30% receive antimalarial drugs (Mudenda et al. 2011; World Health Organization 2021c). Additionally, parasite resistance to artemisinin is present in South East Asia and there is some evidence that it has emerged in Africa (Ikeda et al. 2018; Uwimana et al. 2020). The first malaria vaccine, RTS,S/AS01E, induced 36% protective efficacy for uncomplicated malaria and 29% for severe malaria in children in Phase III clinical trials over four years (Alonso et al. 2004; RTS, S Clinical Trials Partnership 2015) and Phase IV trials of country wide implementation in Malawi, Kenya and Ghana commenced in 2018. Although the vaccine had modest efficacy, a combination of four doses of RTS,S with chemoprophylaxis and insecticide treated bed net access resulted in 63% protective efficacy for clinical malaria and 70% protective efficacy for severe malaria over three years (Chandramohan et al. 2021). In 2022, the vaccine was endorsed by the World Health Organization as a safe and efficacious intervention tool "in the context of comprehensive malaria control" (World Health Organization 2021b; World Health Organization

2022). Further development of RTS,S to improve protective efficacy is ongoing (Datoo et al. 2021), as is the development of alternative vaccines.

Malaria incidence and mortality worldwide have reduced dramatically since the turn of the century, due to vector control efforts including bed net usage and insecticide spraying, use of ACTs, as well as physical and socio-economic changes such as urbanization (including improved access to healthcare) (Tatem et al. 2013; Weiss et al. 2019; World Health Organization 2021c). However, the burden of malaria remains high and incidence reduction has plateaued since 2015 (World Health Organization 2020). Continuation with the current levels of malaria intervention is predicted to reduce but not eradicate malaria in the next 30 years (Feachem et al. 2019). A pull-back of investment and delivery of interventions prior to reaching elimination has historically resulted in a dramatic increase in malaria cases and deaths (Rogerson et al. 2020).

2.2 Clinical manifestations

2.2.1 General spectrum of disease

Clinical manifestations of *Plasmodium ssp.* infection can be asymptomatic, uncomplicated and mild, or severe and life threatening. Symptoms are initially non-specific and are difficult to use as the basis of clinical diagnosis. ‘Uncomplicated malaria’ generally presents as fever, nausea, vomiting, headache and diarrhoea. Progression from the onset of fever to severe disease typically occurs after 5 days in adults or 2-3 days in children (Hawkes et al. 2013; Molyneux et al. 1989). ‘Severe malaria’ is defined by the occurrence of one or more of the severe symptoms summarized in Table 1 (World Health Organization 2014b). In areas of high endemicity, individuals develop protective immunity to clinical malaria after repeat infections (Gupta et al. 1999) although they may still carry the parasite asymptomatically and contribute to transmission. Correspondingly, severe cases typically occur in children under 5 years and non-immune adults. In low transmission settings, adults and children are susceptible to severe disease although symptoms vary with age: renal failure is more common in adults and severe anaemia is more common in children (White 2018; World Health Organization 2014b). Alongside children under the age of 5 and non-immune adults, pregnant women are particularly susceptible to severe malaria, especially severe anaemia, as well as poor pregnancy outcomes (Umbers et al. 2011). The pathogenic mechanisms that lead to different clinical outcomes may differ considerably and hence the broad definition of ‘severe malaria’ is problematic for interpreting and comparing epidemiological data.

Table 1: Diagnosis of severe malaria is based on the presence of one of the following symptoms, signs or laboratory indicators*

Severe malaria symptoms
Impaired consciousness
Respiratory distress
Multiple convulsions
Prostration
Shock
Pulmonary oedema
Abnormal bleeding
Jaundice
Laboratory indicators
Severe anaemia (Haemoglobin < 5 g/dL in children, <7 g/dL in adults)
Hypoglycaemia (<2.2 mmol/L or <40 mg/dL)
Acidosis (plasma bicaronate <15 mmol/L)
Hyperlactataemia (lactate > 5 mmol/L)
Renal impairment (serum creatine > 265 μmol/L)
Hyperparasitaemia (> 250,000/μL or >5%)

Table 1: * Adapted from (World Health Organization 2014b)

2.2.2 Cerebral malaria

Cerebral malaria is an important form of severe malaria defined as unrousable coma, without another known cause, in *P. falciparum* infected individuals. With treatment, an estimated 15-25% of children with cerebral malaria die (Dondorp et al. 2010), accounting for the majority of annual deaths from malaria. A third of survivors of cerebral malaria experience long term neurological sequelae, including motor, visual and language deficits, seizures and behavioral difficulties (Seydel et al. 2015).

In post mortem histology, cerebral malaria can be sub-classified as CM1, identified by sequestration of parasites in the brain capillaries, or CM2, identified by parasite sequestration with additional pathologies, including hemorrhages in the white matter of the cerebral cortex, thrombi (blood clotting) or the presence of haemozoin in the perivascular spaces (Milner et al. 2014; Ponsford et al. 2012). Cerebral malaria (both CM1 and CM2) typically results in swelling of the brain white matter and subsequent pressure on the brain stem that causes fatal respiratory arrest (Seydel et al. 2015).

The mechanisms linking parasite sequestration to brain swelling and death are not entirely clear and are likely multifactorial. Possible factors include decreased cerebral blood flow due to parasite sequestration, and possibly post-ischemic damage, and blood brain barrier disruption leading to vasogenic edema (Taylor et al. 2004). Infected erythrocytes and several parasite factors have been proposed to compromise blood brain barrier integrity by inducing excessive localized inflammation and disruption of coagulation pathways (reviewed in (Jensen et al. 2020)). Clinical trials of anti-TNFαtherapy and inhaled nitric oxide therapy have failed and there are currently no adjunctive therapies for the treatment of cerebral malaria.

The severity of coma in young children can be estimated from the Blantyre coma score, an index from 0 – 5 based on physical and verbal responsiveness, where a score of <5 demonstrates impaired consciousness and a score of <2 demonstrates non-responsiveness and has been used as to classify cerebral malaria (Molyneux et al. 1989). This diagnostic method does not distinguish between cerebral malaria and other causes of coma with incidental parasitaemia, which can result in improper treatment (Taylor et al. 2004). Cerebral malaria pathology correlates strongly with a set of distinct changes to the retina, especially retinal whitening and vessel changes, and retinal examination is the most accurate way to diagnose cerebral malaria (95% sensitivity and 90% specificity) (Beare et al. 2006).

2.2.3 Severe malarial anaemia

In adults, haemoglobin <7 g/dL is considered as severe anaemia whereas haemoglobin <5 g/dL is considered severe anaemia in children (World Health Organization 2021c). Severe anaemia is associated with multiple health issues in Africa including malaria, sickle cell anaemia, bacteremia, infection with hookworm or human immunodeficiency virus, glucose-6-phostphate dehydrogenase deficiency disorder and vitamin A or vitamin B12 deficiency (Calis et al. 2016). Malaria interventions have been estimated to reduce the risk of moderate to severe anaemia (<8 g/dL) by 60% in children under five years (Korenromp et al. 2004), indicating that malaria is a significant contributor to the overall burden of anaemia in Africa. A large-scale multi-country survey of children under five revealed that 79% of malaria infected children were anaemic (haemoglobin <12 g/dL) and 8% had severe anaemia (World Health Organization 2021c). Pregnant women are also at a high risk of severe malarial anaemia.

The mechanisms linking anaemia and malaria in children are multifactorial (White 2018). Parasite infected erythrocytes are destroyed during schizont rupture, however, malaria also results in significant loss of uninfected erythrocytes (White 2018). Destruction of non-infected erythrocytes during malaria may arise from increased erythrocyte rigidity that increases erythrocyte removal by the spleen (Dondorp et al. 1999), and complement deposition on uninfected erythrocytes that may increase lysis or phagocytotic clearance (Dasari et al. 2014; Oyong et al. 2018). Additionally, malaria results in impaired bone marrow production of new erythrocytes, possibly due to direct interactions with parasite factors or systemic inflammation (Lamikanra et al. 2015; Thawani et al. 2014). Interestingly, in high transmission settings, haemaglobin rapidly increases upon antimalarial treatment, at a rate that cannot be accounted for by the production of new erythrocytes (White 2018). This may indicate the release of a hidden reservoir of non-infected erythrocytes, possibly in the spleen, that contributes to malarial anaemia.

3 Molecular manifestations and pathogenesis

3.1 Lifecycle

Plasmodium has a complex lifecycle shared between female anopheline mosquitos and human hosts (Figure 1). In humans, sporozoites are injected into the skin during the mosquito blood feed and then migrate to the liver within hours. Sporozoites are coated in circumsporozoite protein (CSP), which is important for liver invasion and is the target of the RTS,S vaccine (Chatterjee et al. 2021). *P. falciparum* sporozoites invade and replicate within liver hepatocytes over 1 – 2 weeks (although *P. vivax* can remain dormant in the liver for years) to produce up to 40,000 merozoites per hepatocyte. Merozoites are released into the bloodstream, initiating the onset of clinical symptoms. During the blood stage, asexual parasites replicate within erythrocytes over a repeated 24 – 72 hour lifecycle. Within seconds of release, merozoites invade erythrocytes via a process that involves several proteins, including Merozoite Surface Proteins (MSPs), Apical Membrane Proteins (AMAs) and Erythrocyte Binding Antigens (EBA) (Cowman et al. 2012) that are of interest in this thesis. Intraerythrocytic parasites initially resemble a ring under the microscope and remodel the infected erythrocyte for protein trafficking (Warncke et al. 2019). Rings develop into trophozoites that export Knob-associated histidine-rich protein (KHARP), to form knobs on the erythrocyte surface (Pologe et al. 1987) and anchor *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) (Kilejian 1979). Trophozoites progress into schizonts that rupture the erythrocyte and release 16-32 merozoites, recommencing the blood stage cycle, as well as metabolized haemoglobin in the form of haemozoin (Francis et al. 1997). A small number of merozoites develop into male and female gametocytes, to be taken up by the mosquito. Gametocytes undergo sexual reproduction in the mosquito gut and develop into sporozoites that are transmitted from the salivary glands to the next human host (Fujioka et al. 1999; Melancon-Kaplan et al. 1993; Sinden 2015).

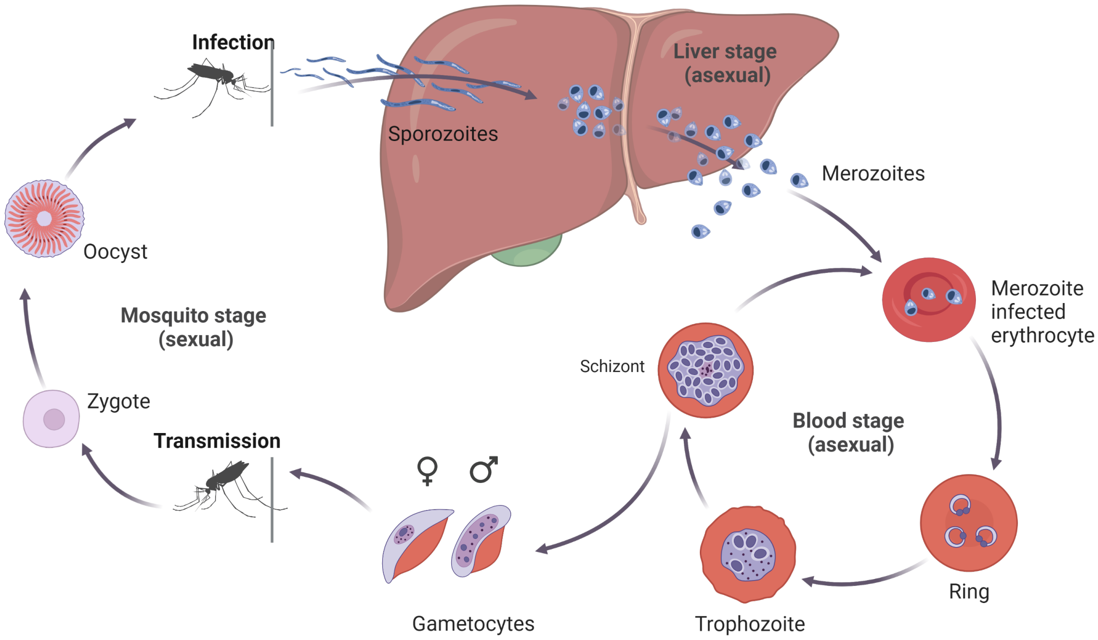


Figure 1: **Life cycle of *Plasmodium falciparum*.** Mosquitos transmit the sporozoite forms of the parasite to humans whilst feeding on human blood and the parasites migrate to the liver. Sporozoites replicate in liver hepatocytes then burst into the bloodstream where they infect erythrocytes. Over 24 hours, the parasites develop from the ring stage to trophozoites, then to schizonts that burst to release merozoites and infect more erythrocytes. A small portion of ring stage parasites commit to becoming gametocytes, the sexual form of the parasites, that are up taken by mosquitoes taking a blood feed. Gametocytes develop into sporozoites in the mosquito part of the life cycle and are transmitted to the next human host. (Image created with BioRender.com)

3.2 Molecular pathogenesis

We have published a review of the key pathogenic factors that contribute to *Plasmodium falciparum* malaria, including genetic factors, drug resistance, epidemiological factors, parasite factors and host immunity (Walker et al. 2022b). Here we summarize the key pathogenic parasite mechanisms that contribute to severe malaria: hyperparasitaemia, cytoadhesion and sequestration, rosetting and Variant Surface Antigen diversity. Naturally acquired immunity, a key determinant of clinical outcomes, will be discussed in detail in the following section.

3.2.1 High Parasitaemia

High parasite load ($>$10% parasitaemia) is strongly linked to poor clinical outcomes, although severe malaria can also occur at low parasitaemia (World Health Organization 2014b). Fatal cerebral malaria in children is associated with a higher mean parasitaemia non-fatal cerebral malaria (McGilvray et al. 2000) and high density parasitaemia is more common in children than adults in holoendemic areas (Owusu-Agyei et al. 2001). High parasite biomass, indicated by PfHRP2 levels, is also associated with severe malaria and cerebral malaria compared to non-severe malaria (Bernabeu et al. 2016; Kessler et al. 2017). High parasitaemia may contribute to other pathological mechanisms such as sequestration– Bernabeu *et al.* found a combination of high parasitaemia and expression of particular PfEMP1 types could distinguish between individuals hospitalized with severe malaria compared to those treated as outpatients (Bernabeu et al. 2016). It is also possible that high parasitaemia directly contributes to endothelial cell damage by release of parasite factors such as haemozoin or histones (Gillrie et al. 2012).

3.2.2 Cytoadhesion and sequestration

During the blood stage of infection, P. falciparum infected erythrocytes adhere to endothelial cells in the vascular periphery and can lead to sequestration in various organs. Cytoadhesion occurs during the trophozoite and schizont stage and has been attributed to the expression of parasite antigens on the surface of infected erythrocytes – predominantly PfEMP1. PfEMP1 is a highly diverse protein family and variants can adhere to a range of endothelial cell receptors, including Intercellular adhesion molecule-1 (ICAM-1), E selectin, Cluster of Differentiation 36 (CD36), P-Selectin, Vascular cell adhesion molecule-1 (VCAM-1), Endothelial protein C receptor (EPCR), chondroitin sulfate-A (CSA), Platelet endothelial cell adhesion molecule-1 (PECAM-1), Integrins and fibronectins (Mahamar et al. 2017). Parasite adhesion via PfEMP1 will be discussed in detail in Section 5. The knobby surface of infected erythrocytes is critical for sequestration (Stanisic et al. 2016; Webster et al. 2021), possibly by decreasing blood flow through the narrow capillaries construction zones to enhance cytoadhesion (Arakawa et al. 2020). Cytoadhesion and sequestration are generally considered to be mechanisms to evade detection by circulating immune cells and to evade splenic clearance (Bachmann et al. 2009). Cytoadhesion can result in organ sequestration of infected erythrocytes, including in the brain (cerebral malaria), placenta (placental malaria), heart, lungs, skin and gastrointestinal tract (Milner et al. 2014). *P. vivax* IE do not express PfEMP1 or adhere to endothelial cells, however, both asexual *P. vivax* and *P. falciparum* sequester in the spleen (Kho et al. 2021). The clinical consequences of splenic sequestration are unclear, although splenomegaly is common in malaria endemic regions (Leoni et al. 2015). Early stage *P. vivax* and *P. falciparum* gametocytes also sequester in the bone marrow parenchyma (Joice et al. 2014), possibly by cytoadhesion to bone marrow stromal cells (Messina et al. 2018a). The mechanisms and consequences of bone marrow sequestration are also unclear but are unlikely to involve PfEMP1, which is not highly expressed at this stage (Messina et al. 2018b).

3.2.3 Rosetting

Rosetting is a phenomenon observed *in vitro* where trophozoite or schizont stage infected erythrocytes adhere to uninfected erythrocytes. Rosetting occurs when variants of PfEMP1 bind to various receptors on the surface of the uninfected erythrocyte, including complement receptor 1, heparan sulfate, and blood groups A and B antigens. (Chen et al. 1998; Rowe et al. 1997; McQuaid et al. 2020), as well as IgM and α_2 macroglobulin (Stevenson et al. 2015a; Stevenson et al. 2015b). Other variant surface antigens STEVORs or RIFINs (Subtelomeric variable open reading frame proteins or Repetitive interspersed family proteins) may also contribute to rosetting (Fernandez et al. 1999; Niang et al. 2014).

Rosetting does not occur in all field isolates and was found to be more common in isolates from individuals with severe malaria in several studies, although it is unclear if it is associated with a particular clinical pathology such as cerebral malaria or severe malaria anaemia (Carlson et al. 1990; Doumbo et al. 2009; Rowe et al. 1995). Others have found no association with clinical presentation (Al-Yaman et al. 1995). Rosetting might contribute to severe malaria by obstructing capillary blood flow (Wang et al. 2015) or by acting as a protective barricade around the IE to inhibit antibody recognition and phagocytosis (Hedberg et al. 2021; Moll et al. 2015; Albrecht et al. 2020).

3.2.4 Variant surface antigen diversity

In the blood stage, variant surface antigens (VSAs) are displayed on the surface of the infected erythrocytes. PfEMP1 is the most well characterized VSA that is encoded by 60 var genes found in each parasite genome. The other known VSA families for *P. falciparum* are RIFINs, STEVORs and Surface-associated interspersed gene family proteins (SURFIN). RIFIN, SURFIN and STEVOR are encoded by 150 – 200 *rif* genes, 10 *surf* genes and 30 - 40 *stevor* genes, respectively (Chan et al. 2014). The diversity PfEMP1 potentially enhances parasite survival by evading antibody recognition in previously exposed human hosts. Indeed, a greater breadth of antibodies to PfEMP1 antigens is acquired with age, although a broad antibody response may not be sufficient for protection from severe disease (Kanoi et al. 2018). Diversity in PfEMP1 VSA also enables cytoadhesion to a diverse range of endothelial cell receptors, despite antibody recognition (Roberts et al. 1992).

4 Immune responses to *P. falciparum* malaria

Early records from the 1900s established that individuals in high transmission settings acquire species specific protective immunity to malaria by adulthood but can still be infected with *Plasmodium ssp.* asymptomatically (reviewed in (Doolan et al. 2009)). The acquisition of immunity is exposure dependent and is lost if exposure is interrupted, accounting for the higher burden of malaria in children under five years of age. Age dependent gains in immune function are also likely to contribute but are not clearly defined (Griffin et al. 2015; Kurtis et al. 2001). Malaria has a complex lifecycle, multiple pathogenic mechanisms and multiple clinical manifestations; therefore it is expected that there are multiple immune pathways that confer protection. Further, the serological responses to malaria are influenced by the local environment of infections, such as the placenta or brain (Jensen et al. 2019; Rogerson et al. 2003). The following is an overview of the immune response to *P. falciparum*, with a particular focus on serological responses.

4.1 Innate immunity

The innate immune system describes the primary, non-antigen specific response to infection, often referred to as the ‘front line’ defense. Innate immune cells, including dendritic cells (DCs), monocytes, macrophages, neutrophils and Natural Killer cells (NK cells) and are activated by pattern recognition reports receptors (PRRs) that recognize non-specific ligands common to many pathogens, known as pathogen associated molecular patterns (PAMPs) (Janeway et al.

2015). Toll like receptors (a PRR) found on monocytes recognize excess GPI on the surface of merozoites (Krishnegowda et al. 2005) and ‘scavenger receptors’ such as CD36 recognize parasite antigens on the surface of infected erythrocytes (McGilvray et al. 2000). Recognition induces phagocytotic clearance of the parasite and release of inflammatory cytokines and chemokines (Gowda et al. 2013). Following internalization, PRRs such as TLR8, TLR9, cGAS and AIM2 recognize parasite DNA that is released from parasites or haemozoin in endosomes. This in turn leads to activation of signaling pathways and caspase 1, and downstream production of cytokines, including Type 1 INFs, IL-1 and IL-8 by phagocytic cells such as monocytes and DCs (Gowda et al. 2018). The innate immune response to blood stage malaria is enhanced by the complement system, (Roestenberg et al. 2007; Wenisch et al. 1997; Garred et al. 2003; Holmberg et al. 2008; Korir et al. 2014), which is comprised of circulating serum proteins and membrane bound proteins that can initiate opsonic phagocytosis or parasite lysis (Garred et al. 2003; Rathnayake et al. 2021; Pawluczkowycz et al. 2007). The complement cascade can be triggered prior to an adaptive immune response by haematin released during cell lysis and platelet factors released from damaged vascular endothelial cells (Silver et al. 2010b).

4.2 Cell mediated adaptive immunity

Unlike the innate immune system, the adaptive immune system is an acquired, antigen specific response to infection that primarily involves lymphocytes, including CD8+ T cells, CD4+ T cells, and B cells. CD8+ T cells recognize antigens presented by Major Histocompatibility Complex (MHC) class I on the surface of most nucleated cells and lyse target cells by creating perforin pores in the target cell membrane (Janeway et al. 2015). CD8+ T cells targeting CSP may protect from liver stage infection or may be a useful activation target for pre-erythrocytic stage vaccines (Cockburn et al. 2013; Ishizuka et al. 2016; Sedegah et al. 1992), however there is little evidence for a role of CD8+ T cells in clearance of blood stage infection. In experimental cerebral malaria in mice models, CD8+ T cells infiltrates drive blood brain barrier breakdown (Swanson et al. 2016), however this is not thought to be the main driver of cerebral malaria in humans (Ghazanfari et al. 2018). CD4+ T cells recognize antigens presented by MHC class II molecules on antigen presenting cells such as B cells, macrophages and DCs. CD4+ T cells aid in CD8+ T cell activation and secrete cytokines that regulate the immune response (Janeway et al. 2015). In malaria, CD4+ T cells are polarized into T helper 1 (Th1) cells that produce pro-inflammatory cytokines including IFNγ and IL-2 and TNF, which activate innate immune cells such as macrophages and NK cells. The presence of CSP specific Th1 cells has been associated with reduced time to parasitaemia following malaria challenge in RTS,S vaccinated individuals (Reece et al. 2004) however there is little direct evidence for an association between naturally acquired malaria specific Th1 cells and protection from severe malaria (Kumar et al.

2020). A strong Th1 response may also be detrimental to immunity because malaria infection hinders the development of T follicular helper cells, which are critical for optimal B cell function and antibody responses (Nurieva et al. 2010; Obeng-Adjei et al. 2015; Pérez-Mazliah et al. 2019).

4.3 Antibody mediated adaptive immunity

4.3.1 Overview

Antibodies, or Immunoglobulins, are circulating molecules produced by B cells. B cells can display 10^5 unique cell surface receptors and upon recognition of a non-self antigen by one of these receptors, B cells secrete soluble antibodies that bind to the same specific antigen (Alberts et al. 2002; Hoehn et al. 2016). Early studies from the 1960s established that antibodies protect from parasitaemia and malarial fever (Cohen et al. 1961) and since then, many targets of antibodies have been identified at the various stages of the parasite lifecycle. Antibodies to the blood stage of infection are most likely to confer protection from severe disease, as this is the symptomatic stage of infection, whereas antibodies to the pre-erythrocytic and sexual stages are more likely to reduce transmission, although sterile protection is not thought to occur naturally. In malaria endemic areas, the breadth of antigens recognized by antibodies increases with age (Cham et al. 2009; Crompton et al. 2010; Kanoi et al. 2018; Nielsen et al. 2002) and transmission intensity (Cham et al. 2009), however the kinetics of antibody acquisition are antigen dependent, with antibodies to some antigens acquired early in life and antibodies to other antigens acquired in a more dynamic process over repeated exposure (Cham et al. 2009; Dent et al. 2015). Although overall low level of total IgG is a risk factor for cerebral malaria (Kessler et al. 2018), protective immunity to severe malaria is acquired in early childhood after a small number of infections (Gupta et al. 1999) and it is hypothesized that antibodies to a small group of target antigens may be sufficient to confer protection from severe disease. The following will provide an overview of the major antigens at the various stages of the parasite life cycle. The diverse mechanisms by which antibodies may confer protection from disease will also be discussed.

4.3.2 Antibody mediated immunity to mosquito/sexual stage antigens

Antibodies targeting gametocytes infected erythrocytes are naturally acquired (Saeed et al. 2008; Chan et al. 2019c) although the functions of antibodies are largely unknown. Antibodies to early stage IE may include inhibition of maturation (Tonwong et al. 2012) or possibly bone marrow sequestration (Messina et al. 2018b). Gametocyte IEs express PfGEXP5, Pfs16 and

Pfg27 (Tibúrcio et al. 2015; Baker et al. 1994; Sharma et al. 2003) but dramatically downregulate expression of PfEMP1 (Tibúrcio et al. 2013) and antibodies to gametocyte IEs are dramatically reduced in comparison to PfEMP1 driven response to IEs (Chan et al. 2019c). Antibodies targeting gametocyte surface antigens (inside the erythrocyte of early stage gametocytes) are naturally acquired and are taken up by mosquitos with a blood meal (Acquah et al. 2019). In the mosquito midgut, antibodies can inhibit parasite development, thereby reducing transmission to the next host (Drakeley et al. 1998; Kolk et al. 2006). The majority of research has focused on abs to Pfs48/45 and P230, that inhibit fertilization and can reduce oocysts density, and contribute to transmission reducing immunity (Stone et al. 2018). Pfd48/25 and Pf230 are strong candidates for monoclonal antibody therapy (Roeffen et al. 2001; Carter et al. 1990) and vaccine immunity. However, it is likely that there are many other targets involved in transmission reducing immunity (Jong et al. 2020). Antigens that develop post-fertilization (in the mosquito gut) do not come in contact with the human immune system but also have potential to be used for vaccine induced immunity to reduce transmission (Jong et al. 2021; Duffy et al. 1997). Pf25 is a post fertilization antigen that is the most developed transmission blocking vaccine target, however phase I clinical trials were halted due to reactogenicity (Wu et al. 2008).

4.3.3 Antibody mediated immunity to pre-erythrocytic stage antigens

The dominant antigen on the surface of sporozoites is circumsporoizte protein (CSP). Antibodies to CSP are naturally acquired, although at a much slower rate than antibodies to blood stage merozoite antigens (Feng et al. 2021). Sporozoites replicate 40,000 times in liver hepatocytes and theoretically, it would be necessary to arrest 100% of sporozoites prior to or during the liver stage to prevent blood stage infection. Interestingly, some studies (but not others) have found an association of naturally acquired antibodies to CSP and reduced risk of symptomatic infection (Bousema et al. 2019; Crompton et al. 2010; Daou et al. 2015; Offeddu et al. 2017). CSP is the antigenic target of RTS,S, the only WHO endorsed vaccine available for prevention of *P. falciparum* malaria in high endemic settings, that has been rolled out in Ghana, Kenya and Malawi (Adepoju 2019). In phase III clinical trials, RTS,S generated 36% protective efficacy for malaria for four years (RTS, S Clinical Trials Partnership 2015). There is current interest in further development of RTS,S to improve immunogenicity and protective efficacy, including altered formulation (Datoo et al. 2021), dosing regimes (Moon et al. 2021) and combination with seasonal chemoprophylaxis (Chandramohan et al. 2021). Naturally acquired antibodies to CSP can mediate opsonic phagocytosis of sporozoites (Feng et al. 2021), block hepatocyte invasion and reduce sporozoite motility (Bousema et al. 2019; Mishra et al. 2012) and RTS,S induced antibodies can mediate opsonic phagocytosis and complement mediated lysis (Kurtovic et al. 2021). Sera depleted of CSP specific IgG still inhibits hepatocyte invasion, suggesting there are non-CSP targets of functional antibodies on sporozoites that may be useful vaccine candidates and have not been thoroughly investigated (Fabra-Garcıa et al. 2022).

4.3.4 Antibody mediated immunity to blood stage merozoite antigens

There is a wide range of antigens on the surface of merozoites (Beeson et al. 2016b; Cowman et al. 2006) and novel antigens continue to be identified (Aniweh et al. 2020; Michelow et al. 2021). Unlike sporozoites, there does not appear to be a dominant target antigen on the surface of merozoites. Antibodies targeting merozoite antigens can block erythrocyte invasion (Chen et al. 2017; Lopaticki et al. 2011; Paul et al. 2018), induce complement mediated lysis, opsonic phagocytosis (Boyle et al. 2015; Feng et al. 2018; Garcia-Senosiain et al. 2021) and neutrophil activation (Murungi et al. 2016; Illingworth et al. 2019) and may confer protection from malaria by reducing parasite growth rates and thereby parasitaemia. Antibodies targeting merozoite antigens (including MSP1, MSP2, MSP3, MSP7, AMA1, RH5, RH2, GLUPRs, RIPR and EBA175)) have been associated with protection from malaria (Chan et al. 2017; Hamre et al. 2020; Kana et al. 2018; Kwenti et al. 2017; Richards et al. 2010; Sakamoto et al. 2018; Weaver et al. 2016) although protective associations have not been mirrored in all studies (Nash et al. 2017; Cummings et al. 2010; Dodoo et al. 1999) partly due to inconsistencies between study protocols (Fowkes et al. 2010). Some studies have reported an association between antibodies targeting merozoite antigens and protection from severe or cerebral malaria (Murungi et al. 2016; Ahmed Ismail et al. 2013; Mbengue et al. 2019a; A-Elgadir et al. 2008) although protective associations have not been found in other studies (Dobaño et al. 2008; Kessler et al. 2018). Single and combinations of merozoite antigens have been trialed as the basis of vaccines in human phase 1/II clinical trials, including for MSP1 (Blank et al. 2020) and AMA1 (Remarque et al. 2021), but so far have shown poor protective efficacy (Beeson et al. 2016a). The dynamics of exposure dependent antibody acquisition varies for different merozoite antigens (McCallum et al. 2017) and several studies have proposed that antibodies targeting merozoite antigens may be useful biomarkers to estimate prior exposure to malaria (Adamou et al. 2019; Nagaoka et al. 2019; Nash et al. 2017; Partey et al. 2018). Sero-conversion rates of antibodies against MSP1-19 and AMA1 have been utilized to identify ‘hot-spots’ of malaria transmission in Tanzania (Bousema et al. 2011), however other target antigens may be more appropriate in low transmission settings (Baum et al. 2013; Elliott et al. 2014).

4.3.5 Antibody mediated immunity to blood stage infected erythrocyte surface antigens

During an infection, children's antibodies towards parasites causing the infection are boosted (and are sustained for a transmission season), whereas there is a weak, transient antibody response towards parasites infecting other children in the community (Bull et al. 1998). This suggests that the immune response is at least in part directed towards variable antigens, such as PfEMP1 and the smaller RIFIN, SURFIN and STEVOR antigens displayed by the parasite on the surface of the infected erythrocytes during the trophozoite stage. PfEMP1 is by far the best characterized IE VSA and knock down of PfEMP1 export proteins dramatically reduced antibody recognition of IE by sera from malaria exposed individuals, suggesting it is the major target of antibodies on the surface of IE (Chan et al. 2019a; Chan et al. 2012). Antibodies to particular variants of PfEMP1 have been associated with protection from severe, cerebral or febrile uncomplicated malaria, as will be discussed in Section 5.3. RIFINs, STEVORs and SURFINs are less well characterized, however antibodies targeting specific variants have been associated with protection from uncomplicated malaria (Kanoi et al. 2020) and severe malaria (Travassos et al. 2018). The functions of antibodies to trophozoite and schizont stage IEs include inhibiting rosetting, blocking adhesion to endothelial cells or activating parasite clearance by innate immune cells, as will be discussed in detail in Section 5.4.

4.4 Function of antibodies in malaria

Antibodies may directly 'neutralize' Plasmodium, by inhibiting mosquito stage development, merozoite invasion of erythrocytes, sporozoites invasion of hepatocytes, IE adhesion to endothelial cells and to non-infected erythrocytes (rosetting). In addition to neutralization, antibodies engage multiple factors of the innate immune system to confer protection, including via interaction with Fc receptors on the surface of innate immune cells.

4.4.1 Antibody Fc receptor interactions

Fc-γ receptors (FcγR) bind to the Fc portion of IgG to activate or inhibit cell signaling pathways that activate defense mechanisms of the innate immune system, such as phagocytosis, antibody dependant cell cytotoxicity (ADCC), reactive oxygen species (ROS) burst and cytokine release. There are 5 types of FcγRs that are well characterized in humans: I, IIa, IIb, IIIa, IIIb that are varyingly displayed on the surface of monocytes, dendritic cells, macrophages, neutrophils, NK cells, B cells, platelets. Upon binding to the Fc portion of IgG, FcγRIIa activates a variety of kinase dependent cell signaling pathways via an intracellular immunoreceptor tyrosine-based activation motif (ITAM motif) on its cytoplasmic chain. FcγRI and IIIa (that do not have an ITAM motif) achieve this induction through interaction of their intracellular chain with an intracellular, membrane bound ITAM containing complex. FcγRIIIb is GPI anchored, with no intracellular chain, and possibly interacts with FcγRIIa to induce activation or act as a decoy for antibody binding to other, activating Fc receptors. In contrast, engagement of the Fc portion of IgG with FcγRIIb inhibits cell activation via an immunoreceptor tyrosine-based inhibition motif (ITIM) on the cytoplasmic chain (that recruits tyrosine and lipid phosphatases) (Hogarth et al. 2012).

FcγRI has the highest affinity for IgG and recognizes monomeric IgG, whereas FcγRII and FcγRIII are low affinity receptors that dimerize to bind to aggregated IgG on the target antigen (Janeway et al. 2015). As mentioned, FcγRIIa and FcγRIIb activate and inhibit cell killing mechanisms, respectively. FcγRIIa are critical for inducing phagocytosis of Plasmodium IEs by monocytes (Tebo et al. 2002) and neutrophil respiratory burst directed against merozoites (Kapelski et al. 2014) and FcγRIIb deficient mice were better able to control parasitemia (Clatworthy et al. 2007a). FcγRI are important for parasite clearance, as shown in mouse studies (McIntosh et al. 2007), and act synergistically with FcγRIIa to induce opsonic phagocytosis of parasite infected erythrocytes (Tebo et al. 2002). FcγRIIIa is highly expressed by Natural Killer cells, and some monocytes, and induces antibody dependent cell cytotoxicity (ADCC) (Jafarshad et al. 2007; McLean et al. 2017; Yeap et al. 2016), whereas FcγRIIIb is highly expressed by neutrophils and contribute to ADNP alongside FcγRIIa (Garcia-Senosiain et al. 2021) but not reactive oxygen species release (ROS burst) (Kapelski et al. 2014).

A single point polymorphism in FcγRIIa and FcγRIIIa influences the interaction of these receptors with different IgG subclasses and may modify host responses to malaria, although there is competing evidence regarding whether particular polymorphisms associate with protection or susceptibility to malaria (Cooke et al. 2003; Nasr et al. 2013; Omi et al. 2002; Zhao et al. 2014). In other studies, polymorphisms in FcγRIIIb have been associated with susceptibility to malaria in children (Adu et al. 2014; Fall et al. 2022) and a polymorphism in FcγRIIb that is more common amongst populations in malaria endemic areas was associated with protection from severe malaria in Kenyan children, possibly by increasing antibody dependent cell phagocytosis (Clatworthy et al. 2007b; Willcocks et al. 2010).

4.4.2 Antibody dependent phagocytosis

Antibody dependent cell phagocytosis (ADCP) is an important cell function induced by engagement of antibody Fc region bound to malaria antigen by Fc receptors (Tebo et al. 2002; Zhou et al. 2015) and can be performed by circulating monocytes and neutrophils, tissue macrophages and dendritic cells. In addition to induction by antibodies, phagocytosis of IE can also be induced by recognition of complement components by complement receptors (including CR1 and CR3), with or without the presence of antibodies (Silver et al. 2010a; Zhou et al. 2015), and non-opsonic phagocytosis can occur by activation of monocyte CD36 receptors (McGilvray et al. 2000). Interestingly, human brain endothelial cells are able to phagocytose IE via that interact with ICAM-1 receptors (Adams et al. 2021). In experimental cerebral malaria in mice (*P. berghei* ANKA), activated brain endothelial cells phagocytose merozoites and cross present antigen to CD8+ T cells (Howland et al. 2015).

ADCP by monocytes has been observed *in vitro* for merozoites, infected erythrocytes and sporozoites (Aitken et al. 2020; Healer et al. 1999). *Ex vivo* evidence of hemozoin in monocytes suggests phagocytosis of blood stage parasites occurs *in vivo* (Stephen J Rogerson et al., 2003). Monocytes are myeloid derived leukocytes that constitute approximately 10% of circulating leukocytes. Monocytes uptake and destroy pathogens intracellularly, secrete inflammatory cytokines that stimulate immune response and present antigen to prime T cells and B cells (Jakubzick et al. 2017; Janeway et al. 2015). Monocytes have high expression of FγRIIa, low expression of FcγRI that increases upon activation, low expression of FcγRIIb, and a subset that is upregulated in severe malarial anaemia (SMA) express FcγRIIIa (CD16) (Hogarth et al. 2012; Janeway et al. 2015; Ogonda et al. 2010). Opsonic phagocytosis activity of IEs is most prominent in intermediate monocytes (CD14++CD16+), compared to classical monocytes (CD14++CD16-) (Dobbs et al. 2017; Zhou et al. 2015). Correspondingly, one study found that FcγRIIIa (CD16), and not FcγIIa nor FcγI, was essential for opsonic phagocytosis by monocytes (Zhou et al. 2015) however another study found that phagocytosis can also be induced by synergistic activation of FcγI and FcγRIIa on THP-1 (a monocytic cell line) (Tebo et al. 2002). Merozoites have also been shown to be phagocytosed by primary monocytes when opsonized with IgM from malaria exposed Malian adults, via Fcμ receptors (Hopp et al. 2021).

The THP-1 cell line is often used to characterize phagocytosis of merozoites and IEs *in vitro* (Osier et al. 2014). *In vivo*, phagocytosis activity is influenced by multiple factors, including: changes in monocyte Fc receptor expression; the activation state of monocytes due to host inflammation (Muniz-Junqueira et al. 2001); exposure to antimalarials (Shalmiev et al. 1996); exposure to parasite factors such as hemozoin (Schwarzer et al. 1992) and repeat exposure to infection (Ortega-Pajares et al. 2018). A recent study found acute *P. falciparum* infection reduced the capacity of monocytes to perform opsonic phagocytosis, although there was no differences in phagocytosis capacity with disease severity (Dobbs et al. 2017).

Opsonic phagocytosis by monocytes has been associated with protection from malaria, particularly for merozoite stage of infection (Ataíde et al. 2011; Chua et al. 2021; Hill et al. 2013;

Osier et al. 2014). In one study, ADCP of merozoites was associated with a reduced risk of symptomatic malaria, whereas total IgG and IgG1 and IgG3 alone did not (Osier et al. 2014). Similarly in malaria in pregnancy, ADCP of pregnancy specific IEs has been associated with protection from placental malaria (Aitken et al. 2021) and correlated positively with birthweight (Ataíde et al. 2011) whereas IgG was not, in both of these studies. In another study, both IgG and ADCP of pregnancy specific IEs were associated with reduced maternal anaemia, but the association was strongest for ADCP (**feng2009**). This suggests that ADCP may be a more effective correlate of clinical outcome than total levels of antibodies.

In addition to monocytes, circulating neutrophils phagocytose opsonized merozoites (Kumaratilake et al. 2000), IEs (Marsh et al. 1989), gametocytes (Steel et al. 2017) and sporozoites (Feng et al. 2021), however the role of antibody dependent neutrophil phagocytosis (ADNP) in protection from severe malaria is unknown (Aitken et al. 2018). Neutrophils, or polymorphonuclear cells, are myeloid derived and are the most abundant (70%) circulating leukocyte (Janeway et al. 2015). Neutrophils perform a range of functions including phagocytosis, ROS release and release of extracellular contents to form extracellular traps (NETs) (Liew et al. 2019), although few studies have investigated the association of neutrophil functions with protective immunity (Aitken et al. 2018). Neutrophils recognize antibody bound to pathogens in general via moderately expressed FcγRIIa, highly expressed FcγRIIIb, low expression of FcγIIb and inducible expression of FcγRI (Hogarth et al. 2012). They may also perform non-antibody dependent phagocytosis, possibly via complement engagement (Kumaratilake et al. 2000). The contribution of ADNP, and other antibody dependent functions of neutrophils to protection from severe malaria requires further investigation.

4.5 Antibody Fc variation

Antibodies are composed of the Fab region (heavy chain) that binds to a target antigen, identical to epitope specific B-cell receptors, and the Fc region (light chain) that is recognized by innate immune cells via Fc receptors. An individual will have at least 10^7 variants of the antigen specific Fab region. The Fc region is more conserved, but can be one of 5 isotypes (IgA, IgD, IgG, IgE and IgM) of which there are multiple subclasses (IgG1-4, IgA1-2) that vary the affinity of antibodies to different antigens, as well as downstream antibody effector functions (Janeway et al. 2015; Torres et al. 2008). For a given Fab region, the Fc region can be altered to different isotypes or subclasses in a process known as 'class switching recombination' in B cells, and combined variation in the Fab and Fc region gives rise to 10^{16} different antibodies (Schroeder Jr et al. 2012). To add an additional layer of diversification for the Fc region, polymorphisms within an antibody subclass give rise to various allotypes, and various carbohydrates can bind to an N-terminal glycan site. Variation in the glycosylation state, and possibly allotype, alters the stability of the antibody, Fc receptor interactions and complement deposition (Bournazos et al. 2017; Lee et al. 2017; Vidarsson et al. 2014).

4.5.1 Antibody isotypes

Of the 5 antibody isotypes, IgG is most prevalent in serum and is almost exclusively investigated as a correlate of protection for malaria (Janeway et al. 2015; Travassos et al. 2018). IgM is produced by naïve by B cells and dominates early in infections then wanes as an IgG response increases (Schroeder et al. 2010). This dynamic appears to be true for malaria in some studies (Kinyanjui et al. 2003; Walker et al. 2020) although others have reported sustained IgM targeting merozoites following infection (Boyle et al. 2019a). IgM is the only subclass able to form pentamers, which can significantly increase antigen avidity (Schroeder Jr et al. 2012). Non-specific IgM binding may contribute to severe malaria pathogenesis by mediating rosetting of particular infected erythrocyte variants (Quintana et al. 2019; Rowe et al. 2002; Stevenson et al. 2015a) and potentially preventing IgG binding and subsequent phagocytosis and C1q deposition (complement) (Barfod et al. 2011). In contrast, IgM has been shown to inhibit merozoite invasion of erythrocytes, activate complement on the merozoite surface (Boyle et al. 2019b) and induce phagocytosis by monocytes *in vitro* (Hopp et al. 2021). Another study found no difference in IgM between mild and cerebral malaria, but significantly higher IgM titers to merozoite antigens in cerebral malaria survivors compared to patients with fatal outcome, suggesting IgM plays a protective role (Mbengue et al. 2019b).

Little is known about the role of the other isotypes in the immune response to malaria. IgA can form dimers in serum and is particularly important for protection in mucosal membranes (Schroeder Jr et al. 2012) however little is known about the role of IgA in the context of malaria infection. In recent studies, IgA2 to a PfEMP1 domain was associated with protection from placental malaria (Aitken et al. 2021). IgA was found to be induced in response to RTS,S vaccination (Suau et al. 2021) and IgA2 correlated with protection against malaria infection following vaccination in a human challenge model (Suscovich et al. 2020). IgE activates Fc receptors on mast cells, basophils and eosinophils and is typically associated with an allergic inflammatory response. An antigen specific IgE response has not been well characterized in malaria and it is unclear if IgE contributes to malaria protection or pathogenesis. Elevated non-specific IgE in patients with co-infection of malaria and other parasites may contribute to protection from cerebral malaria (Nacher et al. 2000). IgE in the serum of individuals from a malaria endemic area induced TNF production by PBMCs, possibly via engagement of FcγRII receptors (CD32) (Perlmann et al. 1997). Another study found upregulated FcγRII receptors in severe malaria patients and suggested IgE may contribute to harmful inflammation, although this may simply be a biomarker of inflammation (Kumsiri et al. 2016). IgD is co-expressed on mature B cells with IgM, although little is known about the function of secreted IgD and there is currently little evidence of a role of IgD in protection or pathogenesis of malaria.

4.5.2 IgG subclasses

There are 4 IgG subclasses, IgG1-4, ranked by their prevalence in healthy human serum (Schroeder Jr et al. 2012). Although the sequence of subclasses is 90% similar, subclass Fc variation affects IgG half life, antigen specificity, Fc receptor affinity and complement interactions. IgG1 is usually the dominant subclass response to pathogens, however other subclasses are usually produced at lower levels (Papadea et al. 1989). The subclass specific response is influenced by different types of T helper cell responses induced by the infection (Stevens et al. 1988). The subclass response varies for different antigens and may be dependent on the flexibility of the subclasses to interact with antigens in different tertiary structure conformations (Irani et al. 2015). IgG has a significantly longer half life than the other antibody isotypes (9-23 days, compared to 2.5-6 days) (Papadea et al. 1989). This is because FcRn binds to antibodies after endocytosis and recycles IgG back into circulation (Lencer et al. 2005). IgG3 has the shortest half life of the IgG subclasses, of around 1 week, because it lacks a key residue required for FcRn binding (Stapleton et al. 2011). However the half life is extended for a specific allotype, G3m15, with a histidine residue mutation H435 (Stapleton et al. 2011). The different subclasses also vary in their affinity for Fc receptors. IgG3 generally has the highest affinity for Fc receptors, followed by IgG1. IgG1, IgG3, and IgG4 have high affinity for FcγRI, whereas IgG2 generally has low affinity for Fc receptors (Bruhns et al. 2009; Hogarth et al. 2012). IgG3 has the highest affinity for complement, followed by IgG1 and IgG2, whereas IgG4 does not fix complement (Schroeder Jr et al. 2012). A single point polymorphisms in Fc receptors can also influence the binding affinity to particular subclasses. For example, IgG2 can bind to the FcγRIIa-H131 allelic variant that induces monocyte phagocytosis and has been reported to correlate with protection from parasitaemia (Saavedra-Langer et al. 2018; Tebo et al. 2002).

4.5.3 IgG subclasses in response to malaria and associations with protection

Some studies have considered the distribution of antibody subclasses in malaria infection, particularly in response to merozoite antigens. Antibodies to merozoite proteins (including MSP1, MSP2, AMA1, EBA175, EBA140, EBA181, Rh5) are predominantly IgG1 and IgG3. IgG2 and 4 have consistently low seroprevalence and are present in relatively low levels, therefore are often excluded from analysis (Nhabomba et al. 2014; Richards et al. 2010; Stanisic et al.

2009; Tongren et al. 2006; Weaver et al. 2016).

Antibodies to multiple merozoite antigens have been associated with protection from infection or severe malaria in some studies (Beeson et al. 2016b; Ismail et al. 2014; Kana et al. 2018), however the dominant subclass varies for different merozoite antigens and is not always the best correlate of protection (Malkin et al. 2005; Stanisic et al. 2009). The subclass response is also likely influenced by repeat exposure (Tongren et al. 2006) and age (Omosun et al. 2005). Further, despite low levels, IgG2 to particular merozoite antigens (EBA-175, RESA, MSP2) has occasionally been reported to correlate with low parasitaemia or low risk of infection (Aucan et al. 2000; Ismail et al. 2014) and IgG4 targeting GLURP was associated against with reduced incidence of clinical malaria (Nebie et al. 2008). However, IgG4 targeting other merozoite antigens has also been associated with enhanced risk of infection (Aucan et al. 2000) and another study reported IgG1 and IgG4 levels to merozoite extract were higher in cerebral malaria compared to mild malaria (Mbengue et al. 2019b), suggesting IgG4 to merozoite antigens may more-so commonly be a marker of infection or disease severity.

Similarly, IgG1 and IgG3 dominate the response to CSP following RTS,S vaccination in CHMI and natural exposure (Chelimo et al. 2005; Seaton et al. 2021). However, IgG1, IgG3 and IgG4 responses to blood stage and pre-erythrocytic antigens have been associated with protection from malaria infection following RTS,S vaccination, particularly in children and in low transmission settings (Dobaño et al. 2019). IgG2 was never not associated with protection. The same study reported IgG subclass specific responses to antigens that were associated with increased risk of malaria following vaccination, suggesting a complex and antigen specific response induced to vaccination (Dobaño et al. 2019).

Responses to VSAs are less well characterized. Small studies have found that IgG1 or IgG3 dominate the response to VSAs or PfEMP1 (Kinyanjui et al. 2003; Megnekou et al. 2005; Piper et al. 1999; Elliott et al. 2005), although this may be influenced by history of exposure - IgG2 and IgG1 to schizont extract dominated in first infection in Swedish adults whereas IgG3 dominated in malaria exposed Liberian adults (Wahlgren et al. 1983). Many of these studies used a single parasite line or involved small numbers of participants. Subclass responses to VSAs are also likely to be age dependent. IgG2 is higher in adults and older children than in younger children (Aucan et al. 2000; Cabrera et al. 2004; Facer 1980) and IgG2 and IgG3 are the dominant subclasses targeting VSAs in Gabonese adults, whereas IgG3 and IgG4 dominated in healthy children (Cabrera et al. 2004).

Few studies have considered association of subclasses targeting VSAs and protective immunity. IgG1 to parasite isolates or crude lysates has been associated with protection from infection (Cabrera et al. 2004; Ndungu et al. 2002) and clinical malaria compared to asymptomatic malaria (Vigan-Womas et al. 2010). Similarly, IgG3 has been associated with survival of severe malaria (Sarthou et al. 1997), and protection from clinical malaria (Vigan-Womas et al. 2010). A recent study found that IgG2, IgG3 and IgG4 responses to domains of the pregnancy specific VSA, VAR2CSA, were associated with protection from placental malaria (Tornyigah et al. 2021). In earlier studies, VSA specific IgG4 was the only isotype to increase in response to severe malaria (Cabrera et al. 2004) and IgG4 to severe malaria isolates was associated with protection from reinfection (Yone et al. 2005). In contrast, IgG2 to asexual lysates has been associated with increased risk of subsequent infection (Ndungu et al. 2002). IgG2 to severe malaria parasite isolates has been associated with severe malaria compared to uncomplicated malaria, and IgG2 to RIFIN antigen has been associated with severe malaria compared to asymptomatic malaria (Schreiber et al. 2006; Yone et al. 2005). It is likely that the subclass bias is variant dependent but this has only been investigated in one study (Cabrera et al. 2005). Only one study has investigated the domain dependent response (Aitken et al. 2021). Further studies are needed to consider the association of PfEMP1 variant specific antibody subclass responses in protection from severe malaria.

4.6 Systems serology

A traditional approach to measuring immunity to vaccine candidates is to measure the titers of antigen specific antibodies. However, studies of several infectious diseases have found that measuring the biophysical properties and Fc interactions of antibodies with components of the innate immune system can reveal better correlates of protective immunity than titers alone. Systems serology describes a process of measuring multiple Fc features of antibodies induced by infection or vaccination and using machine learning analysis to select the best correlates of protective immunity (Arnold et al. 2018). A systems serology approach has been applied to characterize natural and vaccine induced immunity to several infectious diseases, including HIV, Ebola, tuberculosis and SARS-CoV-2 (Gunn et al. 2018; Chung et al. 2017; Selva et al. 2021; Fischinger et al. 2021). In the context of malaria, systems serology has been applied to characterize functional antibody responses following vaccination with merozoite antigen Rh5 (Minassian et al. 2021), where authors reported that vaccination induced IgA1 responses and antibody dependent neutrophil responses that were associated with reduced time to malaria diagnosis following controlled human infection (CHMI) challenge (Minassian et al. 2021). Systems serology was also applied to characterize RTS,S, vaccine induced immunity to CHMI, revealing that antibody dependent phagocytosis and FcγRIIIa engagement were the best correlates of protection (Suscovich et al. 2020). Systems serology was applied by our research group to characterize antibody responses to malaria in pregnancy. Compared to pregnant women with placental infection, pregnant women without placental malaria had antibodies to the pregnancy specific variant surface antigen, VAR2CSA, that induced opsonic phagocytosis by monocytes and neutrophils, as well as IgG3 and IgA2 antibodies (Aitken et al. 2021). Taken together, systems serology provides valuable insights into the best correlates of protective immunity as well as the functions of antigen specific antibodies in the context of multiple infectious diseases, including malaria. So far, no studies have applied a systems serology approach to characterize the naturally acquired immune response to *P. falciparum* antigens in children with severe malaria.

5 *Plasmodium falciparum* erythrocyte membrane protein 1

So far, we have touched on the importance of VSAs in the pathogenesis of *P. falciparum* malaria, primarily as the mediator of sequestration of IEs to vascular endothelial cells. PfEMP1 is the most well characterized VSA on IE and importantly, PfEMP1 is a major target of antibodies. Here we discuss in more detail: the structure and function of PfEMP1; the association of PfEMP1 and particular variants of PfEMP1 with severe disease; and the role of antibodies targeting PfEMP1 in protection from severe malaria. Much of the information in this section was published in our recent review (Walker et al. 2022b).

5.1 Structure, classification, and export of PfEMP1

PfEMP1s are large, 200 – 350 kDa proteins expressed by *P. falciparum* in the trophozoite and schizont stage of the asexual life cycle. PfEMP1s are displayed on the surface of IEs, rooted to clusters of KAHRP that deform the erythrocyte to create a distinct "knobby" surface (Chan et al. 2014). The parasite genome has around 60 *var* genes encoding for PfEMP1. A parasite will generally express a single PfEMP1, however dual PfEMP1 expressing parasites have been reported (Joergensen et al. 2010). *Var* genes can be classified by their upstream promotor sequence type, as group A, B, C, E (specific to *var2csa*), or two intermediate groups B/A and B/C (Lavstsen et al. 2003; Rask et al. 2010). *Var* genes are composed of segments of Duffy binding-like domains (DBLs) and cysteine-rich interdomain regions (CIDRs) (Figure 2) that can be further subclassified into DBL α0.1-2, β1-10, γ1-18, δ1-9, η1-14, 1-6 and CIDR α1-6, β1-7, γ1-12 (Rask et al. 2010; Smith et al. 2000b). *Var* genes are organized into an N-terminal sequence and semi-conserved DBL-CIDR head structure, followed by 2 – 8 DBL and CIDR domains of various subclasses, in various combinations, a transmembrane domain at the erythrocyte surface and an intracellular acidic terminal sequence (ATS region). Combinations of domains often found in tandem have been identified, known as domain cassettes (DC) of which there are 21 described to date (Rask et al. 2010; Smith et al. 2000b; Tonkin-Hill et al.

2018). The sequence similarity of domain subclasses between parasite genomes of laboratory adapted parasite lines ranges from 38% to 98% (Rask et al. 2010). Alternatively, PfEMP1 can be described in terms of homology blocks – 628 short (minimum seven amino acids), conserved sequences that can be lined up to account for approximately 83% of the total PfEMP1 sequence (Rask et al. 2010; Smith et al. 2000b) although this approach is far less common. In addition to the large number of *var* genes encoding PfEMP1, ectopic recombination of *var* genes can occur within a single cycle (Duffy et al. 2009), giving rise to multiple possible *var* gene combinations. Variation in PfEMP1 may be advantageous to the parasite in overwhelming the host immune system with possible epitopes.

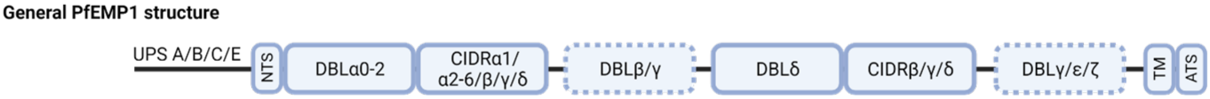


Figure 2: **General structure of *var* genes encoding PfEMP1** PfEMP1 are encoded by an up stream promotor sequence (Groups A/B/C/E), followed by the N-terminal sequence. PfEMP1 are composed of Duffy Binding Like domains (DBL) and Cysteine-rich interdomain Regions (CIDR). The head structure contains a DBLαand CIDR domain and is followed by at least two domains (solid borders) and can be followed by additional domains (dashed borders). The transmembrane region (TM) is followed by the A-terminal sequence (ATS). Figure adapted from (Smith et al. 2013), created with BioRender.com.

Var gene expression does not seem to be random although its regulation is not completely clear, and there is often a dominant transcript within a parasite population (Bachmann et al. 2019; Cham et al. 2009). Several factors may contribute to the selection of *var* genes expressed by parasites. *Var* genes of particular adhesive phenotypes may be selected for in environments displaying particular adhesive receptors. *In vitro*, PfEMP1 variants with a specific binding phenotype can be selected for by exposing parasites to the corresponding adhesion receptor (Smith et al. 2000a). *In vivo*, parasites infecting pregnant women predominantly express PfEMP1 that bind to placental receptors, chondroitin sulfate A (Duffy et al. 2006; Salanti et al. 2003; Tuikue Ndam et al. 2005). Specific adhesive phenotypes may be better able to avoid splenic clearance and therefore persist in circulation.

Host antibody recognition of particular PfEMP1 may also limit the PfEMP1 repertoire of parasites causing an infection, due to antibody mediated clearance by the immune system (Nielsen et al. 2002; Warimwe et al. 2009). Individuals have lower antibodies recognizing the infecting parasite variant than other parasites circulating in the community (Bull et al. 1998) and have lower antibodies to the infecting variant than other individuals in the community (Schieck et al. 2017), in line with the theory that the parasite "exploits holes in the antibody repertoire."

In addition to environmental selection factors such as adhesion phenotype and antibody recognition, different *var* genes appear to switch (turn on or off) at different rates (Bachmann et al. 2011; Paget-McNicol et al. 2002) and switching may be biased by epigenetic modifications (Recker et al. 2011).

5.2 PfEMP1 variants associated with severe malaria

Although PfEMP1 are highly variable, semi-conserved regions may be maintained for functional mechanisms that are evolutionarily important for parasite survival. The most commonly described function of PfEMP1 is to mediate adhesion of IEs to vascular endothelial cells that may lead to sequestration of parasites in the capillaries of various organs, including the brain. Several studies have found an association between particular PfEMP1 subclasses and disease severity, in line with the theory that some PfEMP1 types are more virulent than others.

5.2.1 Group A and B/A PfEMP1

Group A and B/A var gene expression is upregulated by parasites infecting cerebral and non-cerebral severe malaria patients compared to uncomplicated malaria patients (Bertin et al. 2013; Jensen et al. 2004; Kessler et al. 2017; Kyriacou et al. 2006; Rottmann et al. 2006). In line with this, group A and B/A var genes encode for PfEMP1 that bind to EPCR and ICAM-1 and are associated with severe malaria (Jespersen et al. 2016; Lennartz et al. 2017). Group B and C var genes are generally associated with uncomplicated malaria, although some studies have also found associations with severe malaria (Kaestli et al. 2006; Rottmann et al. 2006; Jensen et al. 2004).

5.2.2 Pregnancy specific PfEMP1 - VAR2CSA

It is well established that parasites infecting the placenta during pregnancy predominantly express *var2csa* (Duffy et al. 2006; Salanti et al. 2003; Tuikue Ndam et al. 2005). *Var2csa* genes share 58 – 90% sequence homology between parasite genomes and are encoded by UPS E (Rask et al. 2010). VAR2CSA expressing parasite IE are able to bind to chondroitin sulfate A, a glycosaminoglycan expressed on placental epithelial cells (syncytiotrophoblasts) (Fried et al. 1996; Rogerson et al. 1997). As such, VAR2CSA contributes to sequestration of parasites IE in the placenta, a condition known as placental malaria that leads to poor birth outcomes (Guyatt et al. 2004; Kapisi et al. 2017; World Health Organization 2021c).

5.2.3 CD36 binding phenotype PfEMP1

The most common adhesion receptor for PfEMP1 is CD36. Adhesion to CD36 is mediated by CIDRα2 – 6 domains of Group B or C PfEMP1 head structures (Janes et al. 2011; Robinson et al. 2003), that are present in 70% of *var* genes (Mkumbaye et al. 2017b). Newbold *et al.* and Ndam *et al.* found CD36 adhesion was a property of all parasites isolated from patients with malaria (n = 71, n = 150 respectively) (Newbold et al. 1997; Tuikue Ndam et al. 2017).

Adhesion to CD36 has been more commonly found to be a property of parasites causing uncomplicated, rather than severe malaria. Parasites isolated from uncomplicated malaria cases more commonly bound to CD36 than parasites isolated from severe malaria cases (Mkumbaye et al. 2017b; Rogerson et al. 1999; Tuikue Ndam et al. 2017). CD36 is a widely distributed adhesion receptor but is not ubiquitously expressed in the brain and expression is not upregulated in cases of fatal malaria or cerebral malaria, unlike ICAM-1 and E-selectin (Berendt et al. 1989; Joste et al. 2020; Turner et al. 1994). Correspondingly, Avril *et al.* showed adherence of parasites to HBECs *in vitro* was independent of CD36 (Avril et al. 2016). Therefore it is likely that CD36 binding is a general parasite adhesion phenotype, rather than a key property for parasites causing cerebral malaria. This is in line with the rare occurrence of cerebral malaria and supports the idea that a rarer set of PfEMP1 are involved in cerebral adhesion.

Additionally, CD36 is expressed on platelets and PfEMP1 mediated adhesion of IE to platelets can cause clumping of IE. Clumping is a common phenotype in African field isolates (Pain et al. 2001) although it is unclear if all CD36 binding IE can initiate clumping, or whether it is mediated by specific variants and binding sites. Clumping has been associated with parasites causing severe malaria in some studies (Pain et al. 2001) and with parasitaemia (but not disease severity) in others (Arman et al. 2007), although clumping is difficult to assess *in vitro* (Arman et al. 2008). A few studies have investigated the interaction of parasite infected erythrocytes with CD36 expressed on monocytes. Clusters of CD36 on the surface of activated monocytes play a role in non-opsonic (Fc receptor independent) phagocytosis of infected erythrocytes (McGilvray et al. 2000) and trypsin cleavage of PfEMP1 from the surface of infected erythrocytes significantly reduced non-opsonic phagocytosis, possibly due to less CD36 interaction (Baruch et al. 1997).

5.2.4 Domain Cassette 13, Domain Cassette 8 and EPCR binding PfEMP1

Conserved domain cassette 13 (DC13) and domain cassette 8 (DC8) are upregulated in parasites causing cerebral malaria or severe malaria (Bertin et al. 2013; Lavstsen et al. 2012) and both domain cassettes are upregulated in parasites lines selected for binding to immortalized brain endothelial cells (Avril et al. 2012; Claessens et al. 2012). DC13 consists of a UPS A (group A promotor sequence) followed by a DBLα1.7-CIDRα1.4 and DC8 consists of a UPS B followed by a DBLα2-CIDRα1.1/1.8 – DBLβ12 -DBLγ4/6. Although DC8 is usually a group B var gene, it has a group A-like CIDR domain and contains a group A homology block (HB 141) and so is classified as a hybrid group B/A var gene (Lavstsen et al. 2012; Rask et al. 2010). Parasites expressing DC8 and DC13 adhere to endothelial protein C receptor (EPCR), expressed on brain endothelial cells (Turner et al. 2013) and the interaction site is at the CIDRα1 of the head structure. It is now well accepted that in addition to DC8 CIDRα1.1/1.8 and DC13 CIDRα1.4, group A head structures in general with a CIDRα1.1 or 1.4-1.8 have EPCR binding capabilities, except for CIDRα1.1 and 1.2 of DC1 (Lau et al. 2015; Turner et al. 2013). Although DC8 overall share between 45 – 63% amino acid homology (Lavstsen et al. 2012), Group A CIDRα1 domains (CIDRα1.1/1.4-1.8) are highly diverse. Only 6.5% of amino acids are conserved across all EPCR binding CIDRα1 and these are predominantly internal, structural residues also conserved in non-EPCR binding CIDRα1 (Lau et al. 2015).

Transcription of DC8 PfEMP1 domains has been shown to be upregulated in parasites causing cerebral malaria (Bertin et al. 2013; Jespersen et al. 2016) and several EPCR binding group A and B CIDRα1 domains have been associated with cerebral malaria (Joste et al. 2020; Kessler et al. 2017; Storm et al. 2019; Tuikue Ndam et al. 2017). Some evidence suggests that expression of specific CIDRα may be predictive of a particular manifestation of malaria. Kessler *et al.* found CIDRα1.7 was the dominant transcript in 50% of patients with cerebral malaria and brain swelling (Kessler et al. 2017). Shabani *et al.* found CIDRα1.4/1.6 were higher in patients with retinopathy positive cerebral malaria compared to retinopathy negative cerebral malaria, although no difference was detected by Kessler *et al.* (Shabani et al. 2017). DC8 or group A CIDRα1 domains predicted to bind EPCR have also been associated with severe malarial anaemia (Duffy et al. 2019; Jespersen et al. 2016) and respiratory distress (Lavstsen et al. 2012), although group A CIDRα transcripts are generally higher in cerebral malaria than severe malarial anaemia (Duffy et al. 2019; Shabani et al. 2017).

EPCR is expressed on multiple endothelial cell types, and immune cells including monocytes, neutrophils and placental trophoblasts. DC8 and DC13 expressing parasites are able to bind to brain, lung, heart, dermis and bone marrow endothelial cells (Avril et al. 2012; Mohan Rao et al. 2014; Turner et al. 2013). EPCR is a receptor for Activated Protein C (APC), involved in regulation of blood coagulation, inflammation, and endothelial cell integrity and apoptosis (Mohan Rao et al. 2014). The CIDRα1 domain of DC8 binds to EPCR with similar affinity to APC and can prevent APC binding *in vitro* (Lau et al. 2015; Turner et al. 2013) suggesting potential consequences of parasite adhesion other than sequestration. However, there is some skepticism about whether DC8/DC13/CIDRα1 expressing parasites bind to EPCR under physiological conditions (Azasi et al. 2018). Binding of recombinant DC8 domains to EPCR or EPCR expressing endothelial cells could not be mimicked in whole parasites in the presence of human serum (Azasi et al. 2018) although this result was not replicated in another study (Storm et al. 2019). A recent follow up study proposed that a complement serum factor, C1s, cleaves PfEMP1 at conserved sites in the interdomain regions and prevents adhesion of some parasite lines to endothelial cells, including IT4VAR19 that binds to EPCR. This may dampen sequestration to prevent death of the human host or be a mechanism of immune evasion (Azasi et al. 2021). The contribution of C1s cleavage to sequestration *in vivo* is still unclear. There is nevertheless a clear association between transcription of group A CIDRα1 domains and severe disease.

5.2.5 Domain cassette 5 and PECAM-1 binding PfEMP1

Domain cassette 5 consists of DBLγ12-DBLδ5-CIDRβ3/4-DBLβ7/9 and has been associated with severe malaria. Parasites expressing DC5 PfEMP1 are common (Heddini et al. 2001) and may be more common in severe malaria than uncomplicated malaria (Lavstsen et al. 2012). Parasite transcripts of DC5 were found to be significantly upregulated in patients with cerebral or severe malaria (Storm et al. 2019; Tonkin-Hill et al. 2018) and the DC5 domains DBLγ and DBLβ7/9 were increased in patients with retinopathy-positive cerebral malaria in Malawi

(Kessler et al. 2017).

Lab adapted parasites expressing DC5 are able to bind to platelet endothelial cell adhesion molecule 1 (PECAM-1) on endothelial cells, and PECAM-1 binding is a feature of field isolates (Berger et al. 2013; Heddini et al. 2001; Treutiger et al. 1997). However, parasites are still able to bind to PECAM-1 in the absence of PfEMP1 suggesting other VSA, such as RIFINs, may also be involved in adhesion (Fernandez et al. 1998). Polymorphisms in PECAM-1 may also influence binding of infected erythrocytes to PECAM-1 although it is unclear how this influences susceptibility to cerebral malaria (Hirayama et al. 2002; Ohashi et al. 2016). Glycosaminoglycans and soluble heparin have been shown to inhibit infected erythrocyte adhesion to PECAM-1 (Treutiger et al. 1997).

5.2.6 Domain Cassette 4 and other ICAM-1 binding PfEMP1

Immunohistochemical studies of individuals who died of cerebral malaria showed ICAM-1 mediated adhesion of infected red blood cells to cerebral blood vessels (Turner et al. 1994) and it has been well established that parasites grown in culture with ICAM-1-expressing endothelial cells can adhere via ICAM-1 receptors (Bengtsson et al. 2013; Berendt et al. 1989; Newbold et al. 1997). Inflammatory cytokines such as TNF cause upregulation of ICAM-1 (Avril et al. 2016) and ICAM-1 is more widely expressed in the brain than other known adhesion receptors, CD36 or E-Selectin (Turner et al. 1994).

The DBLβ domains of PfEMP1 were found to mediate adhesion of parasite lines (IT4 derived) to ICAM-1 on endothelial cells *in vitro* (Howell et al. 2007; Smith et al. 2000a), however, the identified DBLβ domains in these studies were group B or C that have high sequence variation, and therefore were considered to have poor prospects as vaccine candidates. Oleinikov *et al.* identified a single group A var gene that was upregulated in 3D7 parasite line selected for ICAM-1 adhesion, Pf110521 (Oleinikov et al. 2009). In a second line of research, Jensen et al. identified a conserved group A var gene, Pfd1235w, that was the most upregulated *var* gene in 3D7 parasites selected on transformed human bone marrow endothelial cells (TrHBMECs) and in parasites selected for recognition by semi-immune sera from African children (Jensen et al. 2004). In a search for Pfd1235w homologues in parasites from Ghanian field isolates, a novel domain cassette was identified, known as DC4. DC4 consists of DBLα1.1/1.4-CIDRα1.6 head structure followed by DBLβ3 domain, that cluster phylogenetically separately from other var gene domains, and have approximately 80% sequence identity between parasite isolates (n=6) (Bengtsson et al. 2013). Parasites expressing Group A PfEMP1 with DC4 - including PfEMP1 encoded by Pfd1235w or the previously identified Pf110521 in the 3D7 parasite line - can bind to ICAM-1. The binding site has been mapped to the DBLβ3 domain directly following the head structure (Bengtsson et al. 2013; Lennartz et al. 2017) and a short sequence of amino acids that comprise the minimum binding motif has been identified by Lennartz *et al.* (Lennartz et al. 2017). The Lennartz ICAM-1 binding motif can be used to predict ICAM-1 binding for all group A and some group B/A DBLβ domains (Lennartz et al. 2017; Madkhali et al. 2014; Olsen et al. 2018; Tessema et al. 2018). Transcripts encoding DBLβ with the binding motif are upregulated in cerebral malaria compared to severe anaemia and uncomplicated malaria (Kessler et al. 2017; Lennartz et al. 2017; Tonkin-Hill et al. 2018).

It is unlikely that a vaccine targeting adhesion blocking antibodies to ICAM-1 alone would be sufficient to protect from severe or cerebral malaria. Whilst some studies found parasites isolated from patients with SM could bind to ICAM-1 more frequently than parasites from uncomplicated malaria, others failed to find an association (Newbold et al. 1997) or found fewer ICAM-1 binding parasites from children with severe malaria (Rogerson et al. 1999). Parasites selected for adhesion to Human Brain Endothelial Cells upregulated expression of PfEMP1 with an ICAM-1 binding phenotype in some studies (Bengtsson et al. 2013) but not in others (Avril et al. 2012; Claessens et al. 2012). Additionally, a point mutation in the ICAM-1 receptor parasite interaction site has inconsistently being associated with susceptibility (Fernandez-Reyes et al. 1997) and protection (Kun et al. 1999) from severe malaria.

5.2.7 Dual binding parasite IE

In light of the inconsistencies in the association of ICAM-1 binding phenotype and severe malaria, several studies have proposed that parasites able to bind multiple adhesion receptors simultaneously may be responsible for severe disease (Avril et al. 2016; Lennartz et al. 2017; Oleinikov et al. 2009). Indeed, parasites expressing the DC13 head structure, were able to bind to both CHO745-EPCR expressing cells, whose ligand was previously mapped to the head structure CIDRα1 domain, and to CHO745-ICAM-1 expressing cells, via their DBLβ3 or DBLβ1 domain directly downstream from the head structure (Avril et al. 2016). Interestingly, all Group A, ICAM-1 binders that contain the ICAM-1 binding motif also bind to EPCR via an upstream CIDRα1 domain. Recombinant DBLβ3 and parasites expressing PfEMP1 with DC4 and the Lennartz motif could bind simultaneously to both ICAM-1 and EPCR and could withstand higher shear stress than single ICAM-1 or EPCR binding parasites (Lennartz et al. 2017). In contrast, Group B or C ICAM-1 binding parasites (that do not have the motif) often bind to both CD36 and ICAM-1 and cannot withstand higher shear stress (Lennartz et al. 2017).

High transcription of dual ICAM-1+ECPR binding PfEMP1 was found in cerebral malaria than non-cerebral severe malaria or uncomplicated malaria, whereas transcription of EPCR binding PfEMP1 was associated with non-cerebral severe malaria (Lennartz et al. 2017). In

Beninese children, dual ICAM-1-EPCR binding parasite IE were more common in cerebral malaria compared to uncomplicated malaria, and CD36 binding IE were more common in uncomplicated malaria (Tuikue Ndam et al. 2017). However, no differences in expression of dual ICAM-1+EPCR binding domains were observed between Beninese children with cerebral and uncomplicated malaria in another study (Joste et al. 2020). This suggests that although this particular parasite phenotype has been associated with severe disease, the presence or absence of the dual ICAM-1-EPCR parasite phenotype alone is not sufficient to determine the severity of disease.

5.2.8 Domains associated with rosetting

Rosetting is mediated via the DBLα1 of some PfEMP1 head structures (Chen et al. 2000; Rowe et al. 1997), binding to complement receptor-1, heparain sulfate or ABO blood group antigens on uninfected erythrocytes (Chen et al. 2000; Cockburn et al. 2004; Rowe et al. 1997; Rowe et al. 2002). IgM positive rosetting variants are characterized by DBLα1.5 and DBLα1.8 domains, followed by three DBL/DBLζ domains, and tend to have CIDRβ/CIDRδ/CIDRγ domains (Ghumra et al. 2012). Rosetting parasites can also adhere to endothelial cells via DBLα1 interaction with heparan sulfate or via adjacent domains binding to other adhesion receptors such as CD36 (Adams et al. 2014; Chen et al. 2000).

5.3 Antibody responses to PfEMP1 associated with protection

As previously described, PfEMP1 is the major VSA on IE targeted by antibodies. Previous studies have found that antibodies to some variants of PfEMP1 are associated with protection from severe or uncomplicated malaria and will be discussed in the following.

5.3.1 Antibodies to Group A and B/A PfEMP1

Antibodies targeting group A or B/A domains are generally acquired earlier in life that antibodies to group B or C domains (Obeng-Adjei et al. 2020; Oleinikov et al. 2012), possibly suggesting group A and B/A PfEMP1 are expressed preferentially to group B and C var genes in naive human hosts, as has been shown in a controlled human infection study (Bachmann et al. 2019). Papuan patients with uncomplicated malaria had antibodies to a greater range of group A and group B PfEMP1s than patients who had severe malaria (Duffy et al. 2016), supporting an associated of group A PfEMP1 and parasite virulence and possibly antibodies to group A PfEMP1 in protection from severe disease.

5.3.2 Antibdoies to pregnancy specific PfEMP1 - VAR2CSA

As VAR2CSA is a pregnancy specific variant, women lack VAR2CSA specific antibodies in their first pregnancies and acquire VAR2CSA specific antibodies with multiple pregnancies (Ricke et al. 2000). It is well established that antibodies targeting VAR2CSA can block IE adhesion to CSA (Doritchamou et al. 2016b; Nielsen et al. 2015b; Ricke et al. 2000) and induce ADCP by monocytes and monocytic cell lines (Feng et al. 2009; Barfod et al. 2010; Ataíde et al. 2011). Antibodies to VAR2CSA expressing IE have been associated with reduced risk of low birthweight babies and maternal anaemia in some studies (Duffy et al. 2003; Mayor et al. 2013; Staalsoe et al. 2004; Teo et al. 2014). However, a meta-analysis found that titers of antibodies to individual domains of VAR2CSA were not associated with low birth weight (Cutts et al. 2020) and it has been difficult to identify the best VAR2CSA fragments for use in a vaccine (Fried et al. 2015). Two vaccines for malaria in pregnancy have undergone phase I clinical trials, PRIMVAC (Sirima et al. 2020) and PAMVAC (Mordmuller et al. 2019), based on VAR2CSA fragments that induce CSA adhesion blocking antibodies to CSA (Doritchamou et al. 2013). Both were considered safe and induced antibodies to the homologous VAR2CSA expressing IE variant in non-pregnant women, however demonstrated poor cross reactive recognition or CSA adhesion blocking activity to heterologous VAR2CSA expressing IE (Mordmuller et al. 2019; Sirima et al. 2020).

5.3.3 Antibodies to CD36 binding PfEMP1

Antibodies directed against CD36 binding PfEMP1 domains and CD36 binding parasites are common and present at early ages (Kessler et al. 2018; Oleinikov et al. 2012). However, antibodies are acquired more slowly and peak later in life compared to Group A antigens (Oleinikov et al. 2012; Turner et al. 2015), in line with idea that Group A PfEMP1 dominate infections at earlier ages and in non-immune hosts.

Antibodies targeting CD36 binding CIDRα have been associated with a reduced risk of severe and uncomplicated malaria (Kanoi et al. 2018; Tessema et al. 2019). In a case-control study, children presenting with uncomplicated malaria had higher reactivity to several non-CD36 binding PfEMP1 proteinss than children with severe malaria (Travassos et al. 2018). In contrast, there was no difference in reactivity to CD36 binding CIDR domains in another study (Rambhatla et al. 2019). After an episode of cerebral malaria or severe malaria anaemia, antibodies directed towards CD36 binding domains were not boosted (Travassos et al. 2018), suggesting CD36 binding parasites were not involved in severe infections or have low immunogenicity. In Papua New Guinea (PNG), antibodies to CD36 binding domains were not boosted following severe or uncomplicated malaria infections (Rambhatla et al. 2019). Taken together, although parasites expressing CD36 are common and have been associated with uncomplicated rather than severe malaria in several studies, it remains unclear if a lack of antibodies to CD36 binding PfEMP1 contributes to a predisposition to severe malaria.

5.3.4 Antibodies to DC13, DC8 and other Group A CIDRα1 domains that bind EPCR

Despite the high sequence diversity across EPCR binding CIDRα1 domains, recombinant CIDRα1 domains from DC8 or DC13 are well recognized by antibodies from patients in malaria endemic areas (Kanoi et al. 2018; Lau et al. 2015; Lavstsen et al. 2012), as are parasites expressing DC8 containing PfEMP1 (Avril et al. 2012; Claessens et al. 2012). One study reported that Beninese children showed little recognition of DC8-PfEMP1 expressing parasite line (IT4VAR19) before or after infection, suggesting these parasites were not infecting this population (Nunes-Silva et al. 2015). In Tanzanian children and older Malian children and adults, the best recognized recombinant domains were EPCR binding CIDRα1 domains. (Lau et al. 2015; Obeng-Adjei et al. 2020). Antibodies to EPCR binding CIDRα1 domains weare acquired prior to CD36 binding domains (Obeng-Adjei et al. 2020), and increased from a young age until at least 10 years old (Turner et al. 2015), in line with age dependent acquisition of immunity. However these findings were not replicated in other studies (Cham et al. 2009; Rambhatla et al. 2019).

Overall, there is good evidence to suggest that antibodies are naturally acquired to diverse CIDRα1 domains associated with EPCR binding, however further studies are needed to validate if EPCR binding/ CIDRα1 domains can induce cross reactive antibodies to other EPCR binding domains, as the ability to induce strain-transcending immunity is a desirable feature for a vaccine. In one study, immunization with one CIDRα1 variant elicited cross reactive antibodies to the same domain subclass, but rarely to other CIDRα1 subclasses (Turner et al. 2018). However, immunization with CIDRα1.4 delivered by Virus Like Particles showed promising ability to induce cross reactive antibodies to recombinant CIDRα1.4-1.7 subclasses (Harmsen et al. 2020), and IgG antibodies purified to CIDRα1.1 from an EPCR binding parasite line were cross reactive with CIDRα1.4 from another parasite line (Lau et al. 2015).

There is evidence to suggest that antibodies against EPCR binding CIDRα1 domains confer protection from uncomplicated malaria or cerebral malaria. Antibodies to DC8 expressing parasite line, IT4VAR19, are elevated in individuals with uncomplicated malaria compared to severe malaria (Chan et al. 2019a) and boosted during severe malaria (Chan et al. 2019a; Rambhatla et al. 2019). Antibodies to EPCR binding CIDRα domains from the 3D7 genome are associated with reduced prospective risk of an episode of uncomplicated malaria (Kanoi et al. 2018). Similarly, Tessema *et al.* identified 17 DBLα domains for which antibodies were predictive of reduced prospective risk of severe malaria in PNG, some of which were part of PfEMP with EPCR binding CIDRα domains (Tessema et al. 2019). In contrast, antibodies to CIDRα1.4 were found to be predictive of higher parasite density in Malian children (Araj et al. 2021). Overall, antibodies to EPCR binding CIDRα1 domains appear to be involved in protection from malaria, however the role of CIDRα1 domain subclasses in different disease pathologies requires further study.

5.3.5 Antibodies to DC5 and PECAM-1 binding PfEMP1

Antibodies recognizing recombinant domains of PF11_0008 (a group A var gene from the 3D7 parasite line that contains DC5) are acquired early in life, peaking at age 5 in high transmission settings and age 10 in moderate transmission settings in Africa (Cham et al. 2009; Magistrado et al. 2007). Antibodies targeting the CIDRβ domain were predicted to reduce the risk of a malaria episode and parasitemia after adjusting for age and bed net usage (Magistrado et al. 2007) and high levels of antibodies to the DBLγ12, DBLδ5 and DBLβ9 domains of this protein reduced the risk of a febrile malaria episode in another study (Berger et al. 2013). In line with this, Travassos *et al.* found children with severe malarial anaemia or cerebral malaria lack antibodies to a portion of PF11_0008, compared to children with uncomplicated malaria (Travassos et al. 2018). In contrast, Duffy *et al.* found no significant difference in antibody levels to DC5 domains in severe and uncomplicated malaria, in patients from Papua, Indonesia (Duffy et al. 2016).

5.3.6 Antibodies to DC4 and other group A ICAM-1 binding PfEMP1

Antibodies to the earliest identified group A DBLβ3 from the var genes Pf110521 and Pfd1235w (commonly referred to as VAR4, which contains DC4), are well recognized by antibodies in African children and can block adhesion to ICAM-1 *in vitro* (Jensen et al. 2004; Oleinikov et al. 2009; Oleinikov et al. 2012). Further to this, the Lennartz motif that predicts ICAM-1 binding for group A DBLβ3 appears to be a well geographically conserved target of naturally acquired antibodies (Lennartz et al. 2017; Madkhali et al. 2014; Olsen et al. 2018; Tessema et al. 2018). The motif is highly conserved across parasite isolates, and antibodies raised against the motif from one parasite line are cross reactive to motif-containing DBLβ3 domains and parasites from other parasite lines (Olsen et al. 2018), but not to parasites with non-ICAM-1 binding DBLβ (Lennartz et al. 2017).

Antibodies to Group B and C ICAM-1 binding DBLβ3 (non-motif) were acquired prior to group A ICAM-1 binding DBLβ3 in one study (Olsen et al. 2019), but not in another study in PNG (Tessema et al. 2018). Further studies are required to determine how the age dependent acquisition of antibody to DBLβ3 domains affects acquisition of antibodies to ICAM-1-binding IEs and how this correlates with protection from severe malaria.

There is evidence to suggest that antibodies targeting IEs that display group A ICAM-1 binding PfEMP1 (with the Lennartz binding motif) play a role in protection from severe, clinical or cerebral malaria. Antibodies to recombinant DBLβ3 domains containing the motif were associated with a 37% reduced prospective risk of high parasitemia and fever in children in PNG, whereas this association was not observed for non-motif containing domains (Tessema et al. 2018). A prospective study by Kanoi *et al.* showed antibodies to DBLβ3 from 3D7 had an estimated 66% potential protective efficacy from clinical malaria in Uganda (based on Cox proportional hazard ratio) (Kanoi et al. 2018). Tanzanian children who did not have antibodies to parasites expressing PF110521 were at a greater risk of developing severe non-cerebral malaria (Oleinikov et al. 2012) and antibodies to the DBLβ-DBLβ couplet of Pfd1235w were boosted after an episode of severe malarial anaemia in another study (Travassos et al. 2018). In contrast, Olsen *et al.* found no evidence of a difference in antibody recognition of DBLβ3 with motif by patients with severe non-cerebral malaria and uncomplicated malaria (Olsen et al. 2018). Further studies are required to determine if antibody responses to group A binding DBLβ domains are associated with protection from cerebral malaria or other forms of severe malaria.

5.3.7 Antibodies to dual ICAM-1-EPCR binding PfEMP1

As previously discussed, all PfEMP1 expressing group A DBLβ3 with the Lennartz motif are predicted to bind to EPCR via an upstream receptor CIDRα1 domain. Therefore the evidence of an association between protection from severe malaria and antibodies to the DBLβ3 domains that contain the Lennartz binding motif also implies an association with antibodies to dual ICAM-1-EPCR binding parasites. In line with this hypothesis, antibodies against each of the domains from 3D7 parasite DC4 (containing the motif) (CIDRα1.3-DBLβ3-DBLβ3) were predictive of clinical protection (Kanoi et al. 2018). Travassos *et al.* found that antibody to the DBLαCIDRα1 head structure of DC4 from Pfd1235w (dual binding) was increased following cerebral malaria, suggesting DC4 expressing IE were the infecting variants involved in cerebral malaria (Travassos et al. 2018) and in another study antibodies to the CIDRα domain of Pfd1235w (encodes a dual binding PfEMP1) were associated with reduced risk parasitemia, reduced risk of anaemia and reduced incidence of malaria fever (Lusingu et al. 2006). However, further studies are needed to determine if having antibodies to one or both binding domains is associated with clinical outcome, potentially by directly blocking parasite adhesion to blood vessels in the brain or by activating protective immune mechanisms.

In Beninese children, antibodies targeting recombinant a ICAM-1 binding DBLβand an ICAM-1+EPCR binding CIDRα1.4-DBLβ3 fragment were elevated in uncomplicated malaria compared to cerebral malaria. However there were no differences in antibodies between children with uncomplicated and cerebral malaria amongst children who were infected with parasites transcribing ICAM-1+EPCR binding PfEMP1 (Joste et al. 2020). This contradicts previous studies to suggest that neither expression of the ICAM-1-EPCR binding phenotype, nor titers of IgG against it, was sufficient to predict clinical outcome, although antibodies were only evaluated for one parasite variant. In another study of Beninese children, overall levels of antibodies targeting recombinant DC13 were boosted following an episode of uncomplicated malaria, whereas cytophilic subclasses IgG1 and IgG3 were boosted following an episode of cerebral malaria (Badaut et al. 2021). Therefore the types of antibodies targeting ICAM-1+EPCR binding IE may be an important determinant of protective immunity to severe forms of malaria.

5.3.8 Antibodies to domains associated with rosetting

A small number of studies have found an association of antibodies targeting PfEMP1 that mediate rosetting and protection from severe malaria. In one study, children with uncomplicated malaria had elevated naturally acquired antibodies targeting rosetting parasite lines compared to children with severe malaria, as well as elevated antibodies to recombinant DBLα1 from rosetting variants (Albrecht et al. 2014). Additionally, antibodies to recombinant DBLα1 were predictive of a reduced risk of severe malaria (Tessema et al. 2019). Although some studies have suggested that rosetting may prevent antibody binding and phagocytosis (Hedberg et al. 2021; Moll et al. 2015; Albrecht et al. 2020), antibodies raised against recombinant DBLα1 could inhibit rosetting and instigate IE phagocytosis. (Angeletti et al. 2013; Ghumra et al. 2011; Quintana et al. 2016). Rosetting DBLα1 are generally followed by CIDRβ, δ or γ and antibodies targeting CIDR3 have been associated with reduced prospective risk of febrile malaria in another study (Obeng-Adjei et al. 2020).

5.4 Functions of antibodies targeting PfEMP1

As discussed, the main function of PfEMP1 is to mediate adhesion of parasitized erythrocytes to blood vessel endothelium and several studies have found that antibodies targeting PfEMP1 can inhibit adhesion to a variety of endothelial cell receptors, including ICAM-1 and EPCR.

However, as discussed, there are multiple functions of antibodies that can be mediated by the antibody Fc region. The following will discuss current evidence for antibody mediated immune activation of innate cells targeting PfEMP1, including antibody dependent phagocytosis, antibody dependent cell cytotoxicity and complement activation.

5.4.1 Antibody dependent phagocytosis by monocytes

Antibody mediated opsonic phagocytosis of IEs by monocytes is well documented *in vitro*. Parasite IE display factors that are usually contained within the inner-erythrocyte membrane, including lipid phosphatidylserines, that can induce non-opsonic phagocytosis of IEs (Fraser et al. 2021). Phagocytosis on non-opsonized IE can also be triggered by increased expression of Band 3 protein on the IE (Giribaldi et al. 2001) or display of PfEMP1 variants that bind to CD36 on monocytes (McGilvray et al. 2000). However, the majority of phagocytosis activity is due to opsonic phagocytosis, at least for some parasite strains (Zhou et al. 2012), and is required to activate the inflammasome pathways that lead to IL1-β (Zhou et al. 2012). Complement components contribute to opsonic phagocytosis, however phagocytosis can also occur for complement depleted sera (heat-inactivated) *in vitro* (Teo et al. 2015; Zhou et al. 2015).

PfEMP1 is the major target of opsonizing antibodies on the surface of the IE, as shown experiments with mutant parasite lines that lack PfEMP1 (Chan et al. 2017). In line with this, opsonizing antibodies are acquired in a variant specific manner, as illustrated by a study of multigravida Malian women who had higher opsonic antibodies to IE expressing the pregnancy specific PfEMP1 variant, VAR2CSA, than Malian men or malaria naïve adults (Lambert et al. 2014). There has been little investigation as to whether different variants or domains of PfEMP1 are better at inducing opsonizing antibodies than others.

Some studies have investigated the contribution of opsonic phagocytosis of IEs to protection from severe malaria or specific malaria pathologies. In malaria in pregnancy, Ataide *et al.* found that antibodies from women with placental malaria that induce THP-1 phagocytosis were associated with infant birthweight (Ataíde et al. 2011). Another study found no associated with improved fetal growth, but found an association with maternal haemoglobin (Chandrasiri et al. 2016). A recent study found that out of 169 measures of antibody features towards VAR2CSA, THP-1 phagocytosis of VAR2CSA expressing parasites (CS2) was amongst the top 6 most important features to predict protection from placental malaria in PNG (Aitken et al. 2021). These studies suggest that opsonizing antibodies that induce monocyte phagocytosis may be in some way protective. In children, opsonizing antibodies targeting EPCR binding IEs (VAR19) were higher in children with uncomplicated malaria than severe malaria in PNG (Chan et al. 2019b), suggesting this may be a protective response, however the authors note that the difference was modest in extent. A study of Cameroon children found no significant differences in opsonic antibodies to a rosetting parasite line (FCR3S1.2) between severe and uncomplicated malaria (Quintana et al. 2018). Taken together, there is strong evidence that opsonic phagocytosis of IE occurs, although further studies are required to evaluate the contribution to protection and how this may vary with parasite variants.

5.4.2 Antibody dependent neutrophil phagocytosis

IEs have been observed inside neutrophils taken from children infected with *P. falciparum* malaria (Brown et al. 1981). Isolated neutrophils *in vitro* also phagocytosed IEs but not uninfected erythrocytes, in the presence of sera from individuals living in an endemic area (Celada et al. 1983b; Salmon et al. 1986) and sera depleted of IgG could not phagocytose IEs (Celada et al. 1983a). However, no phagocytosis of IEs was observed in the presence of sera from American service members with limited prior exposure to malaria (Trubowitz et al. 1968) or European travellers after their first infection with P. falciparum, despite high levels of antibodies (Celada et al. 1983a). This suggests that specific antigen targets of antibodies on IEs are required for phagocytosis by neutrophils and antibodies to such targets may be acquired after multiple infections. Antibody dependent neutrophil phagocytosis has been associated with protection from placental malaria (Aitken et al. 2021) however there is currently no evidence to suggest if phagocytosis by neutrophils protects from severe or cerebral malaria (Aitken et al. 2018).

5.4.3 Antibody dependent cell cytotoxicity

A small number of recent studies have demonstrated NK cell antibody dependent cell cytotoxicity in response to IE. NK cells lyse IE, but not uninfected erythrocytes in the presence of sera from individuals in malaria endemic regions and lysis is sufficient to inhibit growth in cell culture (Arora et al. 2018; Mavoungou et al. 2003). NK cells isolated from both Gambian malaria exposed individuals and malaria naïve individuals responded to IEs with degranulation and cytokine release, when in the presence of Gambian malaria exposed sera but not sera from UK malaria naïve individuals or IgG depleted sera. This suggests the NK cell response is more dependent on IgG targeting the erythrocyte than on other serum factors or the NK cell activation state (Sherratt et al. 2020). Antibodies targeting VAR2CSA and RIFINS induced IE lysis and growth inhibition *in vitro* (Arora et al. 2018). Furthermore, purified IgG to individual domains of VAR2CSA promoted degranulation of NK cells and induced IFN and TNF production (Damelang et al. 2021). There is currently no evidence for NK cell ADCC in protection from severe or uncomplicated malaria (Damelang et al. 2021).

Interestingly, NK cell lysis of IEs also occurs independently of serum antibodies (Mavoungou et al. 2003; Orago et al. 1991) and can be triggered by more than one pathway (Baratin et al. 2007). NK cell activation and secretion of cytokines can be triggred by direct interaction with PfEMP1 DBL1α and VAR2CSA (Baratin et al. 2007; Mavoungou et al. 2003) where as other PfEMP1 variants, and some RIFIN variants, may inhibit NK cell activity (D'Ombrain et al. 2007)(Saito et al. 2017). It would be interesting to know if antibodies targeting these PfEMP1 variants can in turn have an activating or inhibitory effect on NK cells.

5.4.4 Antibody mediated complement fixation

Only a few studies have observed antibody dependent complement fixation on the surface of IEs (Kurtovic et al. 2020; Stanley et al. 1984; Wiesner et al. 1997; Opi et al. 2021). Previous studies have proposed various mechanisms by which IE evade complement fixation and complement mediated lysis (Rathnayake et al. 2021). When complement is activated, the infected erythrocyte appears to be resistant to complement mediated lysis, possibly by upregulating host cell factors such as CD59 (Rathnayake et al. 2021; Wiesner et al. 1997). Larsen *et al.* found that the classical complement cascade was induced by recombinant VAR2CSA protein but not by native VAR2CSA on the surface of IEs, in the presence of pooled polyclonal IgG or monomeric IgG engineered to enhance hexamerization that is required or complement fixation. The authors suggest that the distribution of PfEMP1 on knobs prevents antibody hexamerization (Larsen et al. 2019). However, classical complement fixation may be dependent on different variants of PfEMP1 or variation in antibody Fc properties, which were not considered in this study. Complement can also bind to IgM, however, the VAR2CSA variant of PfEMP1 binds to the same site of IgM as C1q and non-immune IgM cannot active the classical complement cascade on VAR2CSA expressing IEs (Akhouri et al. 2016). A recent study further suggests a more complex dynamic between complement and PfEMP1. Azasi *et al.* found that complement component-1S (C1s) cleaves PfEMP1 at semi-conserved sites between DBL and CIDR domains. C1s is recruited following C1q fixation and is regulated by serum esterases. C1s inhibited IT4VAR19 parasites from binding to EPCR on endothelial cells and inhibited dual binding IT4VAR13 parasites from binding to CD36 but not ICAM-1, matching the predicted cleavage sites for these variants (Azasi et al. 2021). Approximately 80% of PfEMP1 contain at least one C1s cleavage site (not including VAR2CSA), suggesting there is an evolutionary survival advantage for the parasites to conserve these sites. One possibility that the authors propose is that PfEMP1 cleavage may impair cytoadhesion and sequestration when the host is in a hyperinflammatory state, to prevent host death. They also suggest it may be a mechanism for parasites to escape opsonization (Azasi et al. 2021), since both antibodies and complement components induce opsonic phagocytosis of IEs by monocytes (Zhou et al. 2015). Taken together, these studies suggest a complex push and pull between complement mediated protection by the host and adaptions to evade complement by the parasite.

5.4.5 Summary of functions of antibodies targeting IE and PfEMP1

In summary, antibodies targeting IE surface antigens, which are likely dominated by PfEMP1, can induce antibody dependent cell phagocytosis (ADCP), antibody dependent neutrophil phagocytosis (ADNP) and antibody dependent cell cytotoxicity (ADCC), but the contribution of these antibody dependent effector functions to protection from severe malaria in children is largely unknown. It is unclear whether non-VAR2CSA variants of PfEMP1 can fix C1q to induce phagocytosis, and whether C1s cleavage of PfEMP1 contributes to disease severity. There may be several other functions of antibodies targeting IE that have not been explored, including antibody dependent respiratory burst and NETosis by neutrophils (Aitken et al. 2018). Importantly, little is known about the influence of PfEMP1 variants to elicit antibodies that induce these various effector functions targeting IEs.

Part III

Materials and Methods

6 Materials

6.1 Reagents

Table 2: Reagents

Reagent	Manufacturer	Cat.No
AlbuMAX™Lipid-Rich BSA	Gibco	11021-045
Bio-Plex Pro Magnetic COOH Beads	Biorad	MC100XX-01
Human blood	Australian Red Cross Blood Service, Victoria	
BSA	Sigma-Aldrich	A7906
Dihydroethidiumbromide (DHE)	Sigma-Aldrich	D7008
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich	D2650
Dynabeads™ Protein A	ThermoFisher	1001D
EasySep™ Direct Human Neutrophil Isolation Kit	Stemcell Technologies	19257
Ethylenediaminetetraacetic acid (EDTA) 15575	UltraPureTM 0.5M	Invitrogen
EZ-Link™Sulfo-NHS-LC-Biotin, No Weight Format	ThermoFischer	A39257
FACS lysing solution	BD Biosciences	89882
Foetal bovine serum	Gibco	10099141
Gelatin from porcine skin	Sigma-Aldrich	G1890
Giemsa's stain solution R66, GurrTM	VWR chemical	35086
HEPES	Gibco	15630080
Human Sera	Australian Red Cross Blood Service, Victoria	-
MES (C6H13NO4S)	Sigma	M0164-10PAK
MycoAlert™Mycoplasma Detection Kit	Lonza	LT07-318
MycoAlert™positive control	Lonza	LT07-518
Mycoplasma removal agent	MP Biomedicals	3050044
Paraformaldehyde	Sigma-Aldrich	158127
PBS (tablets)	Gibco	18912-014
Penicillin/Streptomycin/Glutamine	Gibco	10378016
Percoll®	GE Healthcare	17-0891-01
Pierce™ Premium Grade EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride)	ThermoFisher	PG82079
RMPI-1640-HEPES Medium, no glutamine	Gibco	21870-076
Sodium azide (NaN3)	Sigma-Aldrich	769320-100G
Sodium Chloride (NaCl)	Sigma	S5886-500G

Continued on next page

Table 2 – continued from previous page

reagent	manufacturer	cat.No
Streptavidin, R-Phycoerythrin Conjugate (SAPE)	ThermoFisher	S866
Sulfo-NHS No-Weight Format (N-hydroxysulfosuccinimide)	ThermoFisher	A39269
Tetanus toxin from Clostridium tetani	Sigma-Aldrich	T3194
Trypan Blue 0.4%	Gibco	15250061
Tween20	Sigma-Aldrich	P9416

Table 3: Plasticware

Product	Supplier	Cat.No.
Petri Dish 150 mm x 15 mm polystyrene	Falcon®	351058
Nunc™ Cell Culture Cryogenic Tubes	Thermo Fischer	377267
Mr. FrostyTM	Thermo Fisher	5100-0001
MiniMACS TM column	Miltenyi Biotec	
Eppendorf® microcentrifuge tube - 1.5 mL	Sigma-Aldrich	T9661
Minisart filter - 0.2 µm	Sigma-Aldrich	16534K
96-Well U-Bottom clear U-bottom, TC-treated plates	Corning®	3799
NuncTM EasYFlasks (T25, T50, T125), TC Surface, Filter Cap	Thermo Fischer	156367
96-well Bio-plex pro flat bottom plate	Biorad	171025001
Grenior Bio-One Microplate, 96/384 well, PS, µCLEAR®, Black, Non-binding	Interpath	781906
Amicon® Ultra-0.5 centrifugal filter device, Ultracel-30	Sigma-Aldrich	UFC5030
Amicon® Ultra-0.5 centrifugal filter device, Ultracel-100	Sigma-Aldrich	UFC5100

Table 4: Equipment

Product	Supplier	Cat.No.
Multiplex reader	LuminexTM FLEXMAP 3D®	
Multiplex reader	Bio-Plex® MAGPIXTM	Bio-Rad
Cell sorter	FACS ARIA-III	Beckman Coulter
Flow cytometer	Cytoflex S	Beckman Coulter
Flow cytometer	Cytoflex LX	Beckman Coulter
Magnet	DynaMag™-2 Magnet	ThermoFisher (12321D)

Continued on next page

Table 4 – continued from previous page

Product	Supplier	Cat.No.
Magnet	MiniMACSTM separator	Miltenyi Biotec (130-042-102)
Magnet	Easy 50 EasySepTM	Stemcell Technologies (18002)
Cell counter	Haemocytometer	
Microplate washer	ELx405TM Select	BioTek
Luminometer	FLUOstar Omega	BMG Labtech
Spectrophotometer	NanoDropTM 2000/2000c	ThermoFisher (ND-2000)

6.2 Antibodies

Table 5: Primary antibodies

Antibody	colour	Supplier	Cat.No.
Mouse α-human CD66b	BV421	Becton Dickinson	562940
Mouse α-human DBLβ3 (*var04*), 24E9		[a]	(Lennartz et al. 2015)
Rat α-human DBLβ3 (*var13*), polyclonal		[a]	(Lennartz et al. 2015)
Rabbit α-erythrocyte membrane proteins, polyclonanl		Cappel	55133

Table 5: ([a]): Anja Jensen, University of Copenhagen

Table 6: Secondary and tertiary antibodies

Targets	colour	Host (clone)	Supplier	Cat.No.
IgG Fc	PE	Mouse α-human (JDC-10)	Southern Biotech	9040-09
IgG1 Hinge	PE	Mouse α-human (HP6001)	Southern Biotech	9052-09
IgG3 Hinge	PE	Mouse α-human (HP6050)	Southern Biotech	9210-09
IgG2 Hinge	PE	Mouse α-human (HP6002)	Southern Biotech	9070-09
IgG4 Hinge	PE	Mouse α-human (HP6025)	Southern Biotech	9200-09
IgM-biotin		Mouse α-human (MT22)	MabTech	3880-6-250
IgG Fc	AF647	Goat α-mouse	Invitrogen	A21235

6.3 Recombinant human Fc receptors and complement component C1q (used in Multiplex assays)

Table 7: Recombinant human Fc receptors and complement component C1q (used in multiplex immunoassays)

Reagent	Supplier	Cat no./Ref
Biotinylated human CD32a (H167), Avitag™, His Tag	Acrobiosystems	CDA-H82E6-25ug
Biotinylated human CD16a (V176) Protein, Avitag™, His Tag	Acrobiosystems	CDA-H82E9-25ug
Biotinylated human CD32b	Acrobiosystems	CDB-H82E0-25ug
Biotinylated human CD16b (NA1)	Acrobiosystems	CDB-H82E4-25ug
Biotinylated Human CD32a (H167)	a	(Wines et al. 2016)
Biotinylated Human CD16a (V176)	a	(Wines et al. 2016)
C1q protein	MP Biomedicals	0219139101

Table 7: (a): Bruce Wines and Mark Hogarth, Burnet Institute

Table 8: Recombinant human proteins I

Protein	Domain	Group	DC/gene	Receptor	Assay
SM1	CIDRα2.4	B			M
SM2	CIDRβ1	B			M
SM3	DBLβ12	B/A		DC8	M
SM4	DBLβ3	A			M
SM5	DBLβ3	A	DC4		M
SM6	DBLδ1	B			M,P
SM8	DBLδ1	B			M
SM9	DBLδ1	B	DC8		M,P
SM11	DBLε3	B	DC11		M
SM12	DBLε9	B			M,P
SM14	DBLγ3	B	DC9?		M,P
SM15	DBLζ4	B	DC9?		M,P
SM17	DBLα1.5	A	DC4		M
SM18	CIDRα1.1	B/A	DC8	EPCR	M
SM24	DBLζ3	B/A			M
SM19	CIDRα1.6	A	DC4	EPCR	M
SM24	DBLζ3	B/A			M
SM22	DBLε5	A	*var1*		M,P
SM24	DBLζ3	B/A			M
SM24	DBLζ3	B/A			M
SM25	DBLβ13	B			M,P

Continued on next page

Table 8 – continued from previous page

Protein	Domain	Group	DC/gene	Receptor	Assay
SM26	CIDRγ12	B			M,P
SM27	DBLδ7	A			M
SM28	CIDRα2.6DBLβ5	B			M
UM1	DBLα0.13	B			M
UM2	DBL1	B			M,P
UM8	DBLγ9	B/A			M
UM14	DBLδ1	B			M
UM19	DBLδ1	B			M
UM20	CIDRα3.1	B/C			M
UM21	DBLα0.9	B			M
UM45	CIDRα1.7	A			M

Table 8: * Proteins with reference code beginning in SM or UM were originally derived from Papuan field isolate sequences (Tonkin-Hill et al. 2018) and expressed in Wheat germ cell-free expression system by Professor Takafumi Tsuboi and Dr. Eizo Takashima, Proteo-Science Center, Ehime University, Matsuyama, Ehime, Japan. Assay: 'M' denotes protein was included in multiplex immunoassays using sera from Malawian children (Part IV), 'P' denotes protein was inlcuded in multiplex immunoassays using sera from Papua New Guinean children (Part V. DC: Domain Cassette.

Table 9: Recombinant human proteins II

Protein	Domain	Group	Gene	Receptor
M1	DBLβ3*	A	PF110521 D4	ICAM-1
M6	DBLβ3*	A	3D7 PFD1235w D4	ICAM-1
M9	DBLβ1*	A	Dd2VAR32 D4	ICAM-1
	CIDRα-DBL$\beta3*$	A	3D7 PFD1235w D3-4	EPCR-ICAM-1
N27	DBLβ3	B	IT4VAR13 D4	ICAM-1
P1021	DBLβ3	C	KOB58843/HB3VAR34	ICAM-1
P1014	DBLβ5	B	KOB63129/HB3VAR21	ICAM-1
P1011	DBLβ5	B	AA75396/Dd2VAR01a	ICAM-1
N30	DBLβ7	A	Dd2VAR52 D4	
N31	DBLβ7	A	HB3VAR01 D4	
N32	DBLβ6	A	BT1983 D4 (JQ691649)	

Table 9: Proteins were expressed in *E-coli* expression system. Supplied by Professor Anja Jensen, University of Copenhagen, Copenhagen, Denmark. (*): contain the DBLβ motif indicative of dual EPCR+ICAM-1 binding PfEMP1 (Olsen et al. 2018)

Table 10: Recombinant human proteins III

Protein	**Domain**	**Group**	**Gene**	**Receptor**
GA007	CIDRα1.1	A	IT4VAR06	EPCR
GA026	CIDRα1.8a	A	GA026	EPCR
GA019	CIDRα1.6b	A	GA019	EPCR
GA020	CIDRα1.4	A	IT4VAR22	EPCR
GA052	CIDRα1.7	A	P2083-1	EPCR
GA012	CIDRα1.5	A	1965-2	EPCR
GA039	CIDRα2.2	B	IT4VAR24	CD36
GA040	CIDRα3.3	B	IT4VAR26	CD36
GA041	CIDRα2.1	B	IT4VAR30	CD36
GA042	CIDRα2.4	B	IT4VAR33	CD36

Table 10: Proteins were expressed in *E-coli* expression system. Supplied by Professor Thomas Lavstsen and Professor Louise Turner, University of Copenhagen, Denmark.

Table 11: Recombinant human proteins I

Protein	**Genome**
MSP2	HEK293
CSP-1	
EBA175-RIII-V	
MSP3	
AMA1	HEK293

Table 11: EBA-175-RIII-V supplied by Dr Ricardo Ataide and Dr jack Richards. Other proteins supplied by Dr Herbert Opi and Professor James Beeson, Burnet Institute, Melbourne.

7 Methods

7.1 Clinical samples

7.1.1 Malaria infected Malawian children

Blood samples were collected from Malawian children as part of a case-control study of cerebral and uncomplicated malaria, conducted over three malaria seasons (from 2015 – 2017) (Harawa et al. 2018). Ethics approval was granted by the College of Medicine Research and Ethics Committee (COMREC) and Melbourne Health Human Research Ethics Committee (HREC 2013.290). Written, informed consent was obtained from guardians of participants at all collection time points. The study included children aged 6 months – 12 years presenting with malaria, to Queen Elizabeth Central Hospital, Blantyre, Malawi. Malaria was diagnosed by light microscopy or a positive rapid diagnostic test and fever >37.5 °C. At enrollment, children with impaired consciousness were classified as having cerebral malaria based on a Blantyre coma score (BCS) of 0, 1 or 2, and children were classified as having uncomplicated malaria based on normal consciousness and a BCS of 5 (Molyneux et al. 1989). Children were excluded from the study if they tested positive to HIV, had recent history of non-malaria illness or appeared severely malnourished. Venous blood samples were collected at enrollment and follow up samples were taken 28 days later, during convalescence. Whole blood was transferred to sodium heparin tubes and plasma was separated from erythrocytes (centrifuged 500 $\times$ g, 5 min). Plasma was shipped to Melbourne, Australia in liquid nitrogen and stored at -80 °C. Plasma samples were used in this study to investigate antibody responses to PfEMP1 by multiplex Luminex assays, neutrophil phagocytosis assay and THP-1 phagocytosis assay (Part IV and VI). For the Luminex assays, children with severe and uncomplicated malaria samples were matched for location under guidance of local knowledge provided by Visopo Harawa. Aliquots of plasma were frozen at -20 °C.

7.1.2 Malaria infected Papua New Guinean children

Blood samples of Papua New Guinean children with malaria were collected as part of an observational study of severe pediatric illness conducted over 3 years (October 2006 – October 2009) (Manning et al. 2011). Ethics approval was granted by the PNG Institute of Medical Research Institutional Review board (IRB number 1103) and Medical Research Advisory Committee of the PNG Health Department (MRAC number 11.12). Written, informed consent was obtained from guardians of all participants. The study included children aged 6 months - 10 years presenting to Modilon Hospital, Madang, Papua New Guinea. Malaria was identified by light microscopy and *Plasmodium* species was confirmed by PCR. For our purposes, we included children with a peripheral parasite density >0, and with confirmed *P. falciparum* or mixed *P. falciparum- P. vivax* infection. Children were classified as having severe malaria based on presence of one or more of the WHO listed criteria (World Health Organization 2014a), Table 1. Children were classified as having uncomplicated malaria if they did not meet the severe malaria criteria. Venous blood samples were collected at enrollment and follow up samples were collected 8 weeks later during convalescence. Plasma was separated from whole blood by centrifugation and shipped to Melbourne, Australia in liquid nitrogen. Samples were stored at – 80 °C. Plasma samples were used in this study to investigate antibody responses to PfEMP1 by multiplex Luminex assays, neutrophil phagocytosis assay and THP-1 phagocytosis assay (Part V and VI). Aliquots were stored at -20 °C.

7.1.3 Malaria positive clinical controls

Plasma samples from Malawian pregnant women that were collected as part of a previous clinical trial (Madanitsa et al. 2016) were pooled to make a positive control for immunity to severe malaria. 150 plasma samples were pre-screened for IgG antibodies to recombinant CIDRα1.1 by Enzyme Linked Immunosorbent Assay and the 6 highest responders were selected for the pooled positive plasma. A second pooled control was made from 10 randomly selected samples of malaria infected Malawian children (from the same cohort as described previously) and was used as a representative for general antibody titers in the Malawi cohort. A third pooled control was made from 10 randomly selected samples of malaria infected Papua New Guinean children (from the same cohort as described previously) and was used as a representative for general antibody titers in the Papua New Guinean cohort.

7.1.4 Sera/plasma heat inactivation

For cell-based assays, sera/plasma was heat inactivated to diminish complement factors. 30 - 50 L aliquots of human sera were heated at 50°C for 45 min in a PCR machine. For all experiments and culture, FBS and NCS were heat inactivated in a water bath at 50 °C for 45 min.

7.2 Parasite culture

7.2.1 Parasite lines

Laboratory adapted *P. falciparum* parasite lines, 3D7 and IT4, were provided by Prof Anja Jensen, Department of Immunology and Microbiology, University of Copenhagen. The 3D7 parasites had been repeatedly selected for expression of *var04*, a group A PfEMP1 that binds to ICAM-1 and EPCR (referred to as 3D7VAR04). The IT4 line had been selected for expression of *var13*, a group B PfEMP1 that binds to ICAM-1 and CD36.

7.3 Maintenance of parasites in culture

Parasites were cultured *in vitro* according to the protocol published by Jensen *et al.* (Jensen 2002). Parasites were cultured in RPMI-HEPES (RPMI-1640 with 25 mM N-2-hydroxyethylpiperazine-N-2-ethane-sulfonic acid), supplemented with 5% heat inactivated human serum and 0.25% AlbuMAXTM lipid rich Bovine serum albumin. Type O+ red cells <3 weeks old were added to cultures at 3 - 5% of the total culture volume. Cultures were maintained between 0.1 – 4% parasitaemia (number of parasites/total number of red blood cells) and monitored by Giemsa's solution stained blood smears under a light microscope. Culture medium was replaced every 1 – 2 days and cultures were incubated at 37 °C, 1% O_2, 5% CO_2 in N_2.

7.3.1 Thawing parasites

Parasites were thawed at 37 °C for 1-2 min. Parasites were rehydrated by dropwise addition of decreasing concentrations of NaCl in PBS at room temperature. 12% NaCl was added at 0.1 times the blood volume and incubated for 5 min. 1.6% NaCl was added at 10 times the blood volume and centrifuged (350 × g, 5 min). 0.9% NaCl with 0.2% glucose was added at 10 times the blood volume and centrifuged. Parasites were resuspended in culture media and transferred to a 10 mL culture dish.

7.3.2 Freezing parasites

Cultured parasites were centrifuged (350 × g, 5 min) and the supernatant removed. Parasites were frozen in a final volume of glycerolyte that was 2 times the culture pellet volume. A third of the total volume of glycerolyte was added, dropwise, and incubated for 5 min. The remaining glycerolyte was added, dropwise, and 300 – 500 µL aliquots were frozen in cryotubes at -80 °C, in a gradual freezing container (Mr. FrostyTM, containing 100% isopropanol). Aliquots were transferred to liquid nitrogen after 24 hours for long term storage (>3 months).

7.3.3 Gelatin selection and synchronization of trophozoite stage parasites

To synchronize cultures and select for ‘knobby’ trophozoite stage parasites, parasite cultures were resuspended in gelatin (0.75% in RPMI-HEPES, 20 mL for 25 mL culture plate) and incubated upright in a centrifuge tube for 40 min, 37 oC. The top layer was collected (15 mL for 25 mL culture plate), diluted 1:1 in culture media and centrifuged (490 × g, 5 min). To return to culture, gelatin selected parasites were resuspended in culture media and fresh blood was added as required.

7.3.4 Mycoplasma detection

Cultures were routinely tested for mycoplasma contamination using a MycoAlertTM detection kit, as per the manufacturer’s instructions. The detection assay converts ADP released from lysed mycoplasma into ATP, and ATP reacts with luciferin to emit luminescence. In brief, 1 mL of parasite culture media was centrifuged (200 × g, 5 min) and 100 µL of supernatant was incubated with MycoAlert Reagent for 5 min. Luminescence was measured (Reading 1) on the FLUOstar Omega luminometer (BMG Labtech). 100 µL MycoAlert substrate was added to the samples and incubated for 10 min. Luminescence was measured a second time (Reading 2). A ratio of Reading 2/Reading 1 > 1 was interpreted as mycoplasma contamination. Contaminated cultures were treated daily with Mycoplasma Removal Agent (0.5 µg/mL) for 7 days and retested for mycoplasma. A positive control (MycoAlert Assay Control) and negative control (MycoAlert Assay Buffer) was included in all assays.

7.3.5 Predicting the dominant PfEMP1 varaint

Parasites were gelatin selected, washed 3 times in 0.1% BSA/PBS (centrifuge 490 × g, 5 min) and resuspended to 0.5% haematocrit (4 µL pellet in 8 µL 0.1% BSA/PBS). A 96 well culture plate was blocked with 0.1% BSA for 1 h, room temperature, and 25 µL of parasites were plated per well. Parasites were incubated with anti-human PfEMP1 antibody (anti-human VAR04 or anti-human VAR13, Table 5) at 0.16 mg/mL for 1 h, room temperature. Parasites were washed 3 times with 0.1% BSA/PBS (100 µL per well, centrifuged 490 × g, 2 min). Parasites were incubated with fluorescent secondary antibody (AF647, 4 µg/mL, 0.1% BSA/PBS) and dihydroethidium (DHE, 25 µg/mL, 0.1% BSA/PBS) for 30 min, room temperature. Parasites were washed 3 times and fixed in 2% PFA in PBS, for >2 h, 4 °C. At least 3,000 DHE positive events (parasite infected erythrocytes) were collected on a flow cytometer (Cytoflex S or Cytoflex LX). A DHE positive gate was set where <1% of events were DHE positive for an antibody free control and a DHE free control. Gating for AF647 positive events was set using an antibody free control, and AF647 free control. The percentage of parasites expressing the PfEMP1 variant of interest was calculated as DHE positive, AF647 positive events out of DHE positive, AF647 positive and negative events.

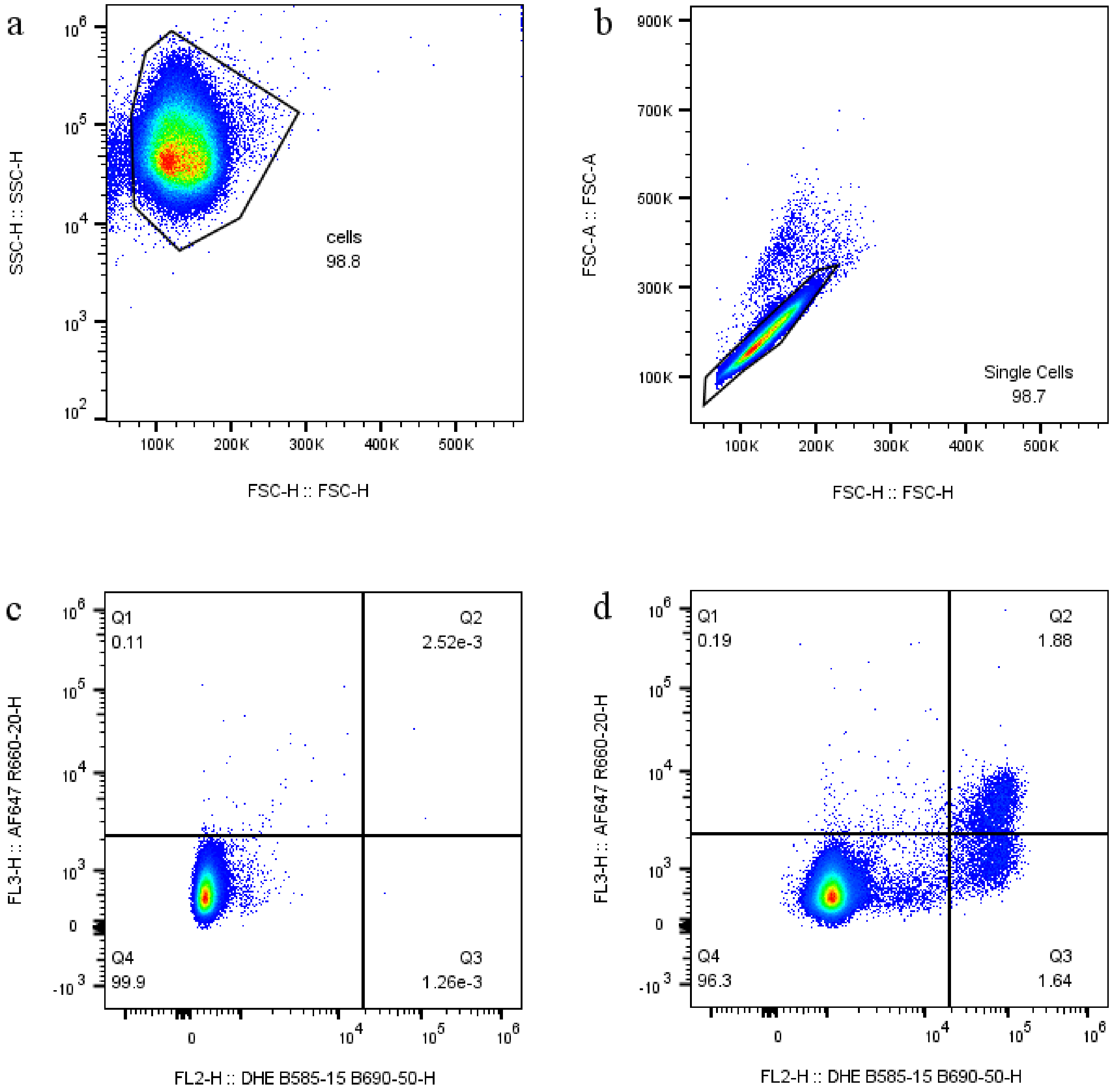


Figure 3: **Gating strategy to determine expression of *var04* on IE.** Isolated trophozoite stage IE were labelled with DHE, primary antibody targeting the DBLβ3 domain of *var04* and AF647 secondary antibody. (a) Single cells were gated on forward scatter and side scatter properties. (b) Duplicates were removed using forward scatter height and area. (c) IE labelled with AF647 (in the absence of primary antibody or DHE) were used to set the cut off for DHE and AF647 positive fluorescence. (d) The percentage of the parasite population expressing *var04* was determined by the frequency of cells in Q2 out of the total frequency of cells in Q2 and Q3, using the formula: 100*Q2/(Q2+Q3).

7.3.6 Selection of parasites expressing specific PfEMP1 variants by Fluorescence-Activated Cell Sorting (FACS)

To enrich cultures for trophozoite and schizont stage parasites, cultures were gelatin selected and resuspended in 2 mL media. To purify trophozoite and schizont stage infected erythrocytes, cultures were passed through a MiniMACSTM separator column on a MiniMACSTM Separator magnet, dropwise. Purified parasites were eluted from the column and washed once with serum-free RMPI-HEPES (500 × g, 10 min). Parasites were incubated with α-human PfEMP1 antibody (α-human VAR04 or α-human VAR13, Table 5) at 0.16 µg/mL for 1 h, room temperature. Parasites were washed 3 times (centrifuged 490 × g, 3 min) and incubated with secondary AF647 antibody at 4 µg/mL, for 30 min, room temperature. Parasites were washed 3 times and sorted on the FACS ARIA III at 37 °C, 85 µm, 45 psi, by Vanta Jamieson at the Melbourne Brain Center. Parasites labelled with AF647 were collected in RPMI-HEPES culture media and transferred directly to a culture dish.

7.3.7 Enrichment of parasites expressing specific PfEMP1 variants using antibody coated beads

To maintain parasite cultures that were selected by FACS for >50% expression of the desired PfEMP1 variant, parasites were re-selected after 2 - 3 weeks with antibody coated magnetic beads. Late trophozoite stage parasites were grown to <4% parasitaemia and were enriched by gelatin selection. Parasites were washed twice in RPMI-HEPES media and 200 µL of pellet was resuspended in 0.5 mL media. 25 µL of DynabeadsTM Protein A magnetic beads were transferred to 1.5 mL tubes and washed twice in RPMI-HEPES. To wash, beads were incubated on a magnet for 1 min and the supernatant was removed. To coat beads with antibody, 15 µg monoclonal α-human PfEMP1 antibody (α-human VAR04 or α-human VAR13, Table 5) was passed through a 0.2 µm sterile filter directly on to the beads and the filter was flushed with 1 mL RPMI-HEPES. Beads were incubated for 1 h, on a plate shaker. To remove unbound antibody, beads were washed 3 times on a magnet and resuspended in 0.5 mL RPMI-HEPES. Gelatin enriched parasites were incubated with antibody-coated beads for 1 h, room temperature, on a plate shaker. To remove unbound parasites, beads were washed 3 times in RPMI-HEPES. Beads were suspended in 10 mL parasite culture media and 100 µl blood and incubated overnight at 37 °C, 1% O_2, 5% CO_2. Culture media was removed and cells were gently resuspended in 2 mL parasite culture media and transferred to a round bottom FACS tube. To remove beads, the tube was incubated on a magnet for 2 min, and the media, containing ring stage parasites, was transfered to a new culture dish.

7.4 Multiplex antibody assays to measure antigen dependent antibody responses

7.4.1 Proteins of interest

For mulitplex immunoassays performed with the Malawian cohort, we included 29 PfEMP1 domain fragments derived from var sequences that were upregulated for severe malaria or uncomplicated malaria in Papuan adults (Table 8, (Tonkin-Hill et al. 2018)). The proteins were expressed in a wheat germ cell-free protein synthesis system as part of a previous study (Rambhatla et al. 2022). Additionally, we included 11 DBLβ proteins derived from various laboratory parasite isolates, including four that bind to ICAM+EPCR (and are associated with cerebral malaria), three that bind to ICAM-1+CD36 and three that do not bind to ICAM-1 (Table 9, (Olsen et al. 2018)). The DBLβproteins were expressed in an *E. coli* system and were supplied by Prof Anja Jensens group, University of Copenhagen. We also included 5 merozoite stage antigens that were provided by Dr Jack Richards, Dr Ricardo Ataide, Dr Herbert Opi and Prof James Beeson, Burnet Institute. For the multiplex immunoassays performed with the PNG cohort, we included proteins listed in Table 8 and 9 as well as 10 CIDRα domains from various parasite isolates, including six that bind to EPCR and four that do not (Table 10). The CIDRαdomains were expressed in an *E. coli* expression system and were supplied by Prof Thomas Lavstsen and Prof Louise Turner, University of Copenhagen.

7.4.2 Coupling recombinant proteins to magnetic carboxylated beads

Each protein of interest was coupled to Bio-Plex magnetic carboxylated beads labelled with a unique fluorescent signal, referred to as a bead 'region'. To resuspend, beads were sonicated for 1 min and vortexed for 30 s. Beads (100 μL per protein) were transferred to 1.5 mL tubes, incubated on a DynaMag^{TM}-2 magnet for 2 min, and storage buffer was removed. Beads were activated with Sulfo-NHS (10 μL, 50 mg/mL) and EDC (10 , 50 mg/mL) diluted in 100 mM monobasic sodium phosphate, pH 6.2, for 30 min, room temperature. To wash, beads were resuspended in 150 μL of 0.1 M MES, pH 5.0, vortexed for 30 s and the supernatant was removed on a magnet. Beads were washed twice and resuspended in 150 μL of 0.1 M MES. Recombinant proteins were added at 1.86 × 10-10 mol (per 100 μL beads) and made up to 250 with 0.1 M MES. Beads were incubated on a rotator for 3 h, room temperature. Protein coupled beads were washed once on the magnet in 500 μL PBS and incubated with 250 μL of blocking buffer (PBS, 0.1% BSA, 0.02% TWEEN-20, 0.05% Azide, pH 7.4) for 30 min. Beads were washed once in storage buffer (PBS, 0.05% sodium azide) and stored in 125 μL of storage buffer, in the dark, 4 C. For storage >2 months, beads were frozen at -80 °C. To scale the coupling procedure for >100 µL of beads, the protein concentration was altered and the final volume of storage buffer was adjusted to an appropriate volume. The final concentration of beads was estimated by counting on a haemocytometer. A protein-free control was included for every coupling procedure and was included in all antibody assays.

7.4.3 Opsonizing protein coated beads with antibodies from sera or plasma

Beads were sonicated for 30 s and vortexed for 30 s immediately before use. A working microsphere solution was prepared by combining each protein coupled bead region at a final concentration of 20 beads/µL/region, in 1% BSA/PBS. 50 µL of combined microsphere solution was added to each well of a black 96-well clear bottom plate (1000 beads per well). Patient serum was diluted 1:50 in PBS and 50 µL was added per well to give a final serum dilution of 1:100. Plates were incubated overnight at 4 C, on a plate shaker. Plates were washed twice on the Elx405^{TM} Magnetic Bead Washer in 1% BSA/PBS. All subsequent incubations (see below) were performed at room temperature, on a plate shaker.

7.4.4 Measuring levels of antibodies bound to protein coated beads

To measure total IgG, IgG1, IgG2, IgG3 or IgG4 antibody bound to protein coated beads, phycoerythrin (PE) conjugated fluorescent detector antibody (Table 6) was diluted to 1.3 µg/mL in 1% BSA/PBS. 50 µL of detector antibody was added per well and incubated for 2 h. To measure total IgE or IgM bound to protein coated beads, beads were incubated with a primary biotinylated detector antibody (α-human IgM or α-human IgE) diluted to 1.3 µg/mL in 1% BSA/PBS, for 2 h. Beads were washed twice in PBS/1%-BSA, and incubated with streptavidin-PE conjugated secondary detector, at 1.3 µg/mL in 1% BSA/PBS for 1 h. Following incubation with primary and secondary fluorescent detectors, plates were washed twice in 1% BSA/PBS. Median fluorescence intensities from a minimum of 40 beads per region were acquired on a Luminex instrument (Bio-Plex®, MAGPIX^{TM} or Flexmap3D).

7.4.5 Measuring Fc receptor engagement with antibodies bound to protein coated beads

To measure antigen dependent antibody binding to Fc receptors, biotinylated, recombinant human Fc receptor monomers (Table 7) were pre-conjugated to streptavidin-PE at a ratio of 4:1 mol to form fluorescent tetramers. Tetramers were diluted to 1.3 µg/mL in 1% BSA/PBS and 50 µL per well was incubated with the serum-bead complexes, for 2 h. For the Malawi cohort (Part IV) FcγRIIa-His131 and FcγRIIIa-Val158 were available as biotinylated, soluble homodimers (Wines et al. 2016), instead of monomers. Dimers were diluted to 1 µg/mL in 1% BSA/PBS and 50 µL per well was incubated with the serum-bead complexes for 2 h. Beads were washed twice in PBS/1%-BSA and incubated with streptavidin-PE conjugated secondary detector (1.3 µg/mL) for 1 h. Following incubation with fluorescent detectors, plates were washed twice in 1% BSA/PBS. Median fluorescence intensities from a minimum of 40 beads per region were acquired on a Luminex instrument.

7.4.6 Measuring C1q engagement with antibodies bound to protein coated beads

To measure antigen dependent antibody binding to C1q, recombinant C1q was biotinylated then conjugated to Streptdavidin-PE. Recombinant C1q buffer was removed using a Amicon Ultra-0.5 centrifugal filter unit with a 30 kDa molecular weight cut off, as per the manufacturer's instructions. In brief, the protein was added to the filter unit with up to 400 µL of PBS, centrifuged (14,000 × g, 20 min), flipped into a new tube and centrifuged (1000 × g, 1 min). The final concentration of protein was estimated using a NanoDrop™ Spectrophotometer. C1q was biotinylated using the EZ-Link™Sulfo-NHS-LC-Biotin, according to the manufacturer's instructions. In brief, 10 mM biotin reagent was prepared in PBS and incubated at a 1:5 ratio of C1q to biotin for 30 min, room temperature. Unreacted biotin was removed using an Amicon Ultra-0.5 centrifugal filter unit with a 100 kDa molecular weight cut off, as per the manufacturer's instructions. The biotinylated C1q was stored overnight at 4 °C. Biotinylated recombinant C1q was conjugated to streptavidin-PE at a ratio of 4:1 mol to form fluorescent tetramers. Tetramers were diluted to 15.92 µg/mL in 1% BSA/PBS and 50 µL per well was incubated with the serum-bead complexes, for 2 h. Plates were washed twice in 1% BSA/PBS. Median fluorescence intensities from a minimum of 40 beads per region were acquired on a Luminex instrument.

7.5 Statistical analysis for multiplex immunoassays

All statistical analysis and graphical representations of data were performed with R studio. The maximum value of 4 serum-free negative control replicates was subtracted from the raw MFI, for each bead region across all individuals, to account for non-specific binding to the antigen. A 'blank' bead region was included for each detector to account for non-specific binding to the microsphere itself and the MFI was subtracted for each individual across all bead regions. To account for plate to plate variation, within each antigen, plates were normalized by fitting a linear or non-linear regression equation to 36 duplicated samples in each plate (24 random samples, 10 malaria negative Melbourne controls, 2 pooled positive serum from immune Malawian adults, 10 standard curve dilution samples from pooled serum of Malawian children). Negative fluorescence intensities (below background fluorescence) were converted to a value of 1.

Seropositive was defined as antibody levels greater than 2 standard deviations above the mean for 10 malaria negative Melbourne controls. To assess antibody responses to grouped variables, the geometric mean was calculated for each individual across the selected group of variables.

7.5.1 Univariate analysis

For characterizing antibody responses in Malawian children, Part IV, to compare the mean difference between the cerebral malaria and uncomplicated malaria antibody levels for each antigen, data was log(x+1) transformed (to adjust for right skewedness), and the means were compared by a two sample Student's t-Test. The results were visualized as a volcano plot (ggplot2). A p value $>$ -log(0.05) and a $\log_2$(fold change) >1 (i.e. $>$ 2 fold change) were considered significant differences.

For characterizing antibody responses to PNG childern, Part V, data was log(x+1) transformed (to adjust for right skewedness), mean centered and scaled to a standard deviation of one. Linear regression was performed between clinical presentation (severe or uncomplicated) and age (as co-variates) with individual antibody features (as the outcome). The results were visualized as a volcano plot (ggplot2). Data was multiplied by the standard deviation for visualization. A p value $>$ -log(0.05) and a $\log_2$(fold change) >1 (i.e. $>$ 2 fold change) were considered significant differences.

7.5.2 Multivariate analysis

For multivariate analysis, each variable was log(x+1) transformed (to adjust for right skewedness), mean centered and scaled to one standard deviation. Missing values were imputed as the mean value for each variable.

7.5.3 Elastic Net regularized logistic regression

Elastic Net regularized logistic regression (ENET) was used to identify key variables that best describe the variation in cerebral and uncomplicated malaria, and was performed with the assistance of Dr Saber Dini, Melbourne School for Population and Global Health. ENET combines the benefits of LASSO regularization, that eliminates highly correlated variables, and ridge regularization, that retains potentially important correlated variables by shrinking the coefficients towards each other. ENET penalization was chosen due to the correlated nature of the data and due to the high number of variables compared to observations (Zou et al. 2005).

Elastic net requires the regularization term, lambda, and the tuning parameter, alpha, to determine the closeness to LASSO ($\alpha = 1$) or ridge ($\alpha = 0$). The alpha term was set to 0.5, the midpoint between ridge and LASSO regularization. Altering alpha to 0.9 (more LASSO-like) did not change the variables that were selected. The glmnet R package was used to generate a range of lambda values. The glmnet and caret package was used to perform 10-fold cross validated models with each value of lambda, to determine the lambda with the best predictive performance, based on AUROC. This was repeated 500 times (with newly selected folds of data) and the coefficients of the selected variable for the best model was recorded on each repeat. Variables that appeared >70% of the time were selected. To assess performance of the selected variables to predict cerebral or uncomplicated malaria outcomes, we performed partial least squares discriminant analysis (PLSDA) using the selected variables. The AUROC was computed from 10-fold cross validation, repeated for 500 randomly generated folds of data. To further demonstrate the relevance of the selected variables to predict outcome, the PLSDA performance was reassessed with the outcomes randomly shuffled.

7.6 Antibody dependent phagocytosis

7.6.1 THP-1 cell culture maintenance and passaging

THP-1 monocytic cells were cultured in RPMI 1640 media supplemented with 10% FBS, 1% penicillin/streptomycin/L-glutamine, 25 mM HEPES. Cultures were stored in NuncTM EasYFlasksTM cell culture flasks in a humidified incubator at 37 °C, 5% CO_2. To passage, cells were gently resuspended and centrifuged at 350 × g, 5 min, room temperature. Cells were counted in 0.4% trypan blue on a haemocytometer. Cells were maintained between 1 – 2 × 10^5 cells/mL and passaged every 2 – 3 days.

Thawing THP-1 cells Frozen vials were thawed at 37 °C in a water bath for 30 s and placed on ice. Approx. 1 mL of thawing media (RPMI 1640 supplemented with 50% FCS) was added, dropwise, and cells were transferred to a 15 ml tube. Approx. 9 mL of thawing media was added, dropwise, on ice. Cells were rested on ice, 10 min. Cells were centrifuged (350 × g, 5 min, room temperature) and gently resuspended in 5 mL culture media.

7.6.2 Freezing THP-1 cells

To freeze for storage, THP-1 cells were grown to approx. 1×10^6 cells/mL and centrifuged at 350 × g, 5 min and cooled on ice. Cells were resuspended at approx. 1×10^7 cells/mL in cold freezing solution (10% dimethyl sulfoxide, 90% FCS) and immediately aliquoted into cold cryovials (400 - 500 µL/vial). Cells were frozen in a gradual freezing container (Mr. Frosty^{TM}, containing 100% isopropanol) at -80 °C and transferred to liquid nitrogen after 2 days for long term storage (>3 months).

7.6.3 Isolation of primary human neutrophils from whole blood

Primary human neutrophils were isolated from whole blood with the EasySep^{TM} Direct Human Neutrophil Isolation Kit according to the manufacturer's instructions. Blood was collected into EDTA and 25 mL was transferred to a 50 mL tube. Isolation Cocktail was added to blood to a final concentration of 50 µL/mL. RapidSpheres were vortexed for 30 s and added to blood to a final concentration of 50 µL/mL. Blood was mixed gently and incubated for 5 min. Blood was topped up to 50 mL with isolation buffer (PBS, 1 mM EDTA) and placed on the Easy 50 magnet for 10 min. Supernatant (containing neutrophils) was transferred to a new tube. RapidSpheres were added to the supernatant at the same volume as previously and incubated for 5 min. Sample was placed on a magnet for 5 min and supernatant transferred to a new tube. Sample was incubated on a magnet for 10 min and the supernatant, containing isolated neutrophils, was collected. Neutrophils were centrifuged at 350 × g for 10 min and resuspended in RPMI 1640 supplemented with 10% FBS, 1% penicillin/streptomycin/L-glutamine. Cells were kept at 37 °C and used within 1 hour of isolation.

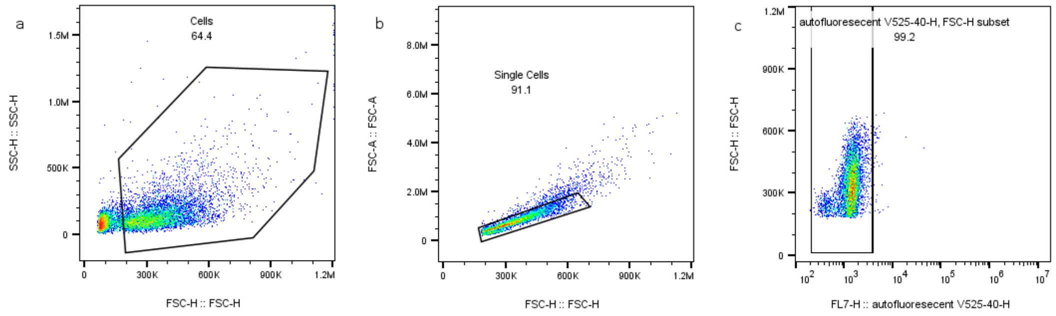
7.6.4 Antibody dependent phagocytosis of parasite infected erythrocytes by primary human neutrophils and THP-1 cells

To assess antibody dependent phagocytosis of trophozoite stage infected erythrocytes (IEs), we followed the method by Ataide et al (**Ataide2010**) for THP-1 cells and made minor adaptations for primary human neutrophils. Parasites were gelatin selected within 1 week prior to phagocytosis assays, to select for 'knobby' IEs. To purify trophozoite stage IEs, cultures were centrifuged (350 × g, 5 min) and loaded onto Percoll gradients made up of 80%-60%-40% Percoll in RPMI-HEPES (layered 3 mL-2 mL-2 mL, respectively). Percoll gradients were centrifuged (1620 × g, 15 min) and the 60% layer containing trophozoite stage IE was collected. Purified IE were washed twice by 4 times dilution with RPMI-HEPES and centrifuge (350 × g, 3 min). To fluorescently label, purified parasites were incubated with DHE at 25 µg/mL of sample for 30 min, room temperature, and washed four times. To opsonize IEs with antibodies, heat inactivated plasma or sera was plated in 96 well culture plates (blocked with 0.1% BSA/PBS, 1 h, room temperature). 5×10^5 IEs were added per well and incubated with sera for 1 h, room temperature. Pooled positive serum from Malawian pregnant women and rabbit α-human red blood cell antibody were used as positive controls for antibody dependent phagocytosis. Sera-free (PBS) and malaria negative Melbourne donor sera were included as controls for non-antibody dependent phagocytosis. Opsonized IEs were washed 3 times (RPMI-HEPES, centrifuged 350 g, 5 min) and resuspended in 50 μL of THP-1 or neutrophil culture media (RPMI-1640, 10% FBS, 1% penicillin/streptomycin/L-glutamine). Parasites were split into duplicates (25 μL/well, 2.5×10^4 parasites per well) in a new 96 well plate (blocked with 0.1% BSA/PBS).

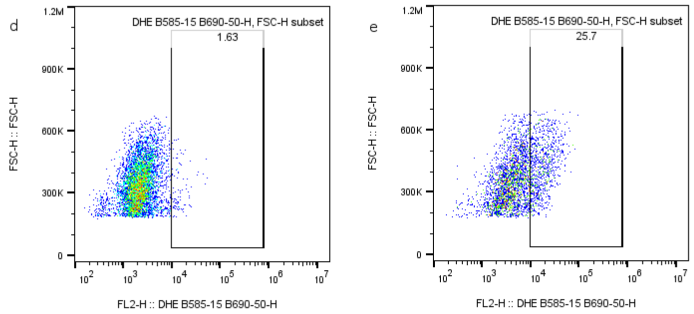
For phagocytosis, THP-1 cells were spun down from culture (centrifuged 300×g, 5 min) and added to opsonized IEs at 2.4×10^4 THP-1 cells per well in THP-1 culture media. THP-1 cells were incubated with IEs for 40 min, 37 °C, 5% CO_2. Primary human neutrophils were isolated from whole blood and added to opsonized IEs at $2.5 \text{ X } 10^4$ neutrophils per well in neutrophil media. Neutrophils were incubated with IEs for 1 h, 37 °C, 5% CO2. To stop phagocytosis, cells were centrifuged at 4 oC (350×g, 5 min). Non-phagocytosed erythrocytes were lysed in 75 μL of FACS lysing solution for 10 min, room temperature and washed 3 times in cold 1% NCS/PBS (100 μL/well, centrifuged 350 × g, 3 min). To check the purity of isolated neutrophils, neutrophils exposed to IEs and neutrophils unexposed to IEs were labelled with fluorescent α-human CD66b antibody (Table 5). To fix, cells were resuspended in 2% PFA, PBS, for >2 h, 4 °C.

7.6.5 Flow cytometry

Cells that had undergone opsonic phagocytosis of infected erythrocytes were identified by DHE fluorescence using flow cytometry (B585-15 laser). A minimum of 10,000 total events were acquired per sample on the CytoFLEX S and CytoFLEX LX. THP-1 cells were gated as the major population on the FSC-H vs. SSC-H graph and neutrophils were gated as the α-CD66b positive population (V450-45 channel). Auto-fluorescent cells were excluded by selecting the negative population in the V525-40 channel. DHE positive cells were gated to <2% of events on the serum free negative control (Figures 4 and 5).



Cells Single cells Autofluoresence



DHE positive cells - No sera control DHE positive cells - Serum sample

Figure 4: **Gating strategy to quantify ADCP.** a) THP-1 cells were gated based on the forward scatter vs. side scatter. b) Single cells were seleced on the forward scatter area vs. height. c) Autofluoresent cells were negated on the V525-40 channel. d) DHE positive cells were gated using the sera-free control. e) Percentage of DHE positive events was recorded.

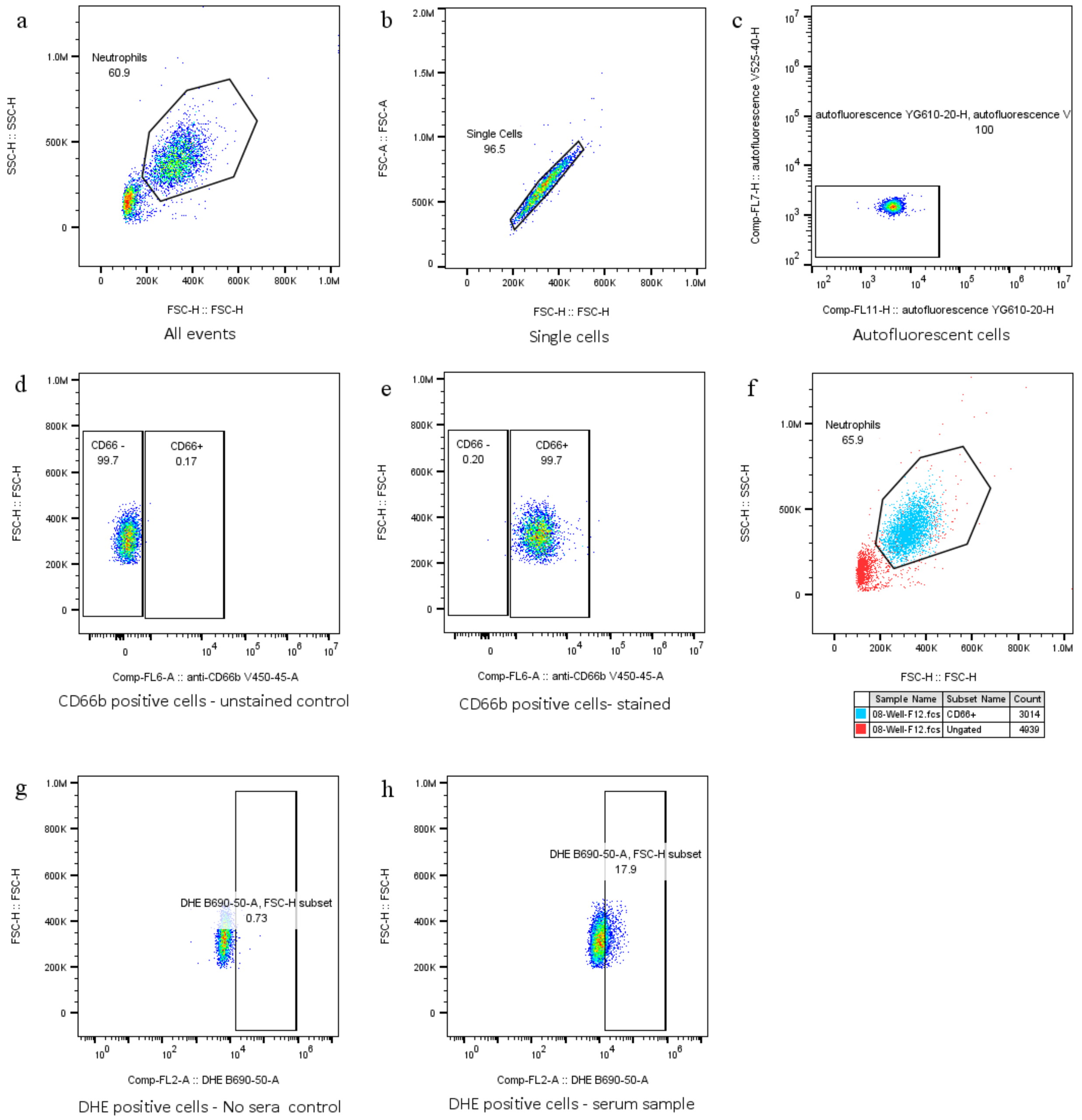


Figure 5: **Gating strategy to quantify ADNP.** a) Neutrophils were gated based on the forward scatter vs. side scatter. b) Single cells were seleced on the forward scatter area vs. height. c) Autofluoresent cells were negated on the V525-40 and YG610-20 channels. d) CD66b positive events were selected using the anti-CD66b free control and e) CD66b stained control. f) Neutorphil cell gating was confirmed by CD66b positive events, shown in blue. g) DHE positive cells were gated using the sera-free control. e) Percentage of DHE positive events was recorded.

7.6.6 Statistical analysis

Technical replicates with $\geq$5 points difference and $\geq$20% variance were removed. ‘Percentage phagocytosis’ was defined as the frequency of THP-1 or neutrophil cell events that were positive for DHE, relative to the rabbit α-human red blood cell positive control: We subtracted the frequency of the no sera control from each 96 well plate to reduce background noise, then divided by the frequency of the rabbit α-human positive control (for each plate) to normalize between plates and multiplied by 100%. Mann-Whitney U test was used to compare between clinical outcomes and time points.

Part IV

Characterizing the PfEMP1 targets and Fc features of antibodies associated with cerebral and uncomplicated malaria in Malawian children

The following chapter is under submission to Cell Reports Medicine and is therefore presented in manuscript format, with associated bibliography, followed by supplementary files.

A systems serology approach to capture the complexity of the immune response to cerebral malaria in Malawian children and identify key correlates of protection.

Authors: Isobel S Walker[1], Saber Dini[2], Elizabeth H Aitken[3,4], Timon Damelang[4], Anja T R Jensen[5], Janavi S Rambhatla[1], Herbert Opi[6], Mark P Hogarth, Bruce D Wines, Michael F Duffy[1,4], Eizo Takashima[7], Visopo Harawa[8], Takafumi Tsuboi[9], Julie A Simpson[2], Wilson Mandala[10], Terrie E Taylor[11,12], Karl B Seydel[11,12], Amy W Chung[4], Stephen J Rogerson[1,3]

Affiliations

1. Department of Medicine, The University of Melbourne, the Peter Doherty Institute for Infection and Immunity, Melbourne VIC 3000, Australia
2. School of Population and Global Health, The University of Melbourne, Parkville VIC 3010, Australia
3. Department of Infectious Diseases, the Peter Doherty Institute for Infection and Immunity, Melbourne VIC 3000, Australia
4. Department of Microbiology and Immunology, the Peter Doherty Institute for Infection and Immunity, Melbourne VIC 3000, Australia
5. Centre for Medical Parasitology, Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
6. The Burnet Institute, Melbourne VIC 3004, Australia
7. Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Japan
8. Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre Malawi
9. Division of Cell-Free Sciences, Proteo-Science Center, Ehime University, Matsuyama, Japan
10. Academy of Medical Sciences, Malawi University of Science and Technology, Thyolo, Malawi
11. Blantyre Malaria Project, Kamuzu University of Health Sciences, Blantyre, Malawi
12. College of Osteopathic Medicine, Michigan State University, East Lansing, USA

Abstract: *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), a variant antigen on the surface of *P. falciparum* infected erythrocytes, mediates parasite sequestration in the deep vasculature, an important component of the pathogenesis of cerebral malaria. PfEMP1 is a major target of protective antibodies, but the features of the protective antibody response are poorly defined. In a case-control study of Malawian children with cerebral or uncomplicated malaria, we used a systems serology approach to undertake a detailed characterization of the antibody response to 39 recombinant PfEMP1 Duffy Binding Like (DBL) domains or Cysteine Rich Interdomain Regions (CIDRs) that are associated with severe or uncomplicated malaria. Machine learning with a logistic regression model was used to identify the combination of antibody targets and features that best distinguish between children with cerebral and uncomplicated malaria. The selected combination of seven features could predict clinical presentation with 87% accuracy. Antibody targets associated with decreased odds of cerebral malaria included well characterized DBLβ3 domains and a less well characterized CIDRγ12 domain. Antigen-specific IgG

antibodies were not associated with protection however their Fc features were, including the ability of antibodies to bind C1q and FcγRIIIb. The results of this study support the hypothesis that specific PfEMP1 variants are involved in the pathogenesis of cerebral malaria and that specific antibody Fc interactions may facilitate clearance of these parasite variants in children with uncomplicated malaria.

Introduction:

There are 247 million cases of malaria annually that result in over half a million deaths, predominantly of children under 5 years of age [1]. Cerebral malaria is a severe and lethal manifestation of *Plasmodium falciparum* malaria that is characterized by impaired consciousness and predominantly occurs in children or adults who lack protective immunity. The pathogenesis of cerebral malaria is complex and only partially understood, however a central component is the adhesion of parasite-infected erythrocytes (IE) to blood vessel endothelial cells in the brain, resulting in sequestration of IE in the cerebral microvasculature [2]. Mostly, malaria illness is uncomplicated, with fever and non-specific symptoms including headache, myalgia and chills, and children do not progress to cerebral malaria.

Adhesion of IE to endothelial cells is mediated by *Plasmodium falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) variant surface antigens expressed on the IE surface. PfEMP1 are encoded by *var* genes, of which there are ~60 variants per parasite genome, with one variant expressed at a time. The majority of *var* genes can be classified as Group A, B or C based on their upstream promotor sequence and encode for PfEMP1s with a combination of two to ten Duffy Binding Like (DBL) domains and Cysteine Rich Interdomain Regions (CIDR), some of which are frequently found in tandem arrangements, known as Domain Cassettes (DCs) [3]. Representative PfEMP1 structures are illustrated in Supplementary Figure 1. Despite enormous diversity in *var* genes, previous studies have identified PfEMP1 domains that are more frequently expressed in different clinical manifestations of malaria, and some of these PfEMP1 domains are known to bind to particular endothelial cell receptors. Generally, Group A *var* genes are associated with severe malaria, whereas Group B and C *var* genes are associated with uncomplicated malaria. PfEMP1 with CIDRα1 domains that bind to endothelial protein C receptor (EPCR), including those within DC8, have been associated with severe and cerebral malaria [4, 5]. CIDRα1 domains are sometimes found adjacent to a DBLβ domain that binds to intercellular adhesion molecule 1 (ICAM-1), including in DC4 and DC13, enabling dual receptor binding of IEs to brain endothelial cells [6]. Dual binding PfEMP1 can be predicted by the presence of a short sequence of amino acids in group A and some group B/A DBLβ domains, referred to as $DBL\beta_{motif}$, and have been associated with cerebral malaria [7, 8].

PfEMP1 is the main target of antibodies on the surface of the IE and antibodies targeting PfEMP1 or recombinant PfEMP1 domains have been associated with protection from severe malaria and uncomplicated malaria in multiple studies [9–11]. However, there does not appear to be a single antigen variant that is associated with cerebral malaria in all cases, and given the high diversity of PfEMP1, it is likely that individuals would have to acquire antibodies to a combination of PfEMP1 target antigens to be protected. Previous studies have shown that antibodies targeting PfEMP1 have diverse functions, including inhibition of adhesion to endothelial cells, promoting phagocytosis by monocytes [12], neutrophil killing of IEs [13], and NK cell cytotoxicity [14]. However, due to the high diversity of PfEMP1, most studies have focused on a small number of domains or parasite lines and have employed a small number of functional immunoassays. Systems serology involves characterizing multiple antibody Fc features and Fab targets, followed by machine learning to identify the most relevant antibody responses [15]. We have recently used this approach to identify antibody responses that best correlate with protection from placental malaria [16].

This study aimed to characterize the antibody responses at the time of hospital presentation in Malawian children with cerebral and uncomplicated malaria and to identify a combination of antibody features that could differentiate between the groups. We measured 11 Fc features or antibody types targeting 39 PfEMP1 domains that have been associated with severe or uncomplicated malaria [17, 18] and used machine learning to select a combination of antibody targets and features that could best discriminate between cerebral and uncomplicated malaria. These responses provide important new insights into the development of protective antibody immunity against cerebral malaria.

Methods:

Clinical samples

Study participants were Malawian children presenting with cerebral or uncomplicated malaria to Queen Elizabeth Central Hospital, Blantyre, Malawi, over three malaria seasons (2015 – 2017) [19]. Written, informed consent was obtained from guardians of participants. Malaria was diagnosed by light microscopy or rapid diagnostic test accompanied by fever >37.5 °C. Participants were classified as having cerebral malaria based on a Blantyre coma score (BCS) of ≤ 2, or as having uncomplicated malaria based on normal consciousness and a BCS of 5 [20]. Participants were aged 6 months – 12 years and were excluded if they tested positive to HIV, had recent history of non-malaria illness or appeared severely malnourished. Venous blood samples were collected at enrollment. Plasma was separated and stored at -80°C. To minimize differences in prior exposure to malaria, we matched individuals with cerebral and uncomplicated malaria based on village of residency and rural or urban environment. Five samples were further removed due to inadequate volumes to complete all assays. Plasma from 10 malaria naïve Melbourne adults were included in each assay as individual negative controls.

Recombinant proteins

The study included recombinant proteins previously associated with severe, cerebral or uncomplicated malaria (Supplementary Table 1). This included 11 PfEMP1 DBLβ domains associated with ICAM-1 or non-ICAM-1 binding [18, 21, 22] and 28 PfEMP1 domains derived from *var* sequences that were upregulated in severe or uncomplicated malaria in Indonesian adults (coded as SM or UM, respectively) [10]. We included four merozoite antigens, Merozoite Surface Protein-2 (MSP2), MSP3, Erythrocyte Binding Antigen-175 (EBA175-RIII-B) and Apical Membrane Antigen-1 (AMA1), and a sporozoite antigen, Circumsporozoite Protein (CSP), as markers of prior exposure. Tetanus toxoid antigen was used as a positive control and an antigen-free bead region was used as a negative control.

Multiplex immunoassay

A multiplex immunoassay was used to assess antibody reactivity and antigen-specific antibody features to the selected recombinant PfEMP1 antigens, as previously described in detail [23]. Recombinant proteins were coupled to Bio-Plex magnetic carboxylated beads (Bio-Rad, Hercules CA, USA) as per the manufacturer's instructions. For each assay, protein coupled beads were combined to a final concentration of 20 beads/μL per bead region in 1% Bovine Serum Albumin in Phosphate Buffered Saline (BSA/PBS). Combined beads were incubated in a 96 well plate (Corning, Corning NY, USA) with participant serum, diluted 1:50 in PBS, overnight at 4°C on a plate shaker. Beads were washed with 1% BSA/PBS and incubated with detector antibody. We compared IgG responses to antigens probed in single format to multiplex array format, to confirm there was minimal antibody competition or interaction between antigens (Supplementary file 1, Figure S2). To detect IgG, IgG1, IgG2, IgG3, and IgG4, beads were incubated with phycoerythrin (PE) conjugated fluorescent anti-human IgG, IgG1, IgG2, IgG3 or IgG4 antibody (1.3 μg/mL in 1% BSA/PBS, Southern Biotech, Birmingham AL, USA). To detect IgM, beads were incubated with a primary biotinylated anti-human IgM antibody (1.3 μg/mL in 1% BSA/PBS, MabTech, Sweden), for 2 h, followed by streptavidin-PE conjugated secondary detector (1.3 μg/mL in 1% BSA/PBS, Thermo Fisher Scientific, Waltham MA, USA), for 1 h. To detect Fc receptor binding to antibodies, biotinylated recombinant human FcγRIIb and FcγRIIIb monomers (ACRObiosystems, Newark DE, USA) were pre-conjugated to streptavidin-PE at a ratio of 4:1 mol to form fluorescent tetramers. FcγRIIa-His^{131} and FcγRIIIa-Val^{158} were available as biotinylated, soluble homodimers [24]. Tetramers or dimers were diluted to 1.3 μg/mL in 1% BSA/PBS and incubated with beads, for 2 h. To detect C1q binding to antibody, recombinant C1q (MP Biochemicals, Irvine CA, USA) was biotinylated using the EZ-Link™Sulfo-NHS-LC-Biotin (Thermo Fisher Scientific, Waltham MA, USA) according to the manufacturer's instructions, using a 1:5 mol ratio of C1q to biotin. Biotinylated recombinant C1q was conjugated to streptavidin-PE at a 4:1 mol ratio to form fluorescent tetramers, which were diluted to 15.92 μg/mL in 1% BSA/PBS and incubated with beads, for 2 h. Median fluorescent intensities (MFI) from a minimum of 40 beads per region were acquired on a Luminex instrument (Bio-Plex® MAGPIX™ or Flexmap3D).

Statistical analysis

Prior to analysis, we subtracted the serum free control and antigen free control region MFI. We normalized between plates by fitting a linear or non-linear equation to 36 duplicated samples from each plate. Negative fluorescence intensities were converted to a value of 1. Four variables were eliminated as all values were below background (FcgRIIIb.SM14_DBLγ3, C1q.SM5_DBLβ3, FcgRIIb.SM26_CIDRγ12, IgG2.SM5_DBLβ3) and two antigens were not included for all Fc detectors due to low protein availability (SM27_DBLδ7 and UM1_DBLα0.13, only probed for IgG, IgG1, IgG2, IgG3 and IgG4).

To compare the mean difference in antibody levels for each antigen between patients with cerebral malaria and uncomplicated malaria, data were log(x+1) transformed (to adjust for right skewedness) and the means were compared by a Welch's t-Test. A p-value < 0.05 and a $\log_2$(fold change) >1 (i.e. > 2 fold change in geometric means of antibody levels) were considered significant differences. To evaluate the influence of age, we divided the population around the median age of 49 months for some analyses. To group responses to multiple proteins the geometric mean of responses was calculated for each individual. To select a combination of features that best distinguish between cerebral and uncomplicated malaria, we performed multivariable logistic regression coupled with machine learning, as previously described with minor changes [16]. Merozoite antigens were excluded from the multivariable analysis. Data were log(x+1) transformed, mean centered and scaled to one standard deviation. Missing values were imputed as the median of five imputed data sets using Multivariate imputations by chained equations [25] with predictive mean matching. Elastic-Net Regularised Logistic regression (ENLR) [26] was used to identify features that best distinguish between cerebral and uncomplicated malaria. We performed 5,000 repeats of ENLR with the α tuning parameter set to 0.5. For each repeat, the data were randomly split into 10 folds, with nine folds used to fit the model across a range of lambda values and one fold to assess the area under the receiver operator curve (AUROC, 10 fold cross validation). The features that appeared in the model with the greatest AUROC and the odds ratio (OR) for each feature were recorded. Features were ranked based on frequency they appeared in the model with the greatest AUROC across 50,000 models (5,000 repeats with 10-fold cross validation). Altering the tuning parameter to 0.25 or 1 did not change the features that were most frequently selected. To determine a minimum set of variables that could be linearly combined by Partial Least Squares Regression (PLSR) to explain the variation in cerebral and uncomplicated malaria, we added one feature at a time to a PLSR model, in order of frequency that they appeared in the ENLR. We performed 500 repeats of 10-fold cross validation to estimate the AUROC after the addition of each feature and selected the top features whose addition resulted in a significant increase in the AUROC. We assessed the performance of the linear combination of selected features to classify samples as cerebral and uncomplicated malaria by fitting the selected features to a PLSR model and computing the AUROC (as above estimation was from 500 repeats of 10-fold cross validation). The PLSR performance was also assessed with randomly shuffled outcome variables and random feature selection.

Results:

1. Malawian children cohort

The final cohort included 51 children with cerebral malaria (median age of 51 months) and 46 with uncomplicated malaria (median age of 48 months). Children with cerebral malaria had a Blantyre Coma Score of 0-2 and 42 (82%) were retinopathy positive [27] (Table 1).

Table 1. Summary of study population categorized by disease severity.

Characteristic	Uncomplicated Malaria (n=46)	Cerebral Malaria (n=51)
Age, median [IQR], months	48 [29 - 88]	51 [28 – 83]
Age group, n %[a]		
0 – 48 months	24 (52%)	25 (49%)
49+ months	22 (48%)	26 (51%)
Sex, n (%)		
Female	19 (41%)	16 (31%)
Male	27 (59%)	35 (69%)
Location, n (%)		
Urban	25 (54%)	25 (49%)
Rural	21 (46%)	26 (51%)
BCS, n (%)		
0	0 (0%)	5 (10%)
1	0 (0%)	15 (29%)
2	0 (0%)	31 (61%)
5	46 (100%)	0 (0%)
Retinopathy positive, n (%)	0	42 (82%)
Severe anaemia, n (%)	0	11 (23%)
Temperature, median [IQR], degrees Celsius	38.6 [37.8- 39.3]	39 [38.5-39.4]
Parasitaemia, per μL		4,599 [396 – 58,880]
Haemoglobin, median [IQR], g/dL	10.4 [9.0-11.7]	8.2 [7.2-9.2]

2. **Univariate analysis of differences in antibody responses in cerebral and uncomplicated malaria**

We measured antibody responses to 39 recombinant PfEMP1 domains that have been associated with severe malaria, cerebral malaria or uncomplicated malaria. We also included 5 recombinant merozoite and sporozoite antigens, an antigen free negative control and tetanus toxoid as a positive control. We measured 11 antigen-specific antibody features: IgG, IgM, IgG subclasses IgG1, IgG2, IgG3 and IgG4, and antibody Fc binding to recombinant FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb and C1q. The final analysis included a total of 451 features, 413 of which were related to PfEMP1 antigens and 38 of which were related to merozoite or sporozoite antigens.

We first used a volcano plot to display univariate analyses comparing antibody responses for each PfEMP1 protein and antibody feature between children with cerebral and uncomplicated malaria. IgG to one PfEMP1 domain, SM9_DBLδ1, was significantly elevated by greater than two-fold in cerebral malaria compared to uncomplicated malaria (Figure 1). IgG, IgG1 and FcγRIIIb responses to the merozoite antigen, MSP2, were significantly higher in cerebral than uncomplicated malaria and there were no other differences for other non-PfEMP1 antigens. Antibody features that were significantly elevated in uncomplicated malaria were IgG2 targeting SM4_DBLβ, IgG4 targeting SM5_DBLβ, IgG4 targeting Dd2VAR32_DBLβ1, FcγRIIa binding antibodies targeting SM5_DBLβ and SM3_DBLβ12, FcγRIIIb binding antibodies targeting PFD1235w_DBLβ, SM26_CIDRγ12, and SM5_DBLβ and C1q fixing antibodies targeting IT4VAR13_DBLβ and PF11_0521_DBLβ (Figure 1).

Given age is an important determinant of PfEMP1 antibody [28], we divided the children into those older or younger than the median age of 49 months. Amongst children under 49 months old, IgG targeting SM9_DBLδ1 remained significantly greater in cerebral malaria, and C1q response to IT4VAR13_DBLβ3 remained significantly greater in uncomplicated malaria. C1q response to SM3_DBLβ12 was also significantly greater in uncomplicated malaria (Figure 2A). In children over 49 months, IgG1 to SM1_CIDRα2.4 was significantly greater in cerebral malaria compared to uncomplicated malaria, although it was not significantly elevated in all children combined. Features that were significantly higher in uncomplicated malaria were IgG4 to Dd2VAR32_DBLb1 and SM5_DBLb3, FcγRIIIb binding antibodies targeting PFD1235w_DBLβ, and FcγRIIa binding antibodies targeting UM8_DBLg9 (Figure 2B).The last of these was not significantly increased in the whole group in Figure 1.

Due to the importance of the expression of DBLβ domains in cerebral malaria, we grouped each individual's responses to DBLβ domains based on binding phenotype: Group A DBLβ that bind ICAM-1 with the DBLβ$_{motif}$ ICAM-1-binding motif, Group A DBLβ that do not bind ICAM-1 and Group B DBLβ that bind ICAM-1, but do not contain DBL$_{motif}$ (Supplementary file 1, Supplementary file 2). Antibodies that engage FcγRIIIb and target Group A and Group B ICAM1-binding DBLβ domains were higher in children with uncomplicated malaria compared to cerebral malaria (Welch's t-test p-value 0.032 and 0.042, respectively) whereas antibodies targeting group A non-ICAM1 binding DBLβ domains did not differ significantly between the two clinical groups. We also grouped responses to domains that were upregulated in severe or uncomplicated malaria in a previous study in Indonesian adults [10], labelled as "SM" or "UM" (Supplementary file 2). Here, antibodies that engage FcγRIIb and target SM proteins were higher in cerebral malaria compared to uncomplicated malaria (p-value 0.004), and IgM antibodies that target UM proteins were higher in cerebral malaria compared to uncomplicated malaria (p-value 0.0092). There were no significant differences between cerebral malaria and uncomplicated malaria in grouped responses to Group A or non-Group A domains, or to domains association with DC8 (Supplementary file 2). All individual comparisons between cerebral and uncomplicated malaria for each protein and feature are included in Supplementary File 3.

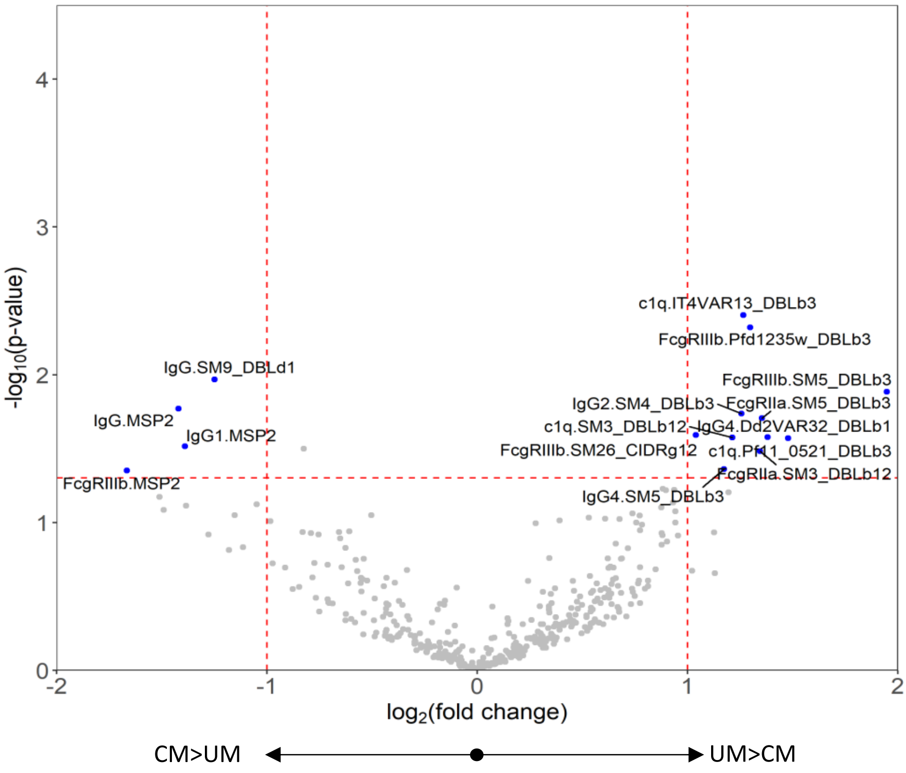


Figure 1. Individual antibody features to recombinant proteins compared between cerebral and uncomplicated malaria for all individuals. X-axis represents the magnitude of difference (log2 transformed) between the geometric mean antibody levels of the cerebral and uncomplicated malaria groups. Vertical lines at log2(2) and log2(0.50) indicate a two-fold elevation in uncomplicated malaria or cerebral malaria, respectively. Y-axis represents -log10 transformed p-value from Welch's t-test comparison of cerebral and uncomplicated malaria. Horizontal line indicates log10(0.05) threshold of statistical significance.

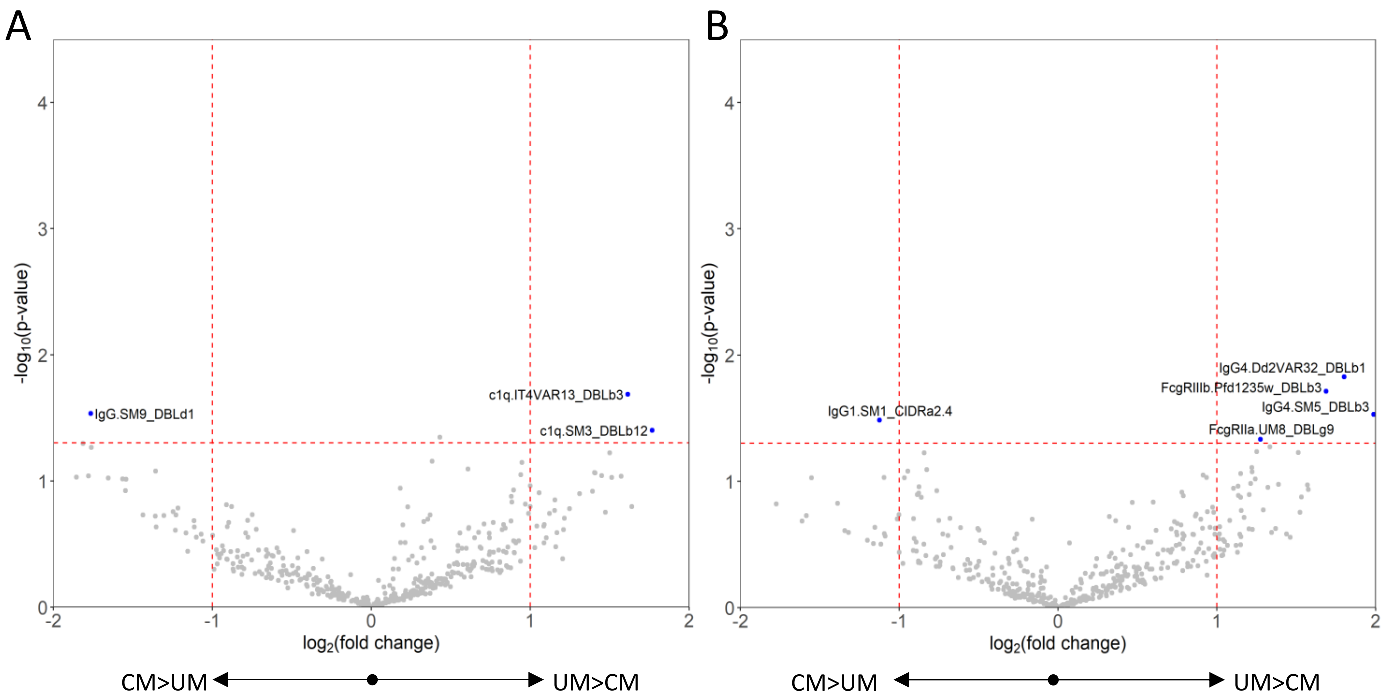


Figure 2. Individual antibody features to recombinant proteins compared between cerebral and uncomplicated malaria for **A)** children under 49 months, **B)** children 49 months and older. X-axis represents the magnitude of difference (log2 transformed) between the geometric mean antibody levels of the cerebral and uncomplicated malaria groups. Vertical lines at log2(2) and log2(0.50) indicate a two-fold elevation in uncomplicated malaria or cerebral malaria, respectively. Y-axis represents -log10 transformed p-value from Welch's t-test comparison of cerebral and uncomplicated malaria. Horizontal line indicates log10(0.05) threshold of statistical significance.

3. Multivariate analysis to select the combination of features that best discriminate between cerebral malaria and uncomplicated malaria.

To identify the antibody features that best differentiate between cerebral and uncomplicated malaria we performed repeated elastic net-regularised logistic regression (ENLR) [26] with 10-fold cross validation and recorded the frequency and odds ratio (OR) of features selected in each model iteration, as described in Gunn *et al.* and Aitken, Ortega, Damelang *et al.* [16, 29]. Area under the ROC (AUROC) was used as the metric of discrimination power. The 20 most frequently selected features included both features associated with an increased odds of cerebral malaria and of uncomplicated malaria. Six features were selected in 100% of model iterations (Figure 3). To select a minimum combination of features that best discriminate between cerebral and uncomplicated malaria we added features one at a time to a PLS regression model, in order of the frequency of selection based on the effect size of the odds ratio from the ENLR (Figure 3) and assessed the AUROC (Figure 4A). After the seventh variable was included in the model, the AUROC did not increase by adding further variables. The top seven most frequently selected features were C1q fixing antibodies targeting IT4VAR13_DBLβ3, IgG1 targeting UM2_DBLδ1, IgG targeting SM9_DBLδ1, IgG2 targeting SM4_DBLβ3, IgG4 targeting Dd2VAR32 DBLβ1, and antibodies targeting SM26_CIDRγ12 or SM5_DBLβ3 that engage FcγRIIIb (Figure 4A). The univariate analyses for these features are shown in Figure 5.

To assess the predictive power of our seven selected features, we compared their ability to correctly classify children to that of seven randomly selected features. Based on the AUROC, the seven selected features could predict clinical presentation with 87% accuracy whereas seven randomly selected features could predict clinical presentation with 50% accuracy, equivalent to chance alone (Figure 4B). The seven selected features did not generally correlate with each other or with the 20 features that frequently appeared in the ENLR model iterations (Figure 6). There were moderate correlations between DBLδ1 domains (IgG.UM2_DBLδ1, IgG_SM9_DBLδ1, IgG.SM6_DBLδ1; Spearman correlation coefficient R = 0.39-0.60), strong correlations between FcγR binding to antibodies targeting group A DBLβ domains (FcγRIIa.SM5_DBLβ3, FcγRIIIb.PFD1235w_DBLβ3, FcγRIIb.SM5_DBLβ3, FcγRIIIb.PFD1235w_DBLβ3, R = 0.63-0.72), and a moderate correlation between IgG4 responses to group A DBLβ domains (IgG4.Dd2VAR32_DBLβ1 with IgG4.SM5_DBLβ3, R = 0.57).

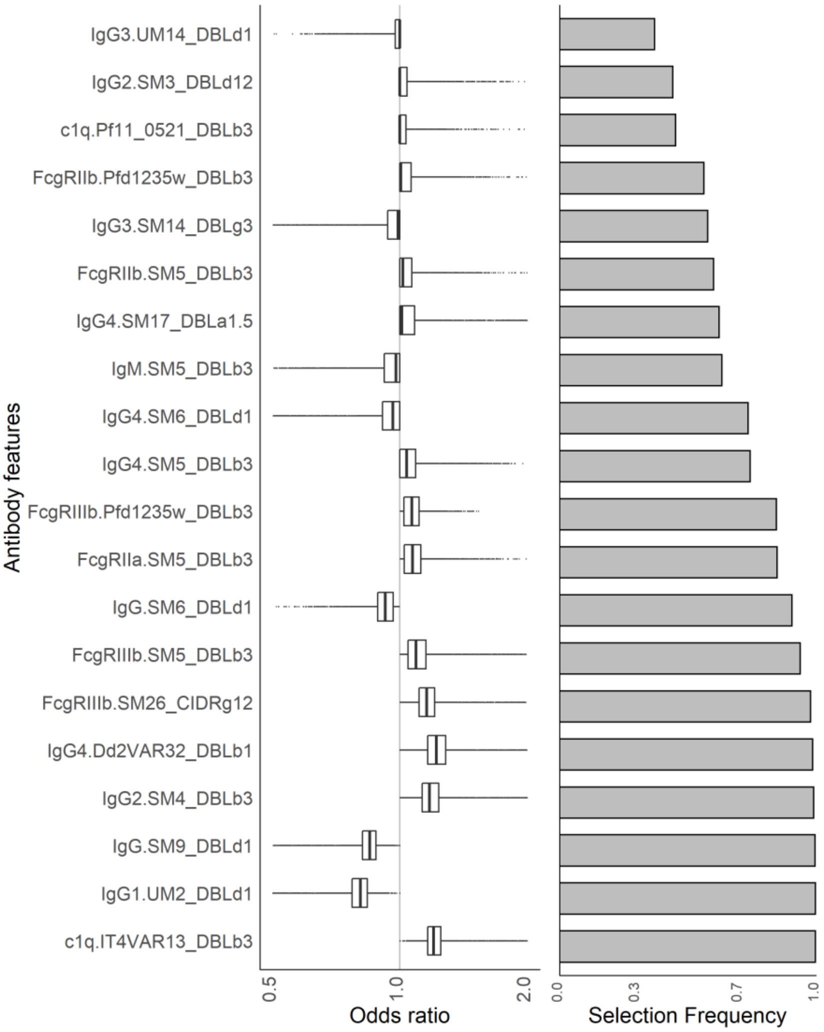


Figure 3. Feature selection by Elastic Net-regularised Logistic Regression (ENLR). Odds ratio of antibody features from 5,000 repeats of 10-fold cross validated ENET regression models, in order of selection frequency (top 20 most selected variables are shown). Features with median odds ratio greater than 1 represents responses associated with increased odds of uncomplicated malaria and features with median odds ratio less than 1 are associated with increased odds of cerebral malaria. Box plots show median and interquartile range (IQR) and whiskers show points within Q1-1.5*IQR and Q3+1.5*IQR

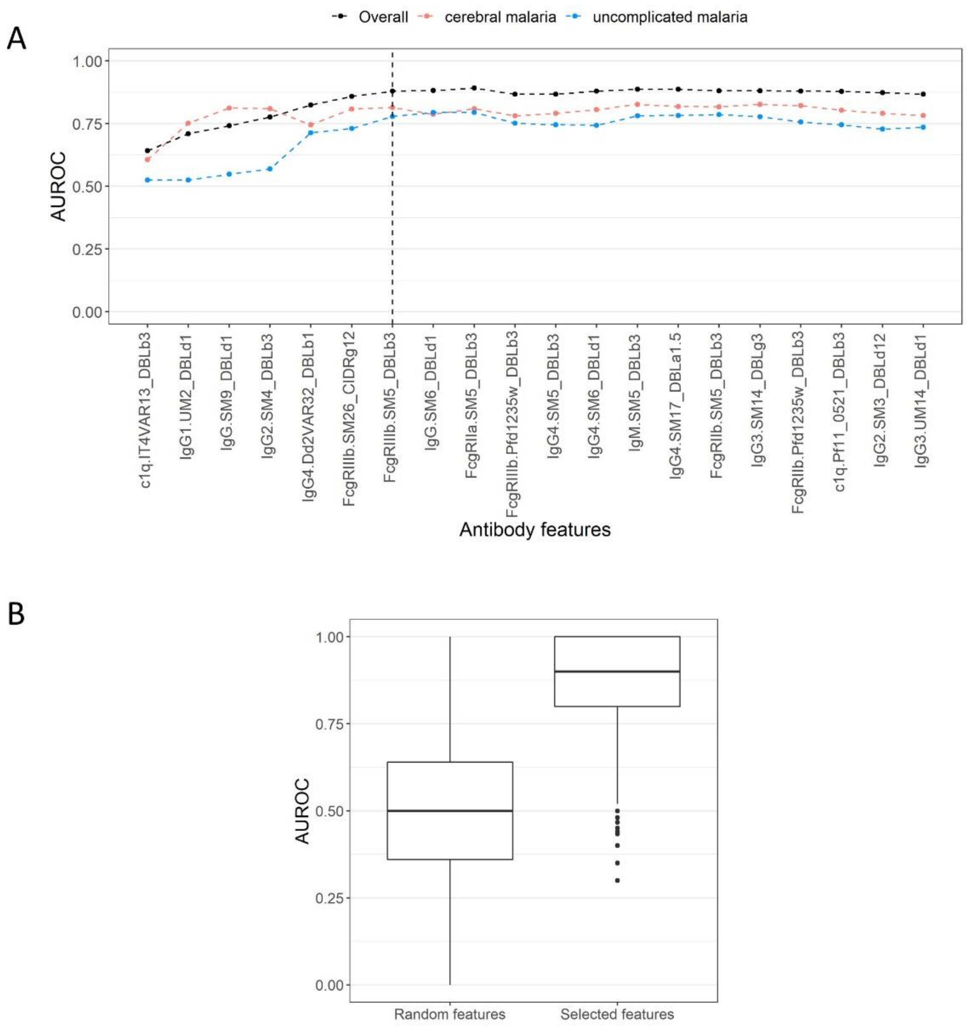


Figure 4. Partial Least Squares (PLS) Regression to select a minimum combination of features that best distinguishes between cerebral and uncomplicated malaria. A) Performance of PLS model after addition of features in order of ENLR selection frequency (left to right), as measured by AUROC from 5,000 repeats of 10-fold cross validated PLS regression. Black line shows AUROC for all children, red line shows the accuracy of classifying children with cerebral malaria only and blue line the accuracy of classifying children with uncomplicated malaria only. Vertical dashed line represents point at which the addition of one or two more features does not significantly increase the AUROC which occurs at seven features (referred to as the 'selected features'). **B)** Performance of PLS regression models using only the seven selected features from ENLR, compared to randomly selected combinations of seven features. AUROCs correspond to 5,000 repeats of 10-fold cross validated PLS regression models. AUROC = Area Under the Receiver Operating characteristic Curve. Box plots show median and interquartile range (IQR) and whiskers show points within Q1-1.5*IQR and Q3+1.5*IQR

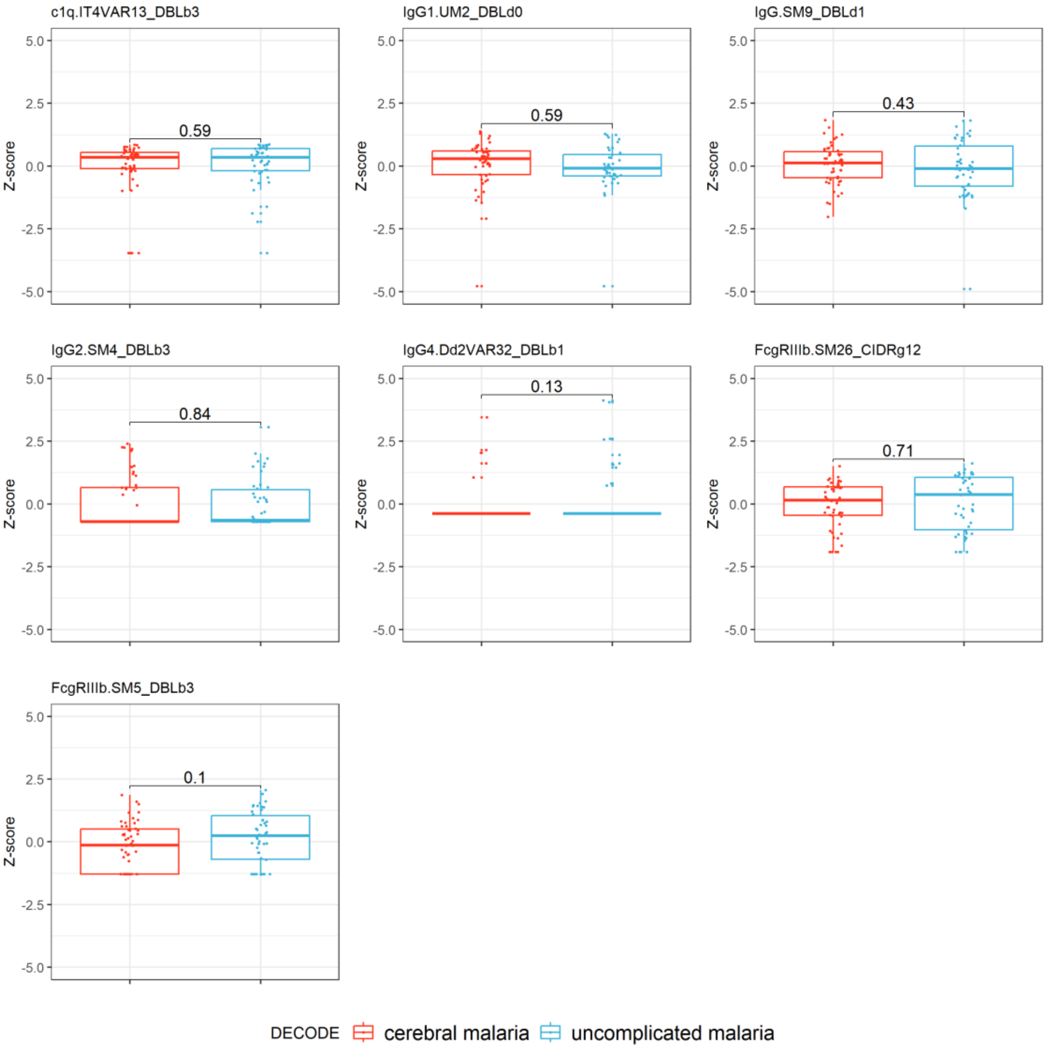
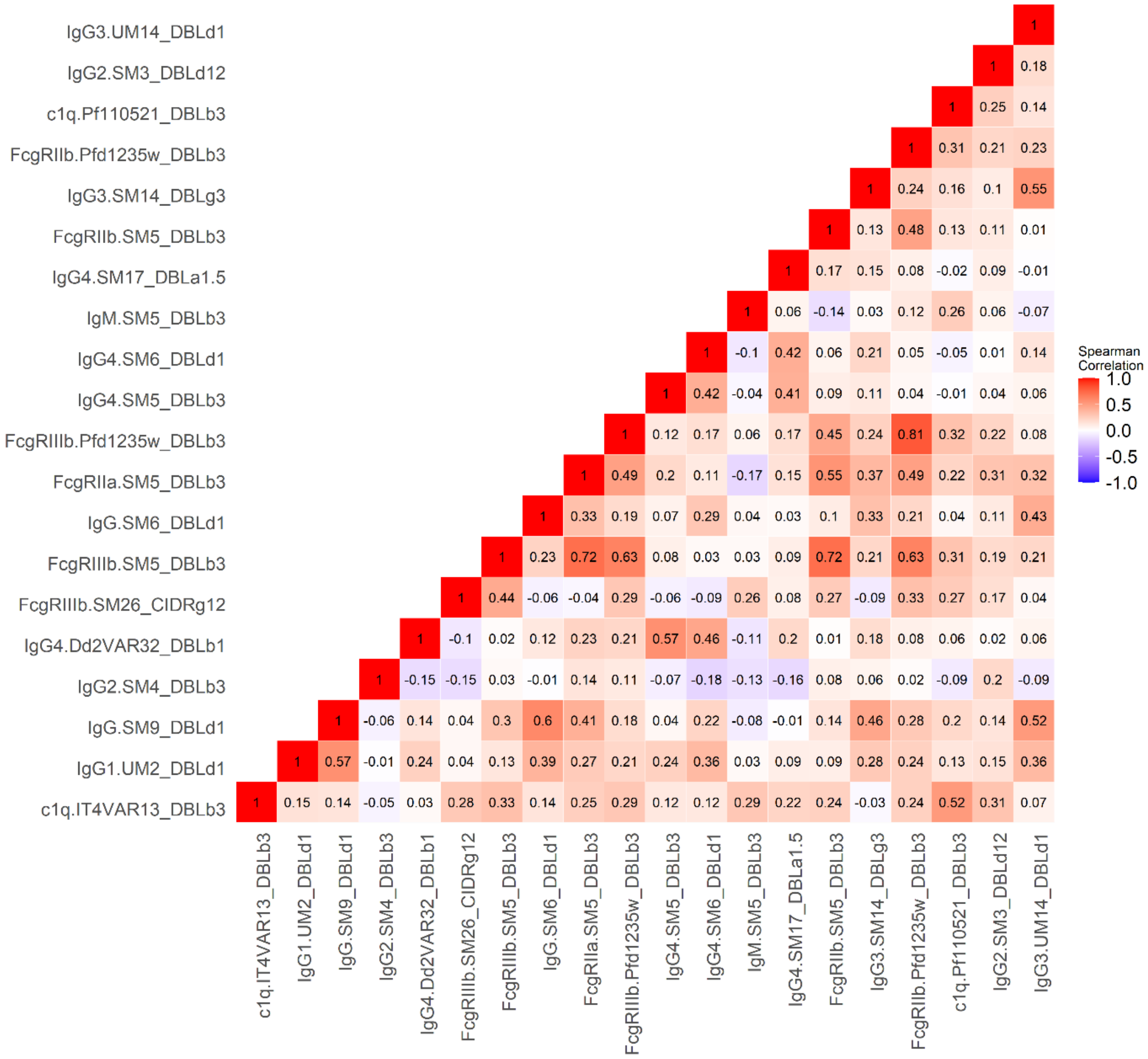


Figure 5. Distribution of seven selected features in children with cerebral malaria (red) and uncomplicated malaria (blue). MFI readouts were log(x+1) transformed, mean centered and scaled to 1 standard deviation (z-score). Box plots show median and interquartile range (IQR) and whiskers show IQR+1.5*IQR. Horizontal bars represent Welch's t-test comparison with p-value shown.



266

Figure 6. Spearman correlation of features that appeared in >70% of ENLR model iterations, including the seven selected features, using non-transformed MFI values.

Discussion:

PfEMP1 plays a key role in the pathogenesis of cerebral malaria, however previous studies have been unable to identify a single PfEMP1 antigen to which IgG antibodies are consistently associated with protection. In this study, we performed a detailed characterization of the targets and Fc features of antibodies to PfEMP1 antigens in Malawian children with cerebral malaria and uncomplicated malaria. We used a previously published statistical approach that combines logistic regression and machine learning [16, 29] as an unbiased method to select a combination of features that could best differentiate antibody responses of children with cerebral and uncomplicated malaria. We found few differences in IgG but identified a combination of seven antigen-specific antibody Fc features that could differentiate between the groups with 87% accuracy, whereas randomly selected antibody features could not predict clinical presentation. This supports the idea that specific PfEMP1 variants are involved in the pathogenesis of cerebral malaria and that specific features of the Fc domains of antibodies to these variants may facilitate their clearance and prevent the development of cerebral malaria.

Four of the seven selected antibody features were targeting DBLβ domains and were associated with decreased odds of cerebral malaria. Upregulated expression of group A $\text{DBL}\beta_{\text{motif}}$ that contain the ICAM-1-binding motif, predictive of dual ICAM-1 and EPCR receptor binding IEs, has been clearly linked to cerebral malaria [5–8], but the role of antibodies targeting these domains in protection from cerebral malaria is less clear. Antibodies targeting Group A ICAM-1 binding DBLβ domains can block cytoadhesion to human brain endothelial cells and have been associated with protection from cerebral malaria in some studies [18, 31] but not in others [32, 33]. An advantage of this study is that we were able to probe multiple examples of DBLβ domains, as well as multiple antibody Fc features to identify correlates of protection. The selected combination of features included a Group A ICAM-1 binding domain with the $\text{DBL}\beta_{\text{motif}}$, as well as two Group A non-ICAM-1 binding domains that lack the motif and a Group B domain that binds to ICAM-1 but lacks the motif. These responses are likely influenced by antibody cross reactivity between DBLβ domains [18] and may reflect current infection with Group B and Group A non-ICAM-1 binding variants in children with uncomplicated malaria, as described in Olsen *et al.* [34].

Other PfEMP1 targets amongst the selected combination of features included two DBLδ1 domains and a CIDRγ12 domain. DBLδ1 is one of the most diverse domains [35] and there is limited data on its functions, which may be equally diverse [36, 37]. In contrast to our study, antibodies to Group B and C DBLδ1 have previously been associated with protection from severe malaria (for UM2_DBLδ1) (13) and reduced prospective risk of hyperparasitaemia (25). In our study, antibody responses to the DBLδ1 domains (UM2 and SM9) were highly correlated and therefore it is difficult to discern whether the antibody response in children with cerebral malaria is driven by proteins more commonly expressed in severe malaria (SM9_DBLδ1) or uncomplicated malaria (UM2_DBLδ1). Similarly, little is known about the function of SM26_CIDRγ12, other than that CIDRγ are part of the head structure of a rosetting parasite line [38]. Antibodies to SM26_CIDRγ12 were elevated in uncomplicated malaria compared to severe malaria in Indonesian adults [10] and may be a novel target of protective antibodies to cerebral malaria.

The selected combination of seven antibody features that could best distinguish between cerebral and uncomplicated malaria included antigen specific antibody engagement with C1q and FcγRIIIb responses and IgG2 and IgG4 responses. Recent work from our group has shown that complement deposition leads to antibody mediated lysis of VAR2CSA IE at high concentrations of antibodies (unpublished). However, another recent study found that complement component 1s (C1s) cleaves PfEMP1 at sites found in interdomain regions, including in IT4VAR13 [39]. Additionally, C1q deposition relies on formation of antibody hexamers and the distribution of PfEMP1 and antibodies on the bead based multiplex platform are unlikely to accurately represent the surface of the IE [40]. Antibodies to malaria sporozoites that fix complement can inhibit their motility and invasion [41], and complement fixation can enhance antibody mediated phagocytosis [42]. Therefore, the relevance of C1q deposition at the DBLβ3 domain to downstream effects on the IE requires further study. FcγRIIIb is highly expressed on neutrophils and can work in concert with FcγRIIa to induce antibody dependent neutrophil phagocytosis (ADNP) [43] and release of reactive oxygen species [44]. ADNP of VAR2CSA expressing IE was identified as a key correlate of protection against placental malaria using a systems serology approach [16] and antibodies enhance neutrophil clearance of ICAM-1 binding IE [13]. Future studies should determine whether neutrophil phagocytosis of IE expressing PfEMP1 with CIDRγ12 or DBLβ3 domains identified in this study is associated with protection from cerebral malaria. The detection of IgG2 and IgG4 specific antibodies as correlates of protection was unexpected. Antibody responses to recombinant Group A and Group B ICAM-1 binders are dominated by the cytophilic subclasses, IgG1 and IgG3 [45], and most children we studied had low or undetectable IgG2 and IgG4. IgG2 and IgG4 could play roles in blocking adhesion of IEs. Alternatively, their selection may reflect greater class switch recombination associated with a more mature antibody response [30] as indicated by their selection in older rather than younger children.

Systems serology is based on the idea that 'neutralizing' antibody titers do not capture the complexity of the immune response. In the context of malaria, systems serology has been applied to characterize functional antibody responses to Rh5 vaccination [48], RTS,S/AS01 vaccination [49], and placental malaria

[16]. Like those studies, we observed that total IgG may not be an accurate predictor of protection from malaria. This finding has been mirrored in a recent study of immunity to IE in pregnant women, in which functional antibodies that promoted phagocytosis by monocytes or neutrophils were better at differentiating between women who were protected from placental malaria or who were susceptible, compared to total IgG levels towards recombinant VAR2CSA proteins or the VAR2CSA-expressing placental binding parasite [16]. Both the present study and the placental malaria study indicate that a broad range of antibody responses contribute to protection from malaria, findings that have implications for the development of new tools to prevent severe malaria syndromes such as cerebral malaria. Similarly, IgG titer against DBLβ domains did not differ in Beninese children with severe or uncomplicated malaria, but opsonic phagocytosis by THP-1 cells induced by a DBLβ3 (Group B, IT4VAR13) was elevated in uncomplicated malaria [45]. Our study supports these findings to suggest that rather than the quantity of antibodies targeting PfEMP1, the Fc features and downstream functional activity of antibodies may be more useful correlates of protection.

Strengths of the study include well-characterised children with cerebral or uncomplicated malaria who were matched for location, and assessment of a broad array of responses to PfEMP1 antigens, including protein products of genes know to be expressed in severe or uncomplicated malaria, and PfEMP1 types known to bind to key endothelial receptors. As we have captured a single time point in single infections, interpretation requires caution. The dynamics of the antibody response do not necessarily align with those of the disease and the observed responses are likely to be influenced by the timing of sampling. We expect there is variation in the duration of infection prior to presentation to hospital, which we partially accounted for by matching for residency location. However, we were unable to account for the possibility that children with uncomplicated malaria may have progressed to severe malaria without timely treatment. Additionally, the antibody responses associated with protection from a single malaria episode do not necessarily represent complete or ongoing protection. Further limitations of this study include the use of recombinant proteins that may not reflect the native protein structure or capture epitopes that span multiple domains, and a relatively small sample size. Stratifying our data by participant age may have limited our power to find PfEMP1 antibody responses that are age dependent. Twenty-eight of the 39 PfEMP1 antigens probed in this study were identified in Papuan adults with severe malaria, and it is possible that these domains may be more relevant to manifestations of severe malaria other than cerebral malaria or may be more relevant in adults compared to children. Validation in other sample sets will be important. Expression of PfEMP1 with EPCR binding CIDRα1 domains has been associated with severe malaria in several studies, however only one CIDRα1 domain (CIDRα1.6) was well recognized in this population and other variants may be more relevant. It also possible that including multiple examples of some domains may have increased the probability of them appearing in the selected features.

We were unable to validate our findings using whole IE expressing the PfEMP1 of interest due to difficulty maintaining parasites that express a uniform and persistent PfEMP1 variant. Future studies should consider development of mutant parasite lines with consistent PfEMP1 expression. The systems serology approach may be used to characterize geographical and age dependent differences in the antibody response to PfEMP1 variants, as well as temporal associations with protection. The majority of PfEMP1 antigens were recognized by less than 25% of children with cerebral or uncomplicated malaria, indicating that children in both clinical groups had many gaps in their antibody repertoire that potentially leave them vulnerable to cerebral malaria in the follow up period. Longitudinal studies have reported that individuals with antibodies targeting DBLβ domains with the DBLβ_{motif} have a reduced prospective risk of uncomplicated malaria [9] and high density parasitaemia [50] and similar studies to determine the risk of cerebral malaria in the follow up period would be informative. Characterizing the antibody response to PfEMP1 may lead to the development of a multivalent PfEMP1 based vaccine or monoclonal antibody cocktail to protect from cerebral malaria, or to the development of more sensitive disease surveillance measures.

Conflict of interest: No authors have any conflict of interest to declare.

Acknowledgements

This work was supported by the National Health and Medical Research Council of Australia (NHMRC) through grants GNT1061993 and GNT1092789 to SJR. JAS and AWC are supported by NHMRC Investigator Awards 1196068 (JAS) and 2008092 (AWC). ISW was supported by a University of Melbourne Research Training Program Scholarship and Rowden White Scholarship. Please advise of other funding or acknowledgements We thank Bruce Wines and Mark Hogarth, Burnet Institute, for the kind provision of Fc dimers. We thank Ricardo Ataíde and Jack Richards for the kind provision of the EBA175-RIII-V protein.

We thank the staff of the MLW Clinical Research Programme and Blantyre Malaria Project in Malawi for assistance in recruiting and caring for study participants, and the children and their families who donated the samples used in this work.

References

1. World Health Organization (2021) Word Malaria Report 2021. https://www.who.int/publications/i/item/9789240040496

2. Taylor TE, Fu WJ, Carr RA, et al (2004) Differentiating the pathologies of cerebral malaria by postmortem parasite counts. Nat Med 10:143–145. https://doi.org/10.1038/nm986

3. Rask TS, Hansen DA, Theander TG, et al (2010) Plasmodium falciparum erythrocyte membrane protein 1 diversity in seven genomes - divide and conquer. PLoS Comput Biol 6:e1000933. https://doi.org/10.1371/journal.pcbi.1000933

4. Lavstsen T, Gilbert MTP, Willerslev E, et al (2012) Plasmodium falciparum erythrocyte membrane protein 1 domain cassettes 8 and 13 are associated with severe malaria in children. Proc Natl Acad Sci 109:e1791–e1800. https://doi.org/10.1073/pnas.1120455109

5. Kessler A, Dankwa S, Bernabeu M, et al (2017) Linking EPCR-Binding PfEMP1 to Brain Swelling in Pediatric Cerebral Malaria. Cell Host Microbe 22:601-614.e5. https://doi.org/10.1016/j.chom.2017.09.009

6. Lennartz F, Adams Y, Bengtsson A, et al (2017) Structure-Guided Identification of a Family of Dual Receptor-Binding PfEMP1 that Is Associated with Cerebral Malaria. Cell Host Microbe 21:403–414. https://doi.org/10.1016/j.chom.2017.02.009

7. Tuikue Ndam N, Moussiliou A, Lavstsen T, et al (2017) Parasites Causing Cerebral Falciparum Malaria Bind Multiple Endothelial Receptors and Express EPCR and ICAM-1-Binding PfEMP1. J Infect Dis 215:1918–1925. https://doi.org/10.1093/infdis/jix230

8. Adams Y, Olsen RW, Bengtsson A, et al (2021) Plasmodium falciparum erythrocyte membrane protein 1 variants induce cell swelling and disrupt the blood–brain barrier in cerebral malaria. J Exp Med 218:e20201266. https://doi.org/10.1084/jem.20201266

9. Kanoi BN, Nagaoka H, Morita M, et al (2018) Comprehensive analysis of antibody responses to Plasmodium falciparum erythrocyte membrane protein 1 domains. Vaccine 36:6826–6833. https://doi.org/10.1016/j.vaccine.2018.08.058

10. Rambhatla JS, Tonkin-hill GQ, Takashima E, et al (2022) Identifying Targets of Protective Antibodies against Severe Malaria in Papua, Indonesia, Using Locally Expressed Domains. Infect Immun 90:e0043521. https://doi.org/10.1128/iai.00435-21

11. Travassos MA, Niangaly A, Bailey JA, et al (2018) Children with cerebral malaria or severe malarial anaemia lack immunity to distinct variant surface antigen subsets. Sci Rep 8:6281. https://doi.org/10.1038/s41598-018-24462-4

12. Chan J-A, Boyle MJ, Moore KA, et al (2019) Antibody Targets on the Surface of Plasmodium falciparum-Infected Erythrocytes That Are Associated With Immunity to Severe Malaria in Young Children. J Infect Dis 219:819–828. https://doi.org/10.1093/infdis/jiy580

13. Zelter T, Strahilevitz J, Simantov K, et al (2022) Neutrophils impose strong immune pressure against PfEMP1 variants implicated in cerebral malaria. EMBO Rep e53641. https://doi.org/10.15252/embr.202153641

14. Arora G, Hart GT, Manzella-Lapeira J, et al (2018) NK cells inhibit Plasmodium falciparum growth

in red blood cells via antibody-dependent cellular cytotoxicity. Elife 7:1–20. https://doi.org/10.7554/elife.36806

15. Arnold KB, Chung AW (2018) Prospects from systems serology research. Immunology 153:279–289. https://doi.org/10.1111/imm.12861

16. Aitken EH, Damelang T, Ortega-Pajares A, et al (2021) Developing a multivariate prediction model of antibody features associated with protection of malaria-infected pregnant women from placental malaria. Elife 10:1–30. https://doi.org/10.7554/eLife.65776

17. Tonkin-Hill GQ, Trianty L, Noviyanti R, et al (2018) The Plasmodium falciparum transcriptome in severe malaria reveals altered expression of genes involved in important processes including surface antigen–encoding var genes. PLoS Biol 16:e2004328. https://doi.org/10.1371/journal.pbio.2004328

18. Olsen RW, Ecklu-Mensah G, Bengtsson A, et al (2018) Natural and Vaccine-Induced Acquisition of Cross-Reactive IgG-Inhibiting ICAM-1-Specific Binding of a Plasmodium falciparum PfEMP1 Subtype Associated Specifically with Cerebral Malaria. Infect Immun 86:1–17. https://doi.org/10.1128/IAI.00622-17

19. Harawa V, Njie M, Kessler A, et al (2018) Brain swelling is independent of peripheral plasma cytokine levels in Malawian children with cerebral malaria. Malar J 17:435. https://doi.org/10.1186/s12936-018-2590-0

20. Molyneux ME, Taylor TE, Wirimaf JJ, Borgsteinj A (1989) Clinical Features and Prognostic Indicators in Paediatric Cerebral Malaria A Study of 131 Comatose Malawian Children. Q J Med 71:441–459

21. Olsen RW, Ecklu-Mensah G, Bengtsson A, et al (2019) Acquisition of IGG to ICAM-1-Binding DBLB Domains in the plasmodium falciparum erythrocyte membrane protein 1 antigen family varies between groups A, B, and C. Infect Immun 87:. https://doi.org/10.1128/IAI.00224-19

22. Bengtsson A, Joergensen L, Rask TS, et al (2013) A Novel Domain Cassette Identifies Plasmodium falciparum PfEMP1 Proteins Binding ICAM-1 and Is a Target of Cross-Reactive, Adhesion-Inhibitory Antibodies. J Immunol 190:240–249. https://doi.org/10.4049/jimmunol.1202578

23. Walker IS, Chung AW, Damelang T, Rogerson SJ (2022) Analysis of Antibody Reactivity to Malaria Antigens by Microsphere-Based Multiplex Immunoassay. Methods Mol Biol 2470:309–325. https://doi.org/10.1007/978-1-0716-2189-9_23

24. Wines BD, Vanderven HA, Esparon SE, et al (2016) Dimeric FcγR Ectodomains as Probes of the Fc Receptor Function of Anti-Influenza Virus IgG. J Immunol 197:1507–16. https://doi.org/10.4049/jimmunol.1502551

25. van Buuren S, Groothuis-Oudshoorn K (2011) mice: Multivariate Imputation by Chained Equations in R. J Stat Softw 45:1–67. https://doi.org/10.18637/jss.v045.i03

26. Zou H, Hastie T (2005) Regularization and variable selection via the elastic net. J R Stat Soc Ser B (Statistical Methodol 67:301–320. https://doi.org/https://doi.org/10.1111/j.1467-9868.2005.00503.x

27. Beare NA V, Taylor TE, Harding SP, et al (2006) Malarial retinopathy: a newly established diagnostic sign in severe malaria. Am J Trop Med Hyg 75:790–7

28. Barry AE, Trieu A, Fowkes FJI, et al (2011) The stability and complexity of antibody responses to the major surface antigen of Plasmodium falciparum are associated with age in a malaria endemic area. Mol Cell Proteomics 10:M111.008326. https://doi.org/10.1074/mcp.M111.008326

29. Gunn BM, Yu WH, Karim MM, et al (2018) A Role for Fc Function in Therapeutic Monoclonal Antibody-Mediated Protection against Ebola Virus. Cell Host Microbe 24:221-233.e5. https://doi.org/10.1016/j.chom.2018.07.009

30. Allen CDC (2022) Features of B Cell Responses Relevant to Allergic Disease. J Immunol 208:257–266. https://doi.org/10.4049/jimmunol.2100988

31. Lusingu JPA, Jensen ATR, Vestergaard LS, et al (2006) Levels of plasma immunoglobulin G with specificity against the cysteine-rich interdomain regions of a semiconserved Plasmodium falciparum erythrocyte membrane protein 1, VAR4, predict protection against malarial anemia and febrile episodes. Infect Immun 74:2867–75. https://doi.org/10.1128/IAI.74.5.2867-2875.2006

32. Kessler A, Campo JJ, Harawa V, et al (2018) Convalescent Plasmodium falciparum-specific seroreactivity does not correlate with paediatric malaria severity or Plasmodium antigen exposure. Malar J 17:178. https://doi.org/10.1186/s12936-018-2323-4

33. Badaut C, Visitdesotrakul P, Chabry A, et al (2021) IgG acquisition against PfEMP1 PF11_0521 domain cassette DC13, DBLβ3_D4 domain, and peptides located within these constructs in children with cerebral malaria. Sci Rep 11:1–12. https://doi.org/10.1038/s41598-021-82444-5

34. Olsen RW, Ecklu-Mensah G, Bengtsson A, et al (2019) Acquisition of IGG to ICAM-1-Binding DBLB Domains in the plasmodium falciparum erythrocyte membrane protein 1 antigen family varies between groups A, B, and C. Infect Immun 87:. https://doi.org/10.1128/IAI.00224-19

35. Otto TD, Assefa SA, Böhme U, et al (2019) Evolutionary analysis of the most polymorphic gene family in falciparum malaria. Wellcome open Res 4:193. https://doi.org/10.12688/wellcomeopenres.15590.1

36. Chesnokov O, Merritt J, Tcherniuk SO, et al (2018) Plasmodium falciparum infected erythrocytes can bind to host receptors integrins αVβ3 and αVβ6 through DBLδ1_D4 domain of PFL2665c PfEMP1 protein. Sci Rep 8:17871. https://doi.org/10.1038/s41598-018-36071-2

37. Gillrie MR, Renaux B, Russell-Goldman E, et al (2016) Thrombin Cleavage of Plasmodium falciparum Erythrocyte Membrane Protein 1 Inhibits Cytoadherence. MBio 7:. https://doi.org/10.1128/mBio.01120-16

38. Ghumra A, Khunrae P, Ataide R, et al (2011) Immunisation with recombinant PfEMP1 domains elicits functional rosette-inhibiting and phagocytosis-inducing antibodies to Plasmodium falciparum. PLoS One 6:e16414. https://doi.org/10.1371/journal.pone.0016414

39. Azasi Y, Low LM, Just AN, et al (2021) Complement C1s cleaves PfEMP1 at interdomain conserved sites inhibiting Plasmodium falciparum cytoadherence. Proc Natl Acad Sci U S A 118:. https://doi.org/10.1073/pnas.2104166118

40. Larsen MD, del Pilar Quintana M, Ditlev SB, et al (2019) Evasion of classical complement pathway activation on Plasmodium falciparum-infected erythrocytes opsonized by PfEMP1-Specific IgG. Front Immunol 10:1–10. https://doi.org/10.3389/fimmu.2018.03088

41. Kurtovic L, Behet MC, Feng G, et al (2018) Human antibodies activate complement against Plasmodium falciparum sporozoites, and are associated with protection against malaria in children. BMC Med 16:61. https://doi.org/10.1186/s12916-018-1054-2

42. Opi DH, Kurtovic L, Chan J-A, et al (2021) Multi-functional antibody profiling for malaria vaccine development and evaluation. Expert Rev Vaccines 20:1257–1272. https://doi.org/10.1080/14760584.2021.1981864

43. Garcia-Senosiain A, Kana IH, Singh S, et al (2021) Neutrophils dominate in opsonic phagocytosis of P. falciparum blood-stage merozoites and protect against febrile malaria. Commun Biol 4:984. https://doi.org/10.1038/s42003-021-02511-5

44. Kapelski S, Klockenbring T, Fischer R, et al (2014) Assessment of the neutrophilic antibody-dependent respiratory burst (ADRB) response to Plasmodium falciparum. J Leukoc Biol 96:1131–42. https://doi.org/10.1189/jlb.4A0614-283RR

45. Suurbaar J, Moussiliou A, Tahar R, et al (2022) ICAM-1-binding Plasmodium falciparum erythrocyte membrane protein 1 variants elicits opsonic-phagocytosis IgG responses in Beninese children. Sci Rep 12:12994. https://doi.org/10.1038/s41598-022-16305-0

46. Chung AW, Alter G (2017) Systems serology: Profiling vaccine induced humoral immunity against HIV. Retrovirology 14:1–11. https://doi.org/10.1186/s12977-017-0380-3

47. Selva KJ, van de Sandt CE, Lemke MM, et al (2021) Systems serology detects functionally distinct coronavirus antibody features in children and elderly. Nat Commun 12:2037. https://doi.org/10.1038/s41467-021-22236-7

48. Minassian AM, Silk SE, Barrett JR, et al (2021) Reduced blood-stage malaria growth and immune correlates in humans following RH5 vaccination. Med 2:701-719.e19. https://doi.org/10.1016/j.medj.2021.03.014

49. Suscovich TJ, Fallon JK, Das J, et al (2020) Mapping functional humoral correlates of protection against malaria challenge following RTS,S/AS01 vaccination. Sci Transl Med 12:. https://doi.org/10.1126/scitranslmed.abb4757

50. Tessema SK, Utama D, Chesnokov O, et al (2018) Antibodies to Intercellular Adhesion Molecule 1-Binding Plasmodium falciparum Erythrocyte Membrane Protein 1-DBLβ Are Biomarkers of Protective Immunity to Malaria in a Cohort of Young Children from Papua New Guinea . Infect Immun 86:1–14. https://doi.org/10.1128/iai.00485-17

Supplementary Table 1. Recombinant PfEMP1 proteins used in multiplex immunoassay

Protein no.	Domain	Group	Associated DC/ gene	Predicted binding receptor	Expression system	Source
SM1	CIDRα2.4	B			WGCFS	*a*
SM2	CIDRβ1	B			WGCFS	*a*
SM3	DBLβ12	B/A	DC8		WGCFS	*a*
SM4	DBLβ3	A			WGCFS	*a*
SM5	DBLβ3	A	DC4		WGCFS	*a*
SM6	DBLδ1	B			WGCFS	*a*
SM8	DBLδ1	B			WGCFS	*a*
SM9	DBLδ1	B	DC8		WGCFS	*a*
SM11	DBLε3	B	DC11		WGCFS	*a*
SM12	DBLε9	B			WGCFS	*a*
SM14	DBLγ3	B	DC9?		WGCFS	*a*
SM15	DBLζ4	B	DC9?		WGCFS	*a*
SM17	DBLα1.5	A	DC4		WGCFS	*a*
SM18	CIDRα1.1	B/A	DC8	EPCR	WGCFS	*a*
SM19	CIDRα1.6	A	DC4	EPCR	WGCFS	*a*
SM22	DBLε5	*A*	*var*1		WGCFS	*a*
SM24	DBLζ3	B/A			WGCFS	*a*
SM25	DBLβ13	B			WGCFS	*a*
SM26	CIDRγ12	B			WGCFS	*a*
SM27	DBLδ7	A			WGCFS	*a*
SM28	CIDRα2.6DBLβ5	B			WGCFS	*a*
UM1	DBLα0.13	B			WGCFS	*a*
UM2	DBLδ1	B			WGCFS	*a*
UM8	DBLγ9	B/A			WGCFS	*a*
UM14	DBLδ1	B			WGCFS	*a*
UM19	DBLδ1	B			WGCFS	*a*

UM20	CIDRα3.1	B/C			WGCFS	*a*
UM45	CIDRα1.7	A			WGCFS	*a*
M1	DBLβ3*	A	PF11_0521_D4	ICAM-1	*Escherichia coli*	*b*
M6	DBLβ3*	A	3D7 Pfd1235w_D4	ICAM-1	*Escherichia coli*	*b*
M9	DBLβ1*	A	Dd2VAR32_D4	ICAM-1	*Escherichia coli*	*b*
	CIDR – DBLβ3*	A	3D7_Pfd1235w_D3-4	EPCR-ICAM-1	*Escherichia coli*	*b*
N27	DBLβ3	B	IT4VAR13_D4	ICAM-1	*Escherichia coli*	*b*
P1021	DBLβ3	B	KOB58843/HB3var34	ICAM-1	*Escherichia coli*	*b*
P1014	DBLβ5	B	KOB63129/HB3var21	ICAM-1	*Escherichia coli*	*b*
P1011	DBLβ5	B	AA75396/Dd2var01a	ICAM-1	*Escherichia coli*	*b*
N30	DBLβ7	A	Dd2VAR52_D4		*Escherichia coli*	*b*
N31	DBLβ7	A	Hb3vAR01_D4		*Escherichia coli*	*b*
N32	DBLβ6	A	BT1983_D4		*Escherichia coli*	*b*

Table 1: (DC) Domain Cassette. (*) contains DBLβ_{motif} associated with dual EPCR-ICAM-1 binding IE. (WGCFS) wheat germ cell free system. (*a)* Proteins labelled SM and UM were derived from sequences of Papuan field isolates [1] and were expressed by Professor Takafumi Tsuboi and Dr. Eizo Takashima, Proteo-Science Center, Ehime University, Matsuyama, Ehime, Japan [2]. (*b)* DBLβ proteins were supplied by Prof Anja Jensen, University of Copenhagen, Copenhagen, Denmark [3–5].

Supplementary Table 2. Recombinant merozoite and sporozoite proteins used in multiplex immunoassay

Protein	**Expression system**
AMA1	HEK293
MSP2 (FC27 allele)	*Escherichia coli*
MSP3	WGCFS
EBA175-RIII-V	*Escherichia coli*
CSP	HEK293

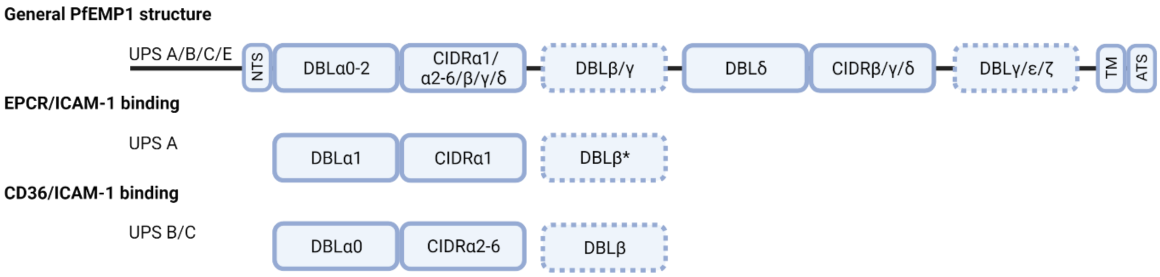


Figure S1. **General structure of PfEMP1.** PfEMP1 are encoded by up stream promotor sequence (Groups A/B/C/E). The NTS is followed by a DBLa and CIDR domain head structure. Head structure CIDRa1 domains bind to EPCR and CIDRa2-6 bind to CD36. The head structure is followed by at least 2 domains, shown with solid borders, and longer PfEMP1 have additional domains, shown with dashed borders. Group A EPCR binding head structures can sometimes be followed by DBLb with the ICAM-1 binding motif (*). Group B CD36 binding head structures can sometimes be followed by DBLb that also bind ICAM-1 (but cannot be predicted by the motif). DBL: Duffy Binding Like; CIDR: Cysteine-rich interdomain Region; NTS: N-terminal sequence, TM: Transmembrane region; ATS: A-terminal sequence. Figure adapted from Smith *et al.* 2013, created with BioRender.com.

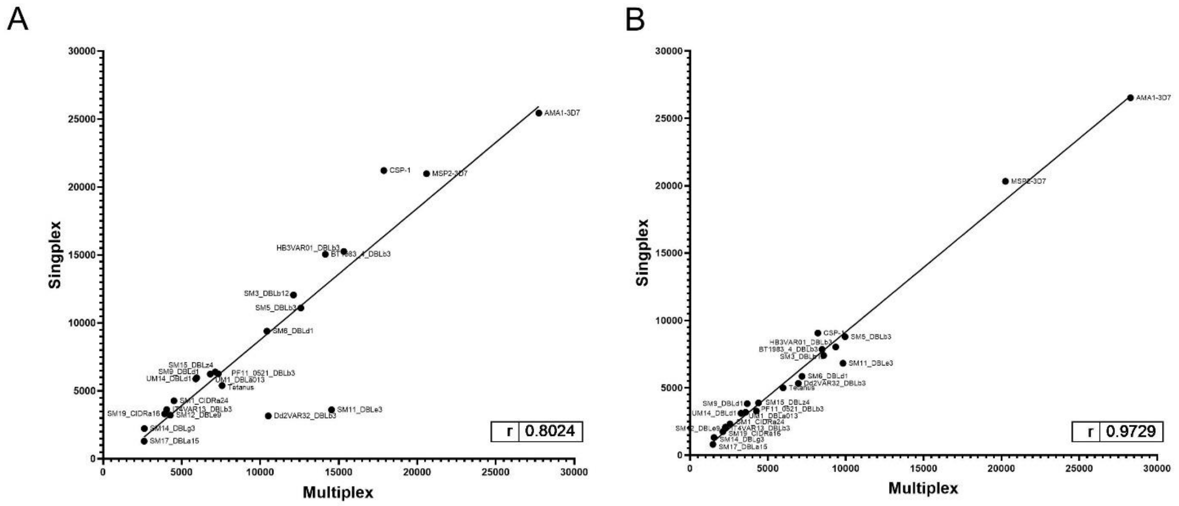


Figure S2. IgG responses for array of 17 antigens (one of two arrays) compared in single and multiplex formats, using serum pooled from 10 Malawian children, diluted by A) 1:50 and B) 1:100. r = Spearman's correlation coefficient.

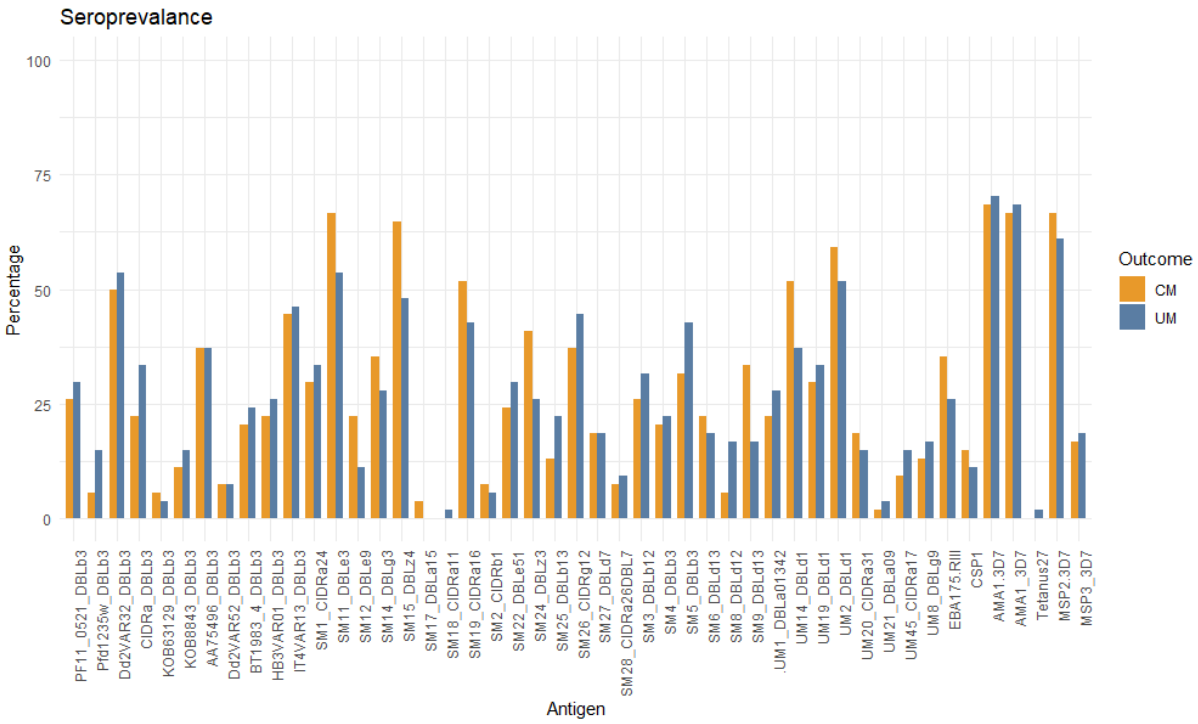


Figure S3. Percentage children with cerebral malaria (orange) or uncomplicated malaria (blue) who are seropositive to PfEMP1 antigens. Seropositivity was defined as IgG titers greater than 2 SD above the mean of malaria naïve individuals from Melbourne, Australia.

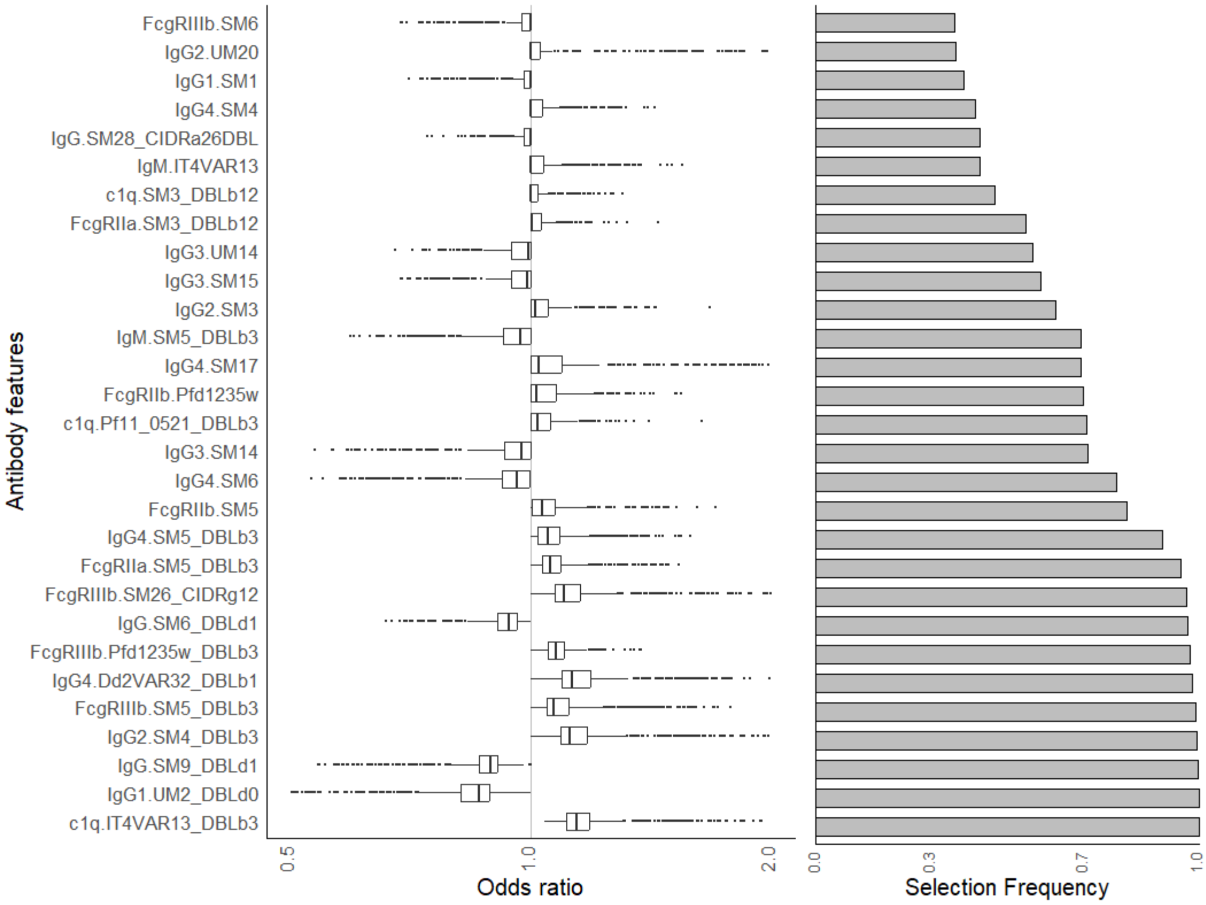


Figure S4. Odds ratio of antibody features from 5,000 repeats of 10-fold cross validated ENET regression models using alpha tuning parameter of 0.25. Variables shown in order of selection frequency (top 29 most selected variables are shown). Features with mean odds ratio greater than 1 represent responses associated with increased odds of uncomplicated malaria and features with mean odds ratio less than 1 are associated with increased odds of cerebral malaria.

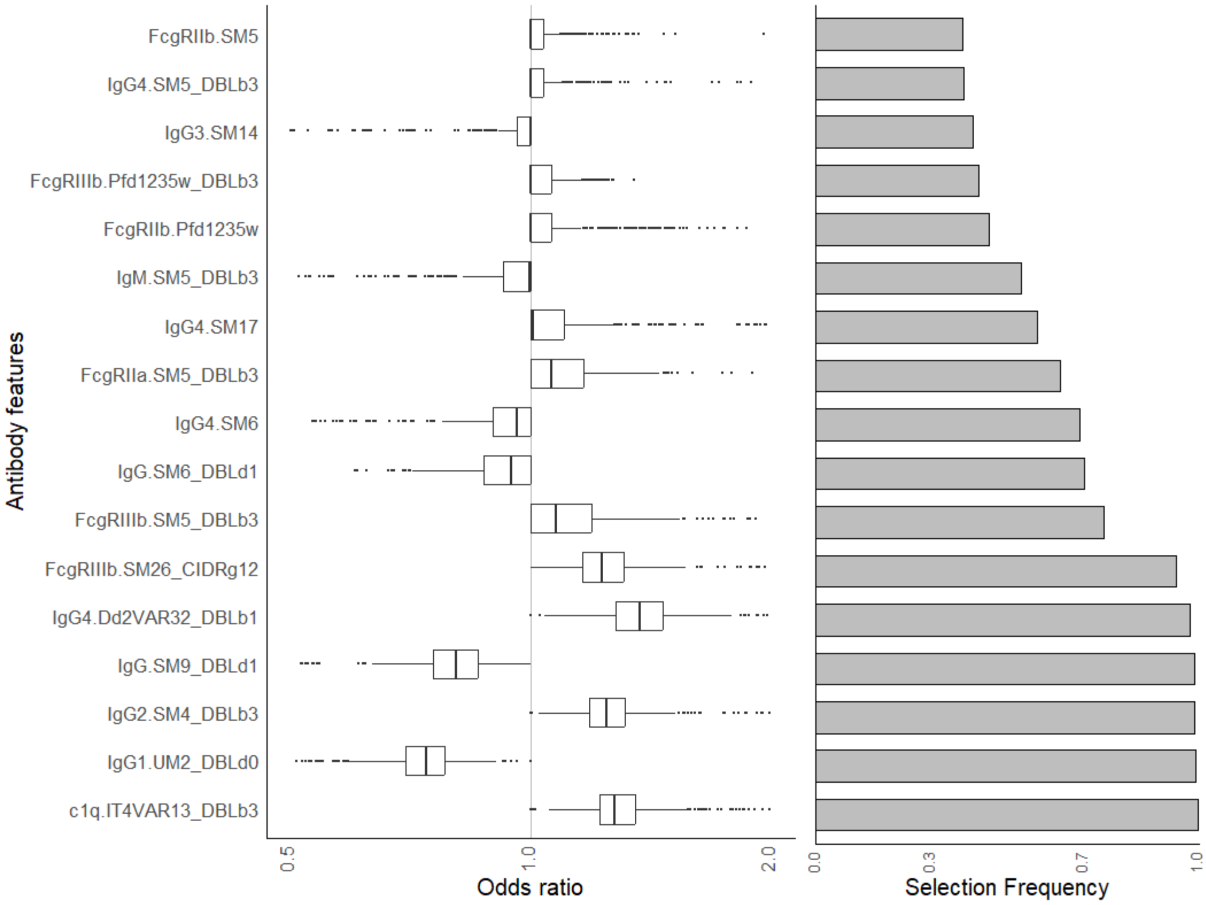


Figure S5. Odds ratio of antibody features from 5,000 repeats of 10-fold cross validated ENET regression models using alpha tuning parameter of 1. Variables shown in order of selection frequency (top 17 most selected variables are shown). Features with mean odds ratio greater than 1 represents responses associated with increased odds of uncomplicated malaria and features with mean odds ratio less than 1 are associated with increased odds of cerebral malaria.

Part V

Characterizing the PfEMP1 targets and Fc features of antibodies associated with severe and uncomplicated malaria in Papua New Guinean children

8 Background

Many countries in South East Asia and the Pacific are approaching elimination of malaria, however the burden of malaria remains high in Papua New Guinea (PNG) (World Health Organization 2021c). The estimated annual incidence of malaria in PNG (2020) ranges from 1 – 2 million annual cases and the estimated deaths range widely from 200 – 6,000 annually, due to the majority of cases being unreported (World Health Organization 2021c). Transmission is highly diverse, ranging from high transmission in the north coastal region of Madang to low and unstable in the surrounding highlands, and both *P. falciparum* and *P. vivax* circulate in the region (Cleary et al. 2022). A household survey of treatment seeking behaviour estimated that 81% of malarial fevers are not treated with antimalarial medicine (Seidahmed et al. 2021). In this setting, serosurveillance tools to identify populations most at risk of severe disease would be beneficial to inform treatment and resource allocation.

'Severe malaria' describes a broad range of epidemiological criteria, including impaired consciousness (cerebral malaria), severe anaemia, acidosis, hypoglycaemia and hyperparasitaemia (World Health Organization 2021a). PfEMP1 is a key pathogenic factor for severe malaria and mediates cytoadhesion of late blood stage infected erythrocytes to blood vessel endothelial cells. Previous studies have identified a broad range of *var* genes encoding for PfEMP1 domains that are differentially transcribed in severe malaria, cerebral malaria, or uncomplicated malaria (Kessler et al. 2017; Lavstsen et al. 2012; Normark et al. 2007; Tuikue Ndam et al. 2017), including a small number of studies outside of Africa (Bernabeu et al. 2016; Duffy et al. 2016; Falk et al. 2009; Tonkin-Hill et al. 2018). Children who lack antibodies to particular pathogenic PfEMP1 variants may be more susceptible to severe disease. From the small number of relevant studies conducted in the Western Pacific region, there is evidence to suggest that antibodies to PfEMP1 CIDRα1 domains that bind to EPCR are associated with protection from severe malaria (Chan et al. 2019b; Rambhatla et al. 2019; Tessema et al. 2019) however antibodies targeting other PfEMP1 variants may also be involved (Rambhatla et al. 2022; Tessema et al. 2018). Additionally, individuals develop antibodies to the infecting parasite phenotypes over the course of an infection and boosting of antibodies over time can be used to identify PfEMP1 variants involved in severe disease (Rambhatla et al. 2019; Travassos et al. 2018). Young children are an ideal study population for detecting correlates of protective immunity and important PfEMP1 variants, because they are seropositive for a small number of PfEMP1 variants compared to adults (Barry et al. 2011) and account for the majority of severe malaria cases (World Health Organization 2021c).

In this study we evaluated antibody responses to PfEMP1 that have previously been associated with severe or uncomplicated malaria, including: antigens whose gene transcription profiles were differentially regulated in Papuan adults with severe and uncomplicated malaria (Tonkin-Hill et al. 2018); a range of CIDRα domains, including EPCR and non-EPCR binding domains (Rambhatla et al. 2019); and a range of DBLβ domains, including ICAM-1 and non-ICAM-1 binding domains (Olsen et al. 2018). Previous studies have only considered the titers of IgG targeting PfEMP1 or the functional activity of antibodies to single parasite variants. Systems serology involves characterizing a broad range of antibody targets and Fc features and using machine learning to select the key features. We applied systems serology in our study of Malawian children (Part IV) and found that PfEMP1-specific IgG titers were not associated with protection from cerebral malaria, but a combination of seven Fc features, including IgG2 and IgG4, and FcγRIIb and c1q binding antibodies to DBLβ domains, could distinguish between cerebral and uncomplicated malaria with 87% accuracy. In this study, we profiled IgG, IgG subclasses 1-4 and Fcγ receptor binding antibodies targeting recombinant PfEMP1 antibodies in a case control study of PNG children with severe and uncomplicated malaria. We identified features that were lacking at presentation or detected in convalescence following severe malaria and used machine learning to identify a combination of antibody targets and features that could differentiate between the groups at presentation (acute disease) and convalescence.

8.1 Hypotheses

8.1.1 One

Antibody features can distinguish between PNG children with severe or uncomplicated malaria at presentation to hospital and at convalescence.

8.2 Aims

(i) Identify PfEMP1 targets for which antibodies are lacking at presentation in PNG children with severe malaria compared to uncomplicated malaria.

(ii) Identify PfEMP1 targets for which antibodies are acquired after an episode of severe or uncomplicated malaria in PNG children.

(iii) Profile the features of antigen specific antibodies in children with severe and uncomplicated malaria in PNG children at presentation and convalescence

(iv) Use machine learning to identify a combination of antibody targets and features that best distinguishes between children with severe and uncomplicated malaria, at presentation and at convalescence.

9 Results

9.1 Cohort of Papua New Guinean (PNG) children

Table 12: **Summary of PNG children cohort categorized by disease severity**

Characteristic	**Uncomp. act.**	**Uncomp. con.**	**Severe act.**	**Severe con.**
n in final assay				
	70	90	142	87
Age, median [IQR], months				
	50 [31-62]	44 [30-58]	43 [30-60]	41 [30-54]
Age group, n %				
0 – 48 months	34 (49%)	51 (57%)	87 (61%)	58 (67%)
49 - 90 months	36 (51%)	39 (43%)	55 (39%)	29 (33%)
Sex, n % [a]				
Female	26 (45%)	39 (43%)	65 (45%)	42 (48%)
Male	32 (55%)	51 (57%)	77 (54%)	45 (52%)
Ethnicity, n % [b]				
Madang	52 (91%)	81 (90%)	106 (75%)	67 (77%)
Other	7 (12%)	9 (10%)	36 (25%)	20 (23%)
Blantyre Coma Score, n (%) [c]				
0-2			18 (13%)	
3			8 (6%)	
4			21 (15%)	
5	70 (100%)		93 (66%)	
Haemoglobin (g/dL) [d]				
	9.0 [7.8-10.8]	10.6 [9.8-11.6]	7.6 [5.0-9.1]	11 [9.6-11.6]
Severe anaemia (<7 g/dl), n (%)				
	0 (0%)	0 (0%)	34 (24%)	0 (0%)
Acidosis, n (%) (e)				
			15 (11%)	
Species, n (%) [f]				
P*f*	33 (89%)		121 (86%)	72 (83%)
P*fv*	4 (9%)		20 (14%)	15 (17%)
Parasitaemia units median [IQR], per µL				
P*f*	23058 [6310-78829]	0 [0-561]	76728 [13494-179260]	0 [0–0]
P*fv*	0 [0–0]	0 [0–0]	0 [0–0]	0 [0–0]

Uncomp. and Severe: Uncomplicated malaria and severe malaria. *act. and conv.*: acute and convalescent. *n*: number of samples. IQR: Inter Quartile Range. (^{a}) 58 uncomplicated acute samples with clinical information available for sex. (^{b}) 59 uncomplicated acute samples with clinical information available for ethnicity. (^{c}) 140 severe acute samples with clinical information available for Blantyre Coma Score (BSC). (^{d}) 66 uncomplicated acute, 84 uncomplicated convalescent and 80 severe convalescent samples with clinical information available for Haemaglobin. (^{e}) 139 severe acute samples with clinical information available for acidosis (^{f}) 141 severe acute samples with clinical information available for species, detected by PCR.

Plasma samples were collected between 2006-2009 from children presenting to Modilon Hospital, Madang, Papua New Guinea, and at 8 weeks follow-up, in convalescence (for details see Materials and Methods, Part III, Section 7.1.2). This study included 70 children with acute uncomplicated malaria and 90 convalescent from uncomplicated malaria, 142 with acute severe malaria and 87 with convalescent severe malaria. Due to limited sample availability, there were paired acute and convalescent samples for 44 of the children with uncomplicated malaria and 75 of the children with severe malaria. The median age of children across the groups and time points was similar, however a greater percentage of children with severe malaria were under 4 years old compared to the children with uncomplicated malaria. There were more males than females across the groups and time points. A greater percentage of children with severe malaria were of non-Madang ethnicity, compared to uncomplicated malaria and a greater percentage of children with severe malaria had mixed *P. falciparum* and *P. vivax* infections compared to uncomplicated malaria. Children classified as having acute severe malaria had a mix of severe etiologies, including severe anaemia (24%), cerebral malaria (13%) or acidosis (11%).

9.1.1 Recognition of PfEMP1 proteins in Papua New Guinean children

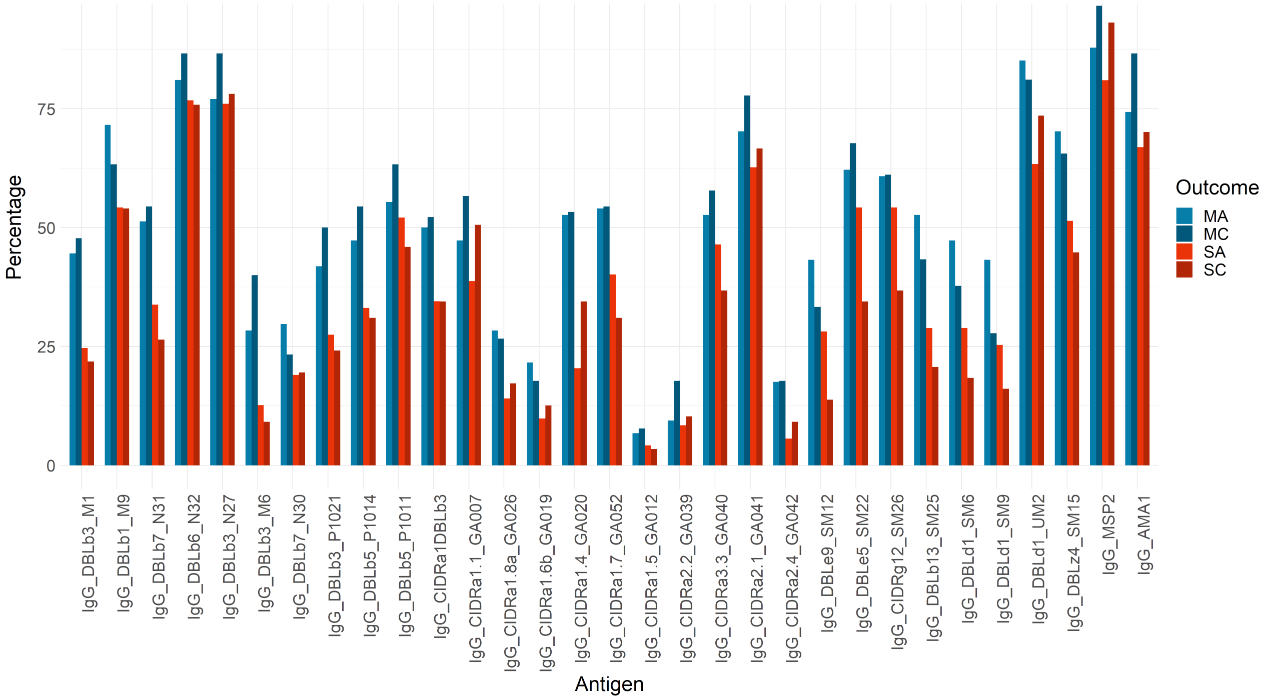


Figure 6: **IgG seroprevalence for PfEMP1 antigens in PNG children.** Percentage of children with IgG titers greater than two standard deviations above the mean of 20 Melbourne controls, shown for children with acute uncomplicated malaria (MA), convalescent uncomplicated malaria (MC), acute severe malaria (SA) and convalescent severe malaria (SC).

The study included 29 recombinant PfEMP1 antigens (see Materials Tables 8, 10, 9) and measured antigen specific IgG, IgG1, IgG3, IgG4, and FcγRIIa, FcγRIIIa, FcγRIIb and FcγRIIIb binding. Due to limited protein availability, there were several antigens for which not all Fc features were measured. SM12, SM25 were not probed for IgG1, IgG2, IgG4, FcγRIIb or FcγRIIIa. GA042 and N31 were not probed for IgG1, IgG2, IgG4, and FcγRIIIa. SM9, SM6, GA026 were not probed for IgG2 and IgG4.

The majority of PfEMP1 antigens were recognized by antibodies from a substantial portion of PNG children, except for CIDRα1.5, CIDRα2.2 and CIDRα2.4, that were recognized by less than 20% of children across all clinical groups (Figure 6). In general, a greater percentage of children with uncomplicated malaria were seropositive compared to children with severe malaria (Figure 6).

9.1.2 Antibody responses to merozoite antigens and controlling for exposure

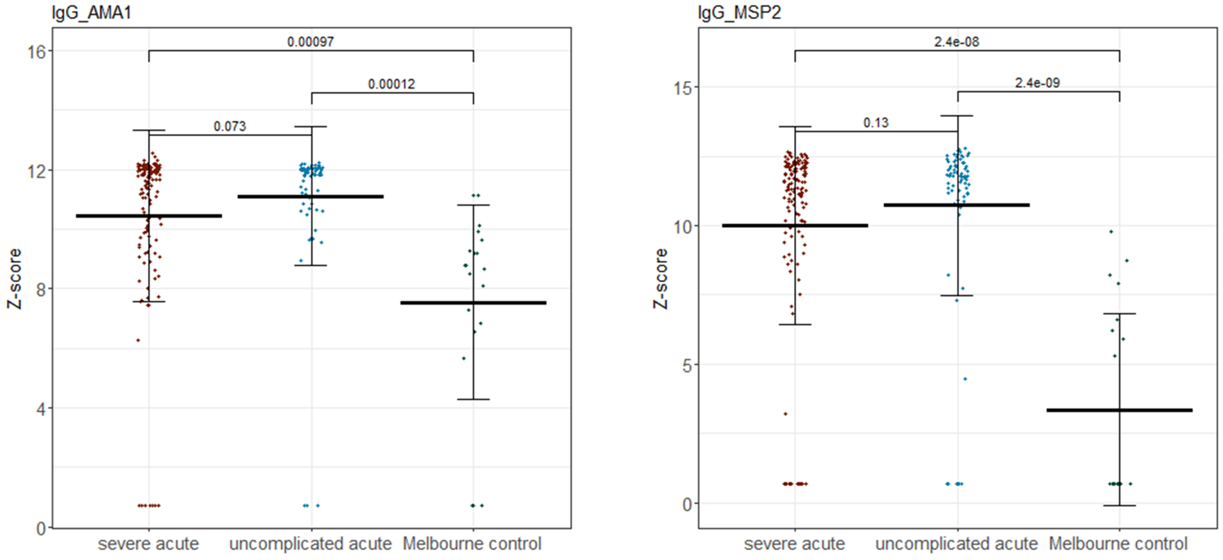


Figure 7: **IgG responses to recombinant AMA1 and MSP2 in acute severe and uncomplicated malaria.** Y axis represents log-transformed Z-score of IgG titers to A) AMA1 and B) MSP2, measured by mutliplex immunoassay. Mean antibody titers were compared between severe malaria (red) and uncomplicated malaria (blue) and healthy Melbourne controls (green) by Welch's t-test, and associated P-values are shown above horizontal bars. Mean and standard deviation are shown.

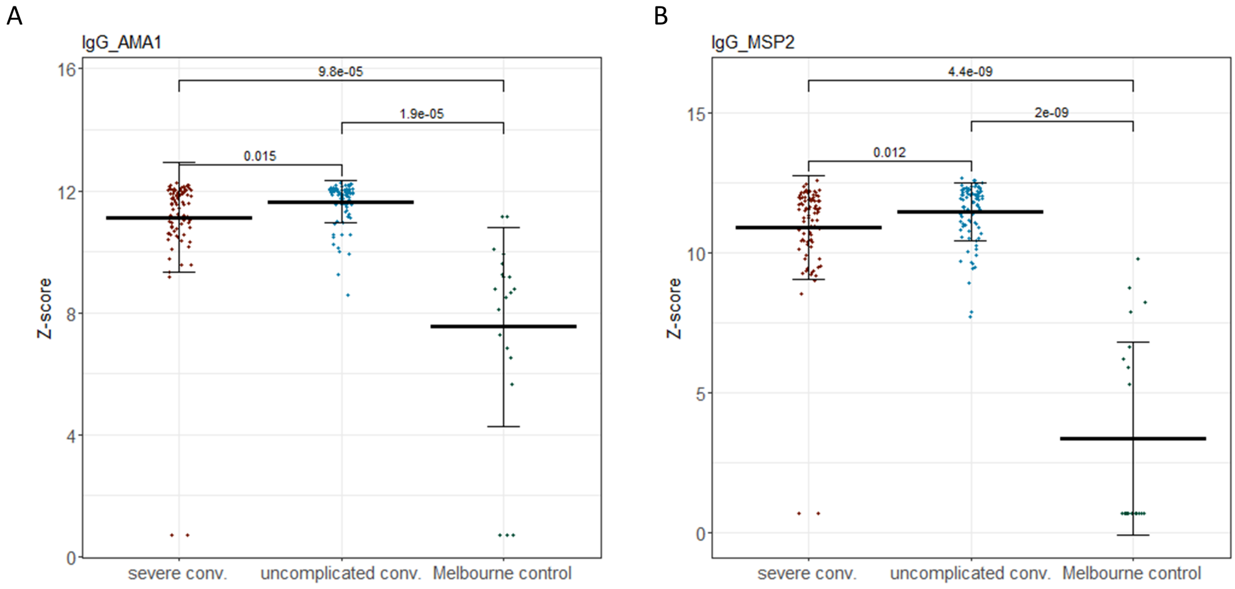


Figure 8: **IgG responses to recombinant AMA1 and MSP2 in convalescent severe and uncomplicated malaria.** Y axis represents log-transformed Z-score of IgG titers to A) AMA1 and B) MSP2, measured by mutliplex immunoassay. Mean antibody titers were compared between severe malaria (red) and uncomplicated malaria (blue) and healthy Melbourne controls (green) by Welch's t-test, and associated P-values are shown above horizontal bars. Mean and standard deviation are shown.

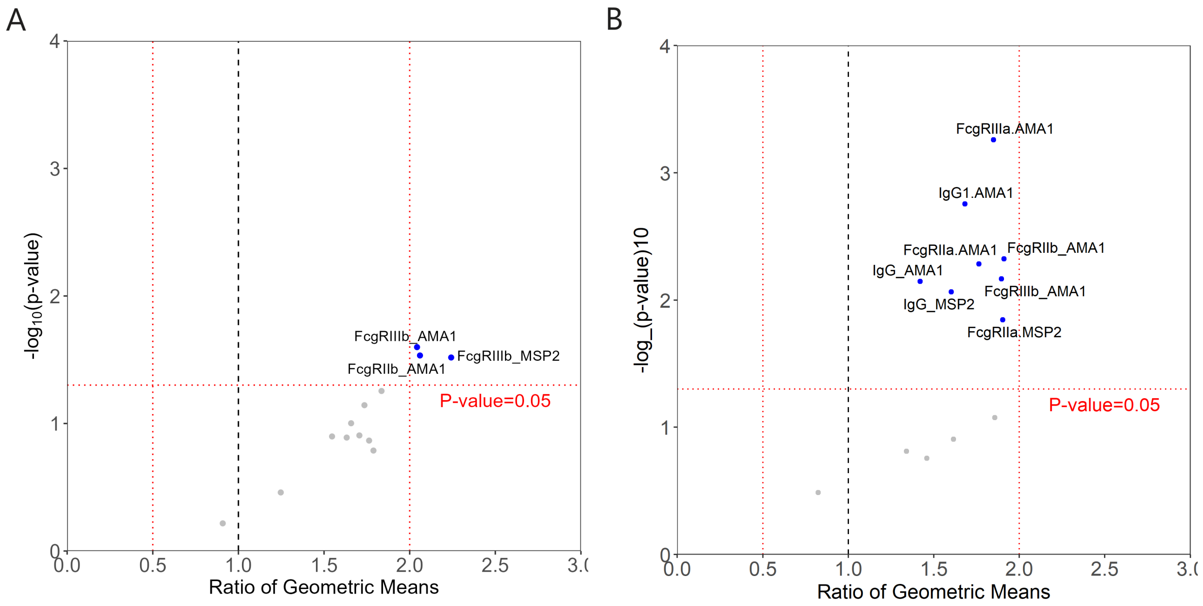


Figure 9: **Linear association between antibody feature to merozoite antigens (AMA1 and MSP2) and uncomplicated malaria compared to severe malaria, after adjusting for age.** Linear regression was performed between clinical presentation (severe or uncomplicated) and age (as covariates) with individual antibody features (as the outcome), at the (A) acute and (B) convalescent time points. X-axis represents the magnitude of difference between the mean antibody levels in children with uncomplicated malaria compared to severe malaria. Y-axis represents -log10 transformed p-value statistic for the estimated mean difference in antibodies between severe and uncomplicated malaria. Right of black vertical line at 1 represents responses elevated in uncomplicated malaria and vertical line at 2 indicates a two-fold elevation in uncomplicated malaria. Left of black vertical line at 1 represents responses elevated in severe malaria and vertical line at 0.5 indicates a two-fold elevation in severe malaria. Horizontal line indicates log10(0.05) threshold of statistical significance. There were no corrections for multiple comparisons.

Total IgG measurements to merozoite antigens were used as surrogate markers for prior exposure to *P. falciparum*. For the acute samples, there were no significant differences in IgG targeting AMA1 or MSP2 between children with severe and uncomplicated malaria (Figure 7). For the convalescent samples, IgG targeting AMA1 and MSP2 were significantly elevated in uncomplicated malaria compared to severe malaria (Figure 8).

Exposure to malaria is correlated with age in malaria endemic settings. Since there were differences in age between the groups, we performed linear regression to assess the association between clinical outcome (severe or uncomplicated malaria) and age with IgG titers to merozoite antigens. After adjusting for age, there were no significant differences in mean IgG targeting AMA1 or MSP2 between children with acute uncomplicated and acute severe malaria (Figure 9A). After adjusting for age, children with convalescent uncomplicated malaria had 1.4-fold higher mean IgG titers to AMA1 compared to children with convalescent severe malaria, and 1.6-fold higher mean IgG titers to MSP2 (P values 0.007 and 0.008, respectively) in uncomplicated compared to severe malaria (Figure 9B).

For other antibody Fc features, mean FcγRIIb and FcγRIIIb binding antibodies targeting

AMA1, and FcγRIIIb binding antibodies targeting MSP2 were significantly and by greater than 2-fold higher in acute uncomplicated malaria compared to acute severe malaria, after adjusting for age (Figure 9A). At convalescence, mean IgG1, FcγRIIa, FcγRIIIa, FcγRIIb and FcγRIIIb binding antibodies targeting AMA1, and FcγRIIa binding antibodies targeting MSP2 were significantly higher in convalescent uncomplicated malaria compared to convalescent severe malaria, after adjusting for age (mean fold differences range from 1.6 – 1.9, P <0.05, Figure 9B) and none of the antibody responses crossed the threshold for a two-fold difference in the means.

All analysis presented from this point was adjusted for age by including age as a covariate in linear regression models.

9.2 Univariable analysis

9.2.1 Association of severe and uncomplicated malaria with individual antibody Fc features.

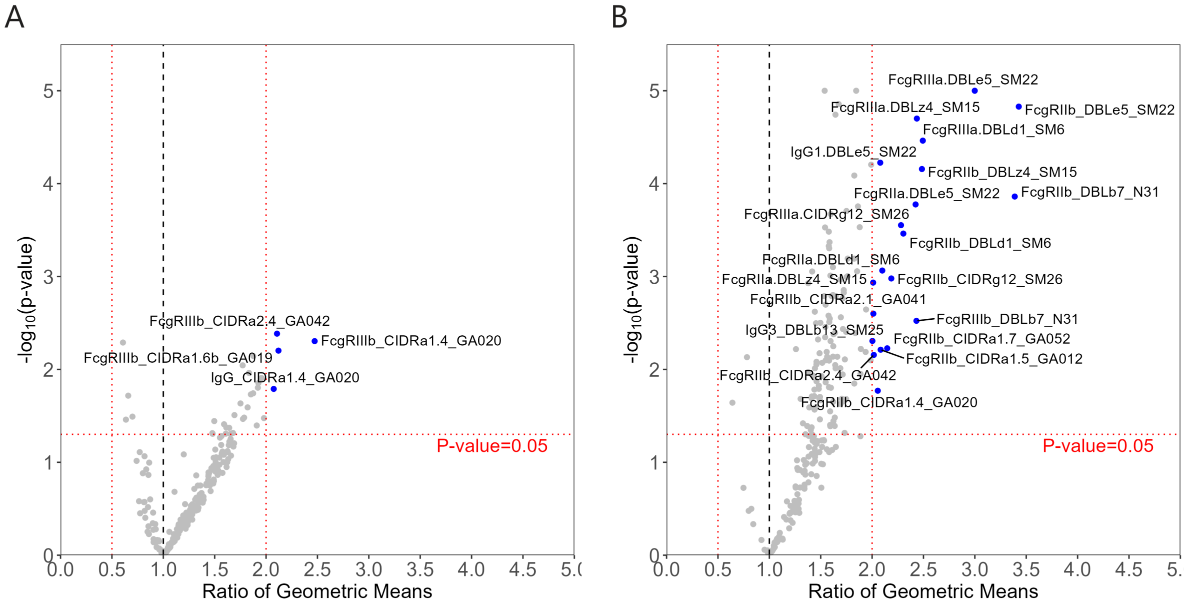


Figure 10: **Linear association between severe and uncomplicated malaria with antibody features targeting PfEMP1.** Linear regression was performed between clinical presentation (severe or uncomplicated) and age (as co-variates) with individual antibody features (as the outcome), at the (A) acute and (B) convalescent time points. X-axis represents the magnitude of difference between the mean antibody levels in children with uncomplicated malaria compared to severe malaria. Y-axis represents -log10 transformed p-value statistic for the estimated mean difference in antibodies between severe and uncomplicated malaria. Right of black vertical line at 1 represents responses elevated in uncomplicated malaria and vertical line at 2 indicates a two-fold elevation in uncomplicated malaria. Left of black vertical line at 1 represents responses elevated in severe malaria and vertical line at 0.5 indicates a two-fold elevation in severe malaria. Horizontal line indicates log10(0.05) threshold of statistical significance. There were no corrections for multiple comparisons

We compared antibody features in uncomplicated and severe malaria, for acute and convalescent time points, by linear regression with age included as a co-variate (Figure 10). There were four antibody features for which the mean levels were significantly and by greater than two folds elevated in uncomplicated malaria compared to severe malaria, after adjusting for age: IgG targeting CIDRα1.4, FcγRIIIb to antibodies targeting CIDRα1.4, CIDRα1.6b and CIDRα2.4. At convalescence, there were 20 antibody features for which the mean levels were significantly and by greater than two folds elevated in uncomplicated malaria compared to severe malaria, after adjusting for age: IgG1 targeting DBLε5, IgG3 targeting DBLβ13; FcγRIIIb binding antibodies targeting DBLβ7 and FcγRIIIa antibodies targeting DBLζ4 and CIDRγ12; and the majority of responses were FcγRIIb binding antibodies, targeting DBLε5, DBLβ7, DBLζ4, DBLδ1, CIDRγ12, CIDRα1.4, CIDRα1.7, CIDRα1.5, CIDRα2.4, CIDRα2.1. The antibody targets were predominantly targeting proteins that are up-regulated in severe malaria in Papua (Table 8) or have been associated with severe malaria (Tables 10 and 9) as well as three proteins associated with uncomplicated malaria: DBLβ7_ N31 (Group A, non-ICAM-1 binder), CIDRα2.4 and CIDRα2.1 (CD36 binders).

9.2.2 Differences in antibody responses between acute and convalescent malaria, amongst children with severe or uncomplicated malaria

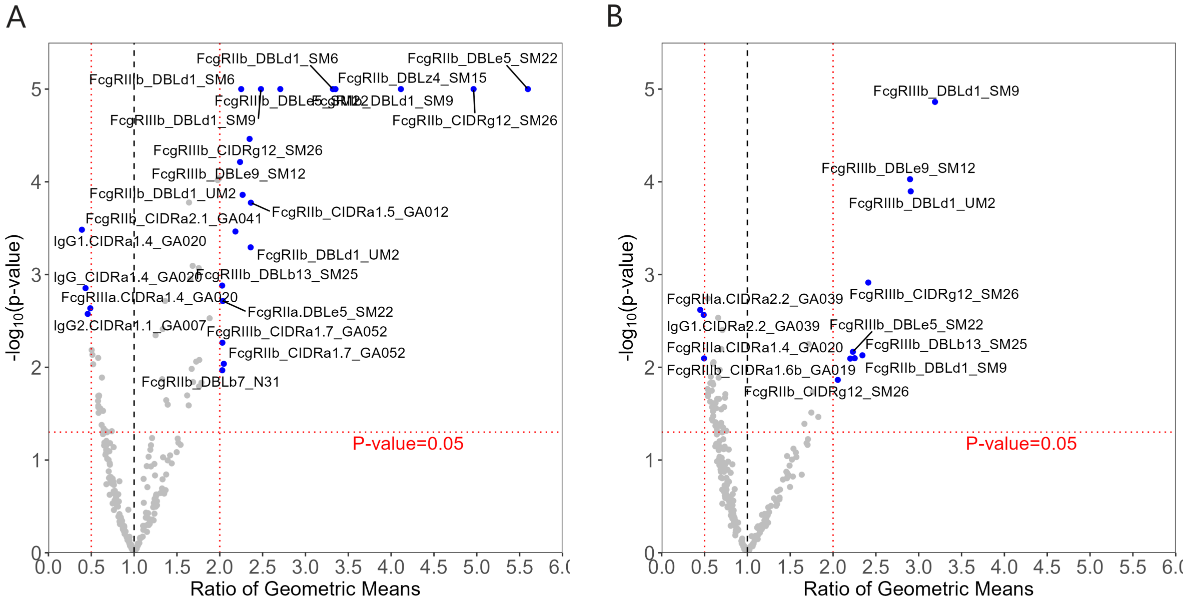


Figure 11: **Differences in mean antibody responses between acute and convalescent malaria, amongst children with severe or uncomplicated malaria** Linear regression was performed between time point (convalescent compared to acute) and age (as co-variates) with individual antibody features (as the outcome), amongst children with (A) severe and (B) uncomplicated malaria. X-axis represents the magnitude of difference between the mean antibody levels at acute compared to convalescent malaria and a mean ratio greater than one represents in favour of acute malaria. Y-axis represents -log10 transformed p-value statistic for the estimated mean difference in antibodies between severe and uncomplicated malaria. Right of black vertical line at 1 represents responses elevated in uncomplicated malaria and vertical line at 2 indicates a two-fold elevation in uncomplicated malaria. Left of black vertical line at 1 represents responses elevated in severe malaria and vertical line at 0.5 indicates a two-fold elevation in severe malaria. Horizontal line indicates log10(0.05) threshold of statistical significance. There were no corrections for multiple comparisons.

We compared antibody features in acute and convalescent malaria, amongst 75 children with severe malaria and amongst 44 children with uncomplicated malaria (only paired samples were used for comparison, Figure 11). Amongst children with severe malaria, there were four antibody features that were significantly and by greater than two folds lower in convalescence compared to acute severe malaria: IgG and IgG1 targeting CIDRα1.4, IgG2 targeting CIDRα1.1 and FcγRIIIa to antibodies targeting CIDRα1.4. There were 19 antibody features that were significantly and by greater than two folds elevated in convalescence compared to acute severe malaria: FcγRIIa to antibodies targeting DBLε5; FcγRIIIb to antibodies targeting CIDRα1.7, DBLβ13, DBLε9, DBLδ1 (SM6, SM9 and UM2), CIDRγ12, and DBLε5; FcγRIIb binding antibodies targeting CIDRα1.7, CIDRα1.5, CIDRα2.1, DBLβ7, DBLδ1 (SM6, SM9 and UM2), DBLζ4, CIDRγ12 and DBLε5.

Amongst children with uncomplicated malaria, there were three antibody features that were significantly and by greater than two folds lower in convalescence compared to acute uncomplicated malaria: IgG1 targeting CIDRα2.2 and FcγRIIIa to antibodies targeting CIDRα1.4 and CIDRα2.2. There were 9 antibody features that were significantly and by greater than two folds elevated in convalescence compared to acute uncomplicated malaria: FcγRIIb binding antibodies targeting CIDRγ12 and DBLδ1 (SM9), and FcγRIIIb binding antibodies targeting CIDRα1.6b, CIDRγ12, DBLε5, DBLβ13, DBLε9 and DBLδ1 (UM2 and SM9).

9.3 Multivariate analysis

9.3.1 Elastic-Net Logistic Regression to identify antibody features that distinguish between severe and uncomplicated malaria

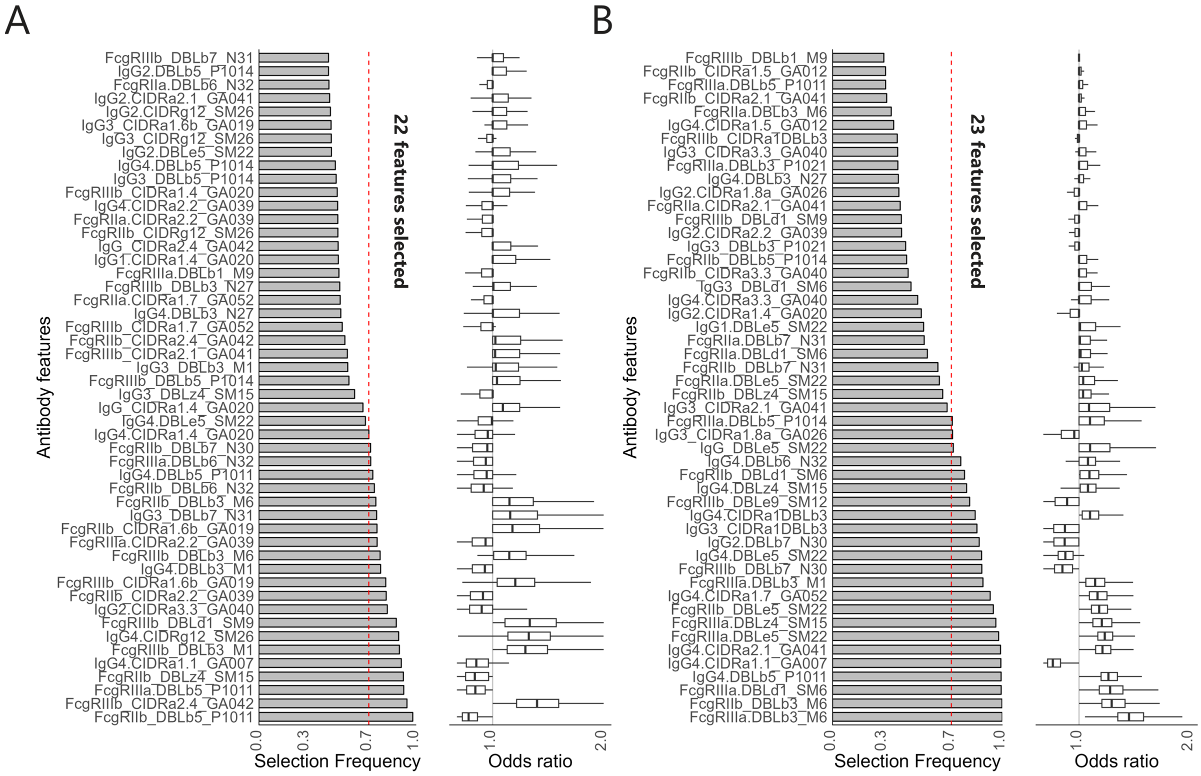


Figure 12: **Antibody features selected by elastic net-regularized logistic regression to distinguish between severe and uncomplicated malaria**. Resampling of elastic net-regularized logistic regression (5,000 times and 10-fold cross validation) was used to record the frequency and Odds ratio of antibody features. The frequency that features appeared in the 5,000 best models is shown as bars. Vertical line shows the chosen cut off of 70% frequency. Box-plots show the Odds ratio median, inter-quartile range (IQR, Q1 to Q3), and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR). Features with median odds ratio greater than one are associated with increased odds of uncomplicated malaria and features with median odds ratio less than one are associated with increased odds of severe malaria.

We used elastic net-regularized logistic regression to identify antibody features that could be combined to best differentiate between severe and uncomplicated malaria, amongst acute and convalescent individuals. We performed 5,000 repeats of 10-fold cross validated elastic net-regularized logistic regression and recorded the features that were selected in the 'best' model (based on AUROC) on each repeat (Figure 12). At the acute time point, 22 features were selected in more than 70% of model iterations. At convalescence, 23 features were selected in more than 70% of model iterations. Selected features at acute and convalescence included features associated with both an increased or decreased odds of uncomplicated malaria compared to severe malaria.

9.3.2 Contribution of selected features to classify children as severe or uncomplicated malaria by Partial Least Squares Discriminant Analysis.

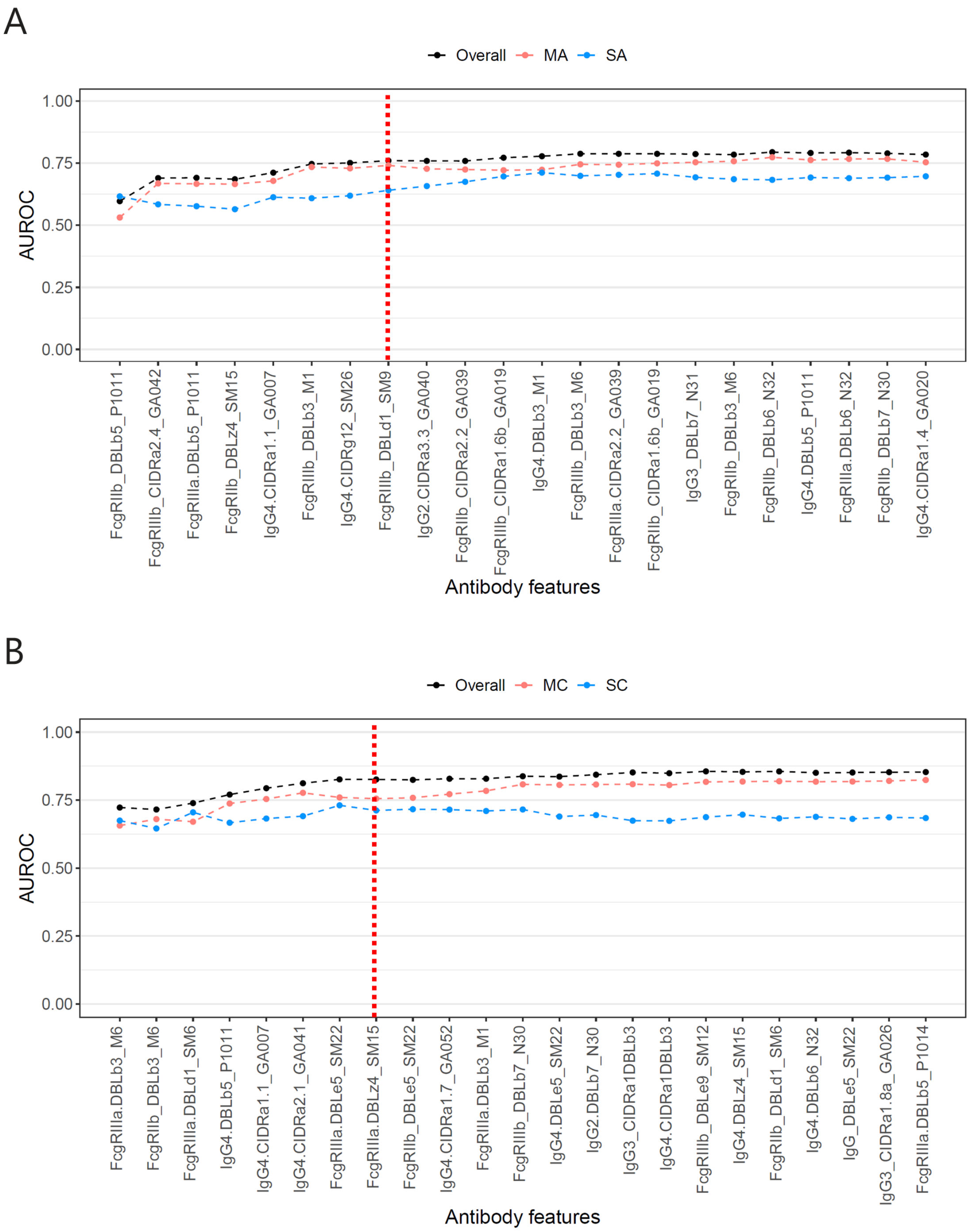


Figure 13: **Contribution of antibody features to classification of severe or uncomplicated malaria by PLS-DA, at (A) acute and (B) convalescent time points.** Y axis shows PLS-DA performance to classify children as severe or uncomplicated malaria (AUROC) when features were added in order of selection frequency that was determined from elastic net-regularized logistic regression modelling. We performed 500 repeats of 10-fold cross-validation to estimate accuracy for each model. Black line represents the overall accuracy for classification of all children, red represents accuracy for classification of children with uncomplicated malaria (MA/MC) and blue for children with severe malaria (SA/SC). Vertical red line represents chosen cut off of eight features.

Next, we assessed the contribution of the most frequently selected features to classify children as severe or uncomplicated malaria, by recording the AUROC as features were added one by one to a PLS-DA regression model (Figure 13), in order of the frequency they appeared in Elastic Net-regularized logistic regression analysis (Figure 12). Amongst acute samples (Figure 13A), the overall accuracy for classification as either uncomplicated or severe malaria plateaued after the inclusion of the sixth feature. The accuracy for classification of uncomplicated malaria samples followed a similar pattern, and the accuracy for classification of severe malaria samples increased until 11 features were included. At convalescence (Figure 13B), the overall accuracy for classification as uncomplicated or severe malaria plateaued after the inclusion of the eighth feature, as did the accuracy of classification of uncomplicated malaria only and severe malaria only.

9.3.3 Performance of antibody features to classify children as having severe or uncomplicated malaria, assessed by PLS-DA

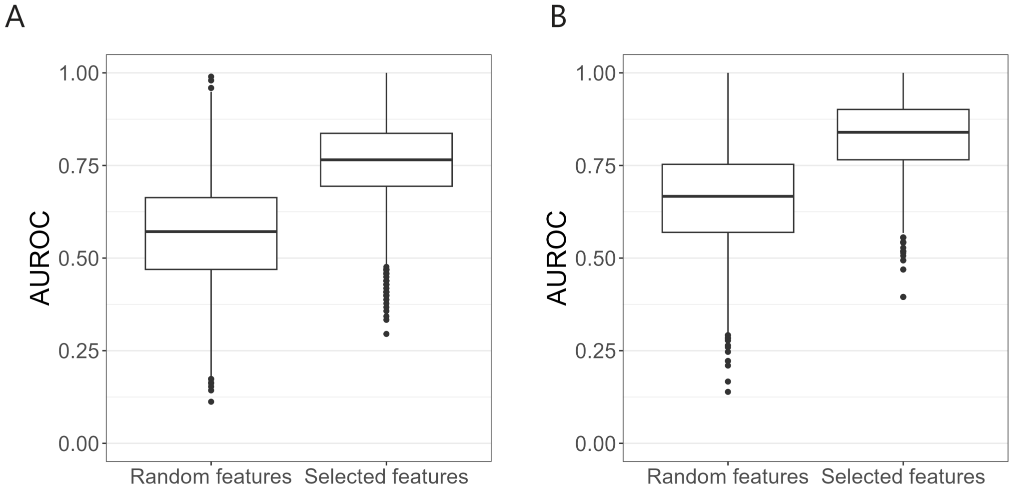


Figure 14: **Performance of eight selected antibody features to classify children as having severe or uncomplicated malaria, at the (A) acute and (B convalescent time points.** PLSDA was fitted with eight features selected by Elastic-Net regularized logistic regression (selected features) or eight random features, and performance (Area under the receiver operator curve, AUROC) was computed for 500 repeats of 10-fold cross-validation resampling. Boxes show median and inter-quartile range (IQR, Q1 to Q3), and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR).

Next, we assessed the performance of eight most frequently selected features (from the Elastic Net-regularized logistic regression analysis, Figure 12) to distinguish between severe and uncomplicated malaria by PLSDA. For the acute time point, the eight selected features could classify children as having severe or uncomplicated malaria with 76% accuracy (69% - 84%), (based on the median AUROC determined from 500 repeats with 10 fold cross validation). Using the eight randomly selected features, the accuracy was 57% (47% - 66%, Figure 14A).

In convalescence, the eight selected features could classify children as having severe or uncomplicated malaria with 84% accuracy (76% - 90%). Using eight randomly selected features, the accuracy was 67% (57% - 78%, Figure 14B).

For the acute time point, the eight selected features were FcγRIIb to antibodies targeting DBLβ5_P1011, DBLζ4_SM15, FcγRIIIb to antibodies targeting CIDRα2.4_GA042, DBLβ3_M1, DBLδ1_SM9, FcγRIIIa to antibodies targeting DBLβ5_P1011, and IgG4 targeting CIDRα1.1_GA007 and CIDRγ12_SM26 (Figure 13A). In convalescence, the eight selected features were FcγRIIIa binding antibodies targeting DBLβ3_M6, DBLδ1_SM6, DBLε5_SM22 and DBLζ4_SM15, FcγRIIb binding antibodies targeting DBLβ3_M6, and IgG4 targeting DBLβ5_P1011, CIDRα1.1_GA007 and CIDRα2.1_GA041 (Figure 13B).

9.3.4 FcγR and IgG4 to a small number of antigens could best distinguish between severe and uncomplicated malaria.

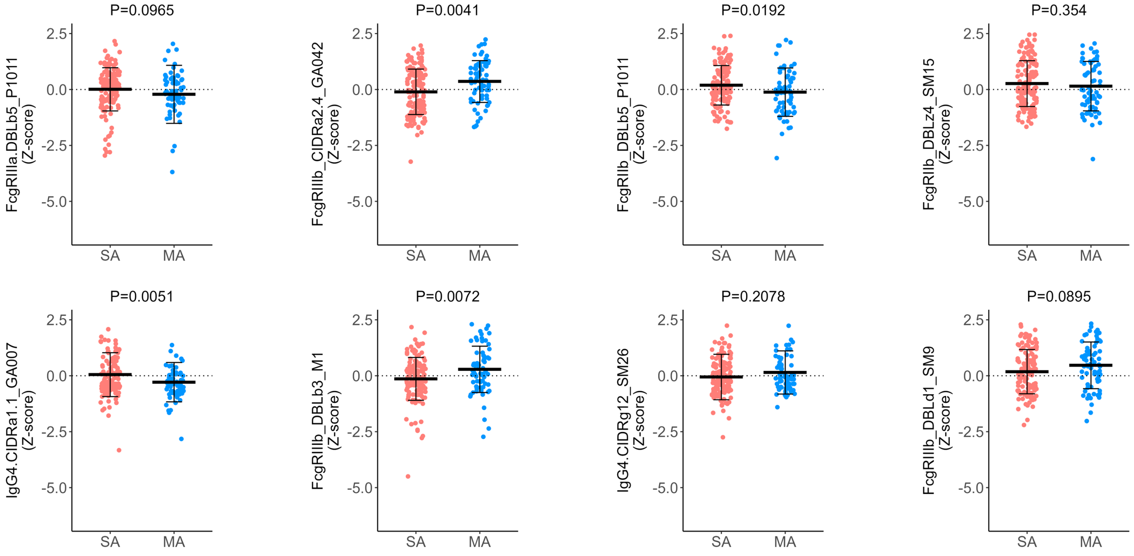


Figure 15: **Distribution of eight selected features that distinguish between severe and uncomplicated malaria at the acute time point.** Comparison of acute severe malaria (SA, red) and uncomplicated malaria (MA, blue) and associated P-values from Welch's T-test are shown. Data was centered to have zero mean and scaled to one standard deviation (Z-score). Error bars show mean and standard deviation.

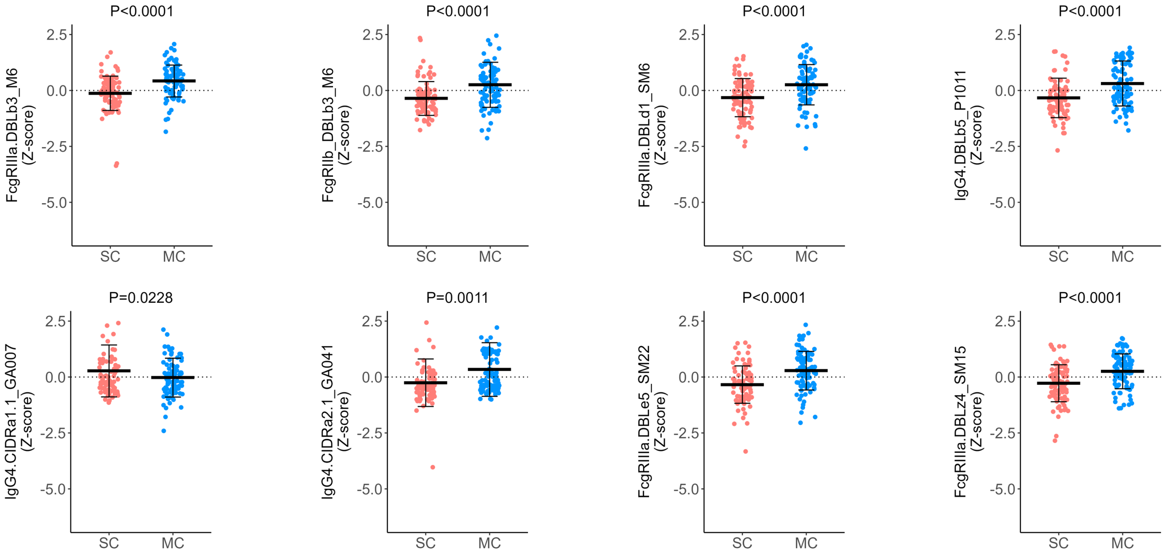


Figure 16: **Distribution of eight selected features that distinguish between severe and uncomplicated malaria at the convalescent time point.** Comparison of convalescent severe malaria (SC, red) and uncomplicated malaria (MC, blue) and associated P-values from Welch's T-test are shown. Data was centered to have zero mean and scaled to one standard deviation (Z-score). Error bars show mean and standard deviation.

For the acute time point, the eight features that were selected from Elastic Net-regularized logistic regression and PLSDA included four features that were elevated in severe malaria and four that were elevated in uncomplicated malaria (Figure 15). Features that were elevated in severe malaria included FcγRIIb and FcγRIIIa to antibodies targeting DBLβ5_P1011, FcγRIIb to antibodies targeting DBLζ4_SM15 and IgG4 targeting CIDRα1.1_GA007. Features that were elevated in uncomplicated malaria were FcγRIIIb to antibodies targeting CIDRα2.4_GA042, DBLδ1_SM6 and DBLβ3_M1, and IgG4 targeting CIDRγ12_SM26. Not all univariate comparisons reached statistical significance (Figure 15).

In convalescence, seven out of the eight features that were selected from Elastic Net-regularized logistic regression were elevated in uncomplicated malaria (Figure 16): FcγRIIa binding antibodies targeting DBLβ3_M6, DBLδ1_SM6, DBLε5_SM22, FcγRIIIa binding antibodies targeting DBLζ4_SM15, FcγRIIb binding antibodies targeting DBLβ3_M6, and IgG4 targeting DBLβ5_P1011 and CIDRα2.1_GA041. IgG4 targeting CIDRα1.1_GA007 was elevated in severe malaria.

9.3.5 Antibody feature seroprevalence of selected PfEMP1 proteins in Papua New Guinean children

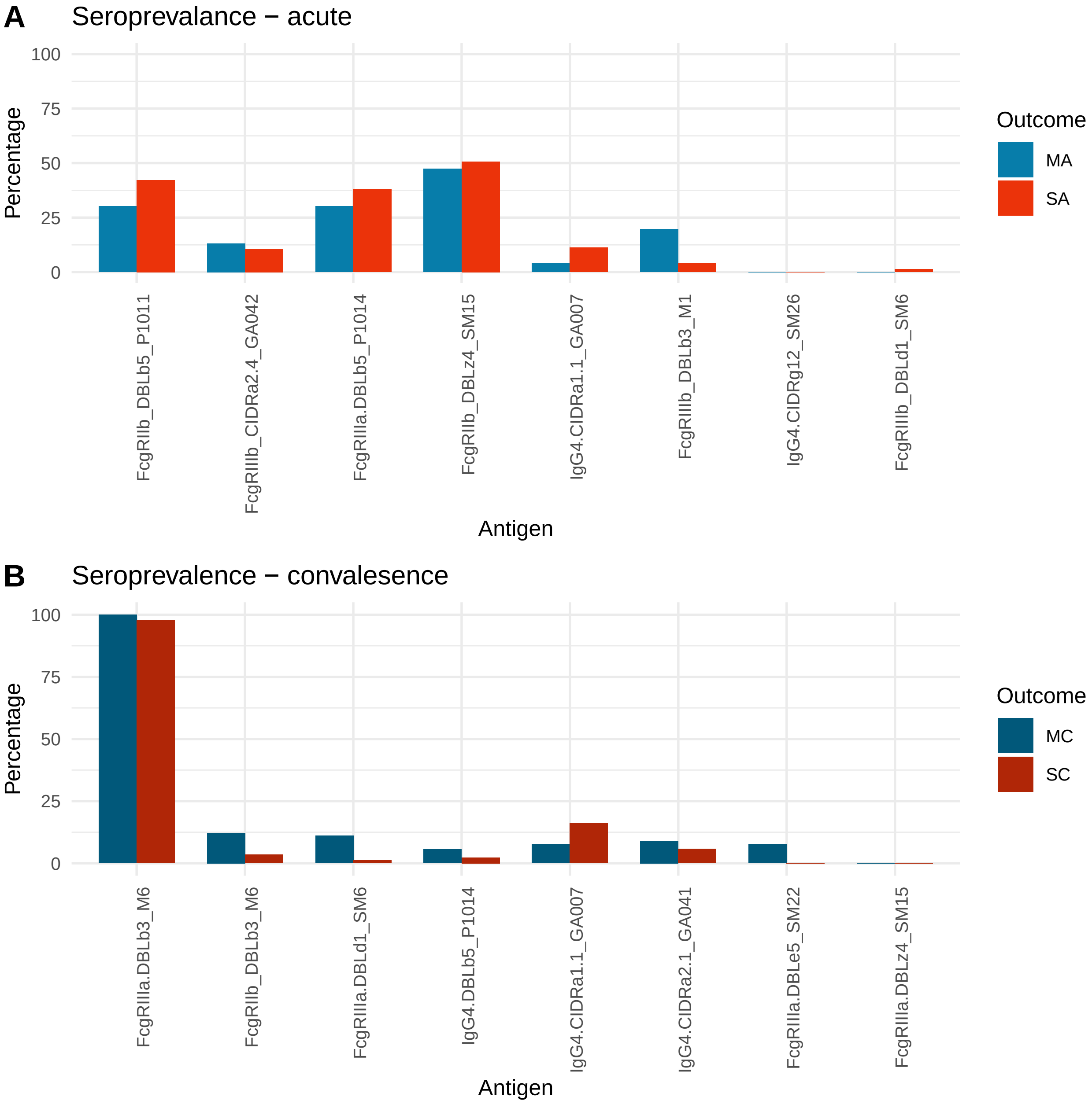


Figure 17: **Antibody feature seroprevalence of eight selected PfEMP1 proteins in PNG children with (A) acute and (B) convalescent malaria.** Percentage of children with antibody feature levels greater than two standard deviations above the mean of 20 Melbourne controls, shown for children with acute uncomplicated malaria (MA), convalescent uncomplicated malaria (MC), acute severe malaria (SA) and convalescent severe malaria (SC).

Amongst the eight selected features at the acute time point, 25 - 50% of children in both groups were seropositive for FcγRIIIa and FcγRIIb to DBLβ5_P1011 and FcγRIIb to DBLζ4_SM15. Less than 25% of children in both groups were seropositive for other features, including IgG4 to CIDRγ12_SM26 and FcγRIIIb to DBLδ1_SM6, for which 0 - 2% of individuals were seropositive.

Amongst the eight selected features at the convalescent time point, 98 - 100% of individuals were seropositive to FcγRIIa binding antibodies targeting DBLβ3_M6. For other features, less than 15% of individuals were seropositive, including FcγRIIIa to DBLζ4_SM15, for which no individuals were seropositive.

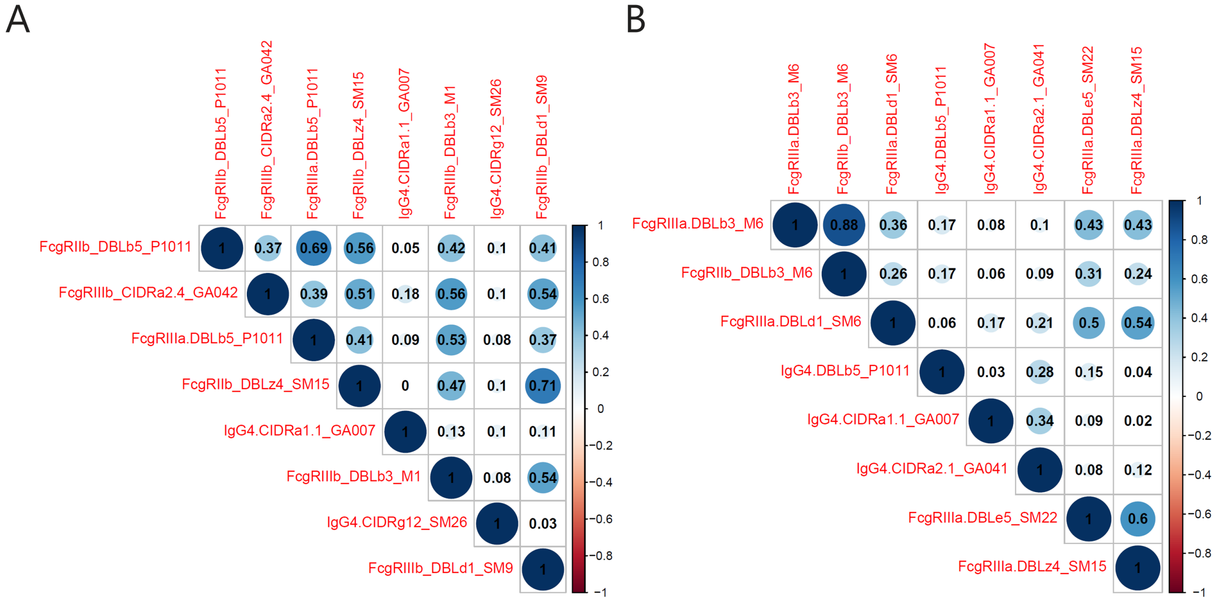


Figure 18: **Correlation of eight selected features that distinguish between severe and uncomplicated malaria at (A) acute and (B) convalescence.** Circle size and shade represents strength of correlation, determined by Spearman's correlation coefficient, R (numbers on circles). Circle colour represents negative (red) or positive (blue) correlation.

Amongst the eight selected features for acute phase analysis (Figure 18A), there were weak - moderate positive correlations between Fc receptors (Spearman correlation coefficient $r = 0.37 - 0.56$) and strong correlations between FcγRIIb and FcγRIIa binding antibodies targeting DBLβ5_P1011 ($r = 0.69$), and FcγRIIb binding antibodies targeting DBLζ4_SM15and FcγRIIIb binding antibodies targeting DBLδ1_SM9 (0.71). There were no correlations between IgG4, or between IgG4 and Fc receptors binding antibodies.

Amongst the eight selected features for the convalescent analysis (Figure 18B), there were weak – moderate positive correlations between Fc receptors (r = 0.26 – 0.54) and strong correlation between FcγRIIIa and FcγRIIb binding antibodies to DBLβ3_M6 (r= 0.88) and between FcγRIIIa binding antibodies to DBLε5_SM22 and DBLzζ4_SM15 (r= 0.6). There were weak correlations between IgG4 to CIDRα2.1_GA041 and CIDRα1.1_GA007 (r= 0.34), and IgG4 to CIDR2.1_GA041 and DBLβ5_P1011 (r= 0.28).

10 Discussion

10.1 Overview

PfEMP1 is a key factor in the pathogenesis of severe malaria and there is evidence that antibodies targeting PfEMP1 are associated with protection. Previous studies have identified domains of PfEMP1 that are more commonly expressed in parasites causing severe malaria or uncomplicated malaria (Lavstsen et al. 2012; Lennartz et al. 2017; Tonkin-Hill et al. 2018) and have identified PfEMP1 target domains for which IgG titers are associated with protection from severe malaria, including in PNG and Papua (Rambhatla et al. 2019; Rambhatla et al. 2022; Tessema et al. 2019). In this study, we probed a broad range of previously identified potential PfEMP1 antibody targets that are associated with severe and uncomplicated malaria and characterized a broad range of antibody Fc features by multiplex immunoassays. We used machine learning analysis to identify a combination of antibody features that best discriminates between severe and uncomplicated malaria in PNG children at the acute stage of infection and at convalescence. We assessed the performance of the selected features to classify individuals using cross validation. Antibodies targeting an ICAM-1 binding DBLβ3 domain, a DBLδ1 domain associated with EPCR binding PfEMP1 and an EPCR binding CIDRα1.1 domain were amongst the top features selected for disease classification at both the acute and convalescent time points. Antibodies targeting CIDRα1 domains were boosted between presentation and convalescence in severe malaria, indicating their involvement in severe disease. However in some cases, different antibody Fc features targeting the same antigen were associated with severe and uncomplicated malaria and we observed complex shifts in antibody features from acute presentation to convalescence. In general, IgG titers did not differ between severe and uncomplicated malaria, whereas FcγRIIb, FcγRIIIb and FcγRIIIa binding and IgG4 were the best features selected by machine learning to distinguish between the groups. The Fc receptors identified are abundant on neutrophils and NK cells, suggesting an important role of these cells in the immune response to pediatric malaria.

10.2 Cohort of PNG children

Children are a valuable cohort to study antibodies to VSAs as they have limited prior exposure compared to adults and make up the majority of severe malaria cases. The advantages of access to samples from this cohort are that it enabled us to study antibody response to PfEMP1 in a region outside of Africa and that there were samples taken at two-time points available (presentation and convalescence). The original study cohort were matched for age and sex (Manning et al. 2011), however, samples for this study were chosen based on availability and there were important differences in children with severe and uncomplicated malaria that may affect the results.

First, children with severe malaria were younger than children with uncomplicated malaria. This is important because antibodies to VSAs are acquired with age in high transmission settings (Barry et al. 2011; Cham et al. 2009) therefore age may confound the association of antibodies with uncomplicated and severe malaria. We adjusted for age in both univariate and multivariate analysis by including age as a covariate. The coastal area surrounding the sample collection site (Modilon hospital, Madang) has high transmission of malaria (estimated entomological inoculation rate (EIR) of 37 per year for *P. falciparum* at the time of study collection (Michon et al. 2007)), although children with severe malaria may have been referred to Modilon Hospital from a broader catchment area. However, PNG has highly heterogeneous transmission dynamics (Cleary et al. 2022) that can vary between nearby villages as well as within villages (Greenwood 1989). Therefore, there may also be differences in prior exposure that are not captured by differences in age.

To assess non-age related variation in exposure, we measured IgG to MSP2 and AMA1. Antibodies targeting AMA1 and MSP2 are acquired early in life in high transmissions settings (McCallum et al. 2017; Stanisic et al. 2015) and are useful to evaluate differences in prior exposure at a population level (Drakeley et al. 2005; McCallum et al. 2017). There is some evidence they can be used at an individual level to evaluate recent exposure (including in PNG) (McCallum et al. 2017; Stanisic et al. 2015; Yman et al. 2022) but this may be limited to young children, who have low levels of antibodies that do not contribute to protection (Stanisic et al. 2015). At the acute timepoint, there were no statistically significant differences in the mean IgG titers to AMA1 or MSP2 in children with severe or uncomplicated malaria. There were some differences in antibody Fc features that remained significant after adjusting for age (FcγRIIIb, FcγRIIIb to AMA1 and FcγRIIIb to MSP2), although the effect of exposure on these responses is unknown. At convalescence, the mean IgG titers (as well as other Fc features) to AMA1 and MSP2 were significantly higher in uncomplicated malaria compared to severe malaria and the difference remained statistically significant after adjusting for age. The differences in IgG responses to merozoite antigens suggests greater prior exposure in children with uncomplicated malaria compared to severe malaria amongst the convalescent samples, independent of age. This may confound the association between antibodies to PfEMP1 antigens and severe malaria in our analysis.

Additionally, individuals were not matched for ethnicity in the original study and a greater portion of children with severe malaria were of non-Madang ethnicity (included Sepik and other ethnicities). There are a large number of genetic polymorphisms that have been selected for in populations living in malaria endemic areas, including PNG. Polymorphisms that have a high prevalence in PNG include α-thalassemia, Southeast Asian ovalocytosis and complement receptor 1 deficiency. These polymorphisms have been associated with protection from severe malaria (Allen et al. 1999; Cockburn et al. 2004; Genton et al. 1995; Manning et al. 2011) but not uncomplicated malaria (Rosanas-Urgell et al. 2012). Due to the diverse transmission dynamics in PNG, polymorphisms have been shown to be prevalent in individuals in high transmission settings and absent in those in low transmission settings (Cockburn et al. 2004; Oppenheimer et al. 1984; Yenchitsomanus et al. 1985; Yenchitsomanus et al. 1986) and this is likely to lead to ethnicity linked differences in prevalence. The presence of genetic variants is not known to affect the age specific acquisition of antibodies to VSAs (Fowkes et al. 2008) but may still contribute to variation between the SM and UM groups of our study that is not explained by antibodies to VSAs.

10.3 Targets of antibodies associated with severe and uncomplicated malaria.

We hypothesized that children with severe malaria lack antibodies to specific PfEMP1 and aimed to identify a combination of antibody features that can be used to identify children with severe or uncomplicated malaria. The difficulty with samples taken at the acute phase of infection is that antibody levels may reflect both prior exposure and the current infection. Nevertheless, in Part IV, we were able to use acute phase samples to identify a combination of antibody features that correlated with clinical presentation in Malawian children. Here, the combination of 8 features chosen to predict clinical outcome at the acute time point included features associated with both increased odds of severe and uncomplicated malaria. The targets of antibodies that were associated with an increased odds of uncomplicated malaria included a group A DBLβ3, that has the ICAM-1+EPCR dual binding motif, a group B CIDRα2.4 that binds CD36, CIDRγ12 and DBLδ1 that are upregulated in severe malaria.

Expression of DBLβ3 that contain the dual ICAM-1+EPCR binding motif has been associated with cerebral malaria in some studies (Tuikue Ndam et al. 2017; Lennartz et al. 2017) but not all studies (Joste et al. 2020). In our previous study of Malawian children, antibodies to DBLβ3 were represented in four out of five features were associated with increased odds of uncomplicated malaria compared to cerebral malaria. In this PNG cohort, 13% of children are predicted to have had cerebral malaria (Blantyre coma score of 0 – 2) and it is possible that antibodies targeting DBLβ3 are included in the top selected features because they accurately describe this subset of children. In other studies, antibodies to DBLβ3 domains have been associated with protection from severe malaria (Oleinikov et al. 2012), severe anaemia (Badaut et al. 2021) and uncomplicated malaria (Kanoi et al. 2018) and can block adhesion to ICAM-1 *in vitro* (Jensen et al. 2004). In line with our results, a previous study in PNG found that antibodies to DBLβ3 that contain the dual binding motif are associated with protection from high parasitaemia (Tessema et al. 2018). Therefore both the current study and prior studies suggest that antibodies to DBLβ3 play a role in protection from severe malaria pathologies beyond cerebral malaria in PNG.

Expression of CD36 binding domains is generally associated with uncomplicated malaria (Mkumbaye et al. 2017a; Rogerson et al. 1999; Tuikue Ndam et al. 2017), however, antibodies to both EPCR binding and CD36 binding CIDRα have been associated with prospective protection from febrile malaria in Uganda (Kanoi et al. 2018) and severe malaria in PNG (Tessema et al. 2019) and are elevated in uncomplicated malaria compared to cerebral malaria in Mali (Travassos et al. 2018). Other studies have found no evidence of an association with protection (Rambhatla et al. 2019; Travassos et al. 2018). CD36 binding domains can bind platelets that form a bridge to human brain endothelial cells (Pain et al. 2001) and it is possible that antibodies may protect from severe disease by disrupting this process. The great majority of PfEMP1s contain CD36 binding CIDR domains (Mkumbaye et al. 2017a) and it is possible that antibodies may contribute to a general reduction in parasite sequestration to prevent progression to severe malaria.

Little is known about the other targets of antibodies that were associated with increased odds of uncomplicated malaria at the acute time point. A CIDRγ12 was found in the head structure of a rosetting parasite line (Ghumra et al. 2011) but little is known of its functions. Antibodies to CIDRγ12 were amongst the most useful correlates of protection in Malawian children with cerebral and uncomplicated malaria and in Papua adults with severe and uncomplicated malaria (Rambhatla et al. 2022), suggesting they are a novel important target of protective antibodies. Antibodies to a DBLδ1 variant were associated with a decreased parasite burden in Malian children (Araj et al. 2021) whereas in Malawi, antibodies to a DBLδ1 were associated with cerebral malaria (Part IV). DBLδ1 are difficult to study due to their high sequence diversity (Otto et al. 2019) and the limited studies of DBLδ1 suggest they have diverse functions. One study identified variants of DBLδ1 that bind αVβ3 and αVβ6 integrins and may strengthen adhesion to endothelial cells or innate immune cells (Chesnokov et al. 2018). In contrast, another study identified a DBLδ1 with a thrombin cleavage site that may reduce sequestration in cerebral vasculature (Gillrie et al. 2016). The DBLδ1 that appeared amongst the top correlates in our study (SM9) is a part of DC8 and may be an immunogenic marker of upstream EPCR binding PfEMP1.

The protein array included several examples of EPCR and CD36 binding CIDRα domains. The antibody targets associated with an increased odds of severe malaria included an EPCR binding domain, CIDRα1.1. By univariate comparison, there were three CIDRα domains for which antibody features were elevated in uncomplicated malaria compared to severe malaria at the acute timepoint, including two EPCR binding domains (CIDRα1.4 and 1.6) and one CD36 binding domain (CIDRα2.4). CIDRα1.1 can be found in DC13, CIDRα1.4 can be found in DC8 and CIDRα1.6 can be found in DC4. Transcription of DC8, DC13 and individual EPCR binding domains are elevated in severe malaria (Bertin et al. 2013; Jespersen et al. 2016), including in PNG (Duffy et al. 2016), as well as in specific severe pathologies including cerebral malaria (Joste et al. 2020; Kessler et al. 2017; Storm et al. 2019; Tuikue Ndam et al. 2017), severe malarial anaemia (Duffy et al. 2019; Jespersen et al. 2016) and respiratory distress (Lavstsen et al. 2012). In PNG, antibodies to EPCR binding domains have been associated with uncomplicated malaria compared to severe malaria (Rambhatla et al. 2019) and antibodies to DBLα domains upstream of EPCR binding CIDRα domains have been associated with reduced risk of severe malaria (Tessema et al. 2019). In Papua, IgG to CDIRα1.1 was amongst the three antibody targets that best correlated with uncomplicated malaria (Rambhatla et al. 2022). In this study, the inconsistency in the association of EPCR binding domains with either susceptibility to or protection from severe malaria may be dependent on the Fc feature measured, as will be discussed in the following section.

Of the eight selected features that differentiated between severe and uncomplicated malaria at convalescence, seven were associated with increased odds of uncomplicated malaria. Similarly to the acute phase, antibodies targeting DBLβ3, DBLδ1 and a CIDRα2.1 were associated with increased odds of uncomplicated malaria. That antibodies were not acquired to these domains following an infection may reflect a poor response to these targets in children with severe malaria, or a lack of exposure to these targets in children with severe malaria that may have left them vulnerable to future infection with these variants. Surprisingly, the two additional targets, DBLβ5 and DBLζ4, were associated with severe malaria at the acute phase and associated with uncomplicated malaria at convalescence. DBLζ4 is a group B domain that is part of DC9 (DBLγ3-DBLζ4). The function of DBLζ is unknown, other than that DBLζ of specific *var* genes can bind the Fcμ region of IgM to mediate rosetting (Stevenson et al. 2015a). Expression of DBLζ are upregulated in adult severe malaria (Bernabeu et al. 2016; Tonkin-Hill et al. 2018), pediatric severe malaria (Mkumbaye et al. 2017b) and also pediatric uncomplicated malaria compared to severe anaemia (Lavstsen et al. 2012). These differences may depend on whether the DBLζ is downstream to an EPCR or CD36 binding region. Antibodies targeting DBLζ4 were higher in uncomplicated malaria compared to severe malaria in Papuan adults (Rambhatla et al. 2022). In our study, features targeting DBLζ4 decreased from acute to convalescence amongst children with severe malaria but not uncomplicated malaria, which may explain the changing associations with severe malaria at the acute phase, and uncomplicated malaria at convalescence. DBLβ5 is a group B ICAM-1 binding domain that is expected to be downstream to a CD36 binding CIDR and associated with uncomplicated malaria (Olsen et al. 2018). There were no detectable differences in features targeting DBLβ5 from acute to convalescence however there are differences in the Fc features targeting these domains at the different time points, as will be discussed in the following section.

Given that children are likely to acquire antibodies to infecting variants between presentation and convalescence, we aimed to use changes in antibodies over time to identify variants associated with severe disease. Univariable comparison revealed that antibodies to EPCR binding CIDRα1 domains (1.1 and 1.4) were boosted by greater than two folds from acute severe malaria to convalescence. In uncomplicated malaria, antibodies to both a CD36 binding CIDRα2.2 and an EPCR binding CIDRα1.4 were boosted from acute to convalescence. This suggests that severe malaria is caused by a more restricted phenotype than uncomplicated malaria and is in line with a role of EPCR binding CIDRα1 domains in severe pathology. Interestingly, a broad range of antibody features decayed from the acute phase to convalescence in both groups and many of the features that decayed were the same in severe and uncomplicated malaria. A decay in antibodies targeting CIDRα1.7, CIDRα1.5, CIDRα2.2, DBLβ7 and DBLζ4 was unique to children with severe malaria and a decay in antibodies to CIDRα1.6 was unique to children with uncomplicated malaria. The decay in a greater number antibody features amongst children with severe malaria compared to uncomplicated malaria may contribute to the greater number of univariate differences between severe and uncomplicated malaria at convalescence, compared to acute malaria. The significance of the target antigens for which antibodies decayed from acute to convalescence is unclear. One explanation is that the decay in antibodies may represent low affinity antibodies to non-infecting variants that are cross reactive to other infecting variants (Ofori et al. 2002). Despite high sequence diversity, there is some evidence that naturally acquired antibodies targeting CIDRα1 domains are cross reactive (shown between CIDRα1.1 and CIDRα1.4) (Lau et al. 2015). Alternatively, the decay may represent a transient antibody response to infecting variants. Previous studies have reported short lived antibodies to PfEMP1 (Kinyanjui et al. 2003; Olsen et al. 2019) and other *Plasmodium* antigens (Akpogheneta et al. 2008; Crompton et al. 2010), relative to decades long lived antibodies developed in some viral and bacterial pathogens (Amanna et al. 2007). Following expansion of antibody secreting cells in the acute phase of infection, B cells quickly undergo contraction once the pathogen is controlled for, and surviving antibody secreting cells are converted to long lived plasma cells and memory B cells (Pérez-Mazliah et al. 2020). The decay in antibodies over 8 weeks may be representative of the contraction phase and loss of short lived antibody secreting cells (Pérez-Mazliah et al. 2020). A loss of antibodies following infection may also be indicative of poor induction of long lived plasmablasts and memory B cells, although maintenance of memory B cells does not necessarily correlate with detectable antibodies (Amanna et al. 2007; Ndungu et al. 2012). Anaemia is associated with impaired memory B cell and plasmablast formation and iron availability enhances B cell proliferation in vitro (Hill et al. 2020). It is possible that a lack of memory B cell formation due to anaemia contributes to the greater decay in antibodies amongst children with severe malaria in this study. Young age (Akpogheneta et al. 2008; White et al. 2014) and altered cytokine profiles that impact B cell survival (Nduati et al. 2011) can also affect antibody longevity. Without understanding the mechanisms of antibody decay, it is difficult to conclude whether examining changes in antibodies from acute to convalescent can be used to accurately identify variants associated with severe malaria.

In summary, the antibody targets that were selected by machine learning to differentiate between children with severe or uncomplicated malaria included DBLβ3, CIDRγ12, DBLδ1 and CIDRα2, CIDRα1.1, as well as DBLβ5 and DBLζ4. EPCR binding CIDRα1 are also likely to be involved in infection with severe malaria, as evidenced by a lack of antibodies to CIDRα1 domains at the acute phase of infection and acquisition of antibodies to CIDRα1 domains in convalescence. However, antibodies to domains associated with both ECPR and CD36 binding PfEMP1 might contribute to protection from severe malaria. Antibody responses to some antigens were associated with both severe and uncomplicated malaria, possibly due to differences in the type of antibody response, as will be discussed below. Antibodies to multiple target antigens decayed over 8 weeks in both groups, particularly amongst children with severe malaria, making it difficult to identify infecting variants based on antibodies.

10.4 Fc features associated with severe and uncomplicated malaria.

Systems serology is based on the idea that Fc features may be better correlates of protection than antibody titers and we hypothesized that this would be true for protection from severe malaria in children. In this study, IgG to only one antigen was elevated in uncomplicated malaria at the acute phase and (in line with our hypothesis) the majority of univariable differences between severe and uncomplicated malaria were antibody Fc features. Amongst the top features selected to classify disease severity, FcγRIIIb was in three of the four features associated with acute uncomplicated malaria. At convalescence, three of the seven features associated with increased odds of uncomplicated malaria were FcγRIIIa. When comparing acute to convalescence, FcγRIIIb and FcγRIIb features decayed and FcγRIIIa features were boosted, in both the severe and uncomplicated malaria groups. The switch from favouring FcγRIIIb to FcγRIIIa between acute and convalescence was surprising, given that FcγRIIIb and FcγRIIIa share 97% sequence identify in the extracellular region (Roberts et al. 2018) and that there was a strong correlation between Fc receptor responses amongst the top selected features. One possible explanation is that there is a shift in antigen specific IgG subclass towards IgG2 and IgG4 that have higher affinity for FcγRIIIa than FcγRIIIb (Gillis et al. 2014). When comparing acute to convalescence, IgG, IgG1 and IgG2 were boosted to CIDRα1 amongst children with severe malaria, and IgG1 to CIDRα2.2 was boosted in uncomplicated malaria, but otherwise there were no detectable changes in antibody subclasses by our chosen analysis method.

An alternative explanation for the changes in antibody features over the course of infection is that there are changes in the glycosylation patterns on the antibody Fc region. Post transcriptional modification of antibody Fc glycosylation also influences affinity to FcγRIII and may change over the course of infection. Antibodies to membrane bound antigens, including the VAR2CSA variant of PfEMP1 (Larsen et al. 2021), become afucosylated with exposure and this enhances FcγRIII affinity (Oosterhoff et al. 2022). The enhanced affinity is more apparent in FcγRIIIb than FcγRIIIa (Subedi et al. 2016), although the addition of galactose enhances the effect of afucosylation in FcγRIIIa more than FcγRIIIb (Dekkers et al. 2017). It is possible that a specific glycosylation pattern may explain the affinity for FcγRIIIb at the acute phase and FcγRIIIb following exposure. FcγRIIIa/b affinity also varies with Fc receptor glycosylation patterns (Roberts et al. 2018) but the recombinant FcγRIIIa and FcγRIIIb used in this study were produced in the same expression system and are likely to have consistent glycosylation patterns. Given that changes in FcγRIIIa and FcγRIIIb occurred in both uncomplicated and severe malaria, this Fc feature signature may not necessarily be a ‘protective’ signature but rather a trend in innate immune function over the course of infection.

Although the reason for the shift in FcγRIIIa/b binding affinity is unclear, the selection of these Fc features has interesting implications. FcγRIIIa is predominantly expressed on NK cells and has an immunoreceptor tyrosine-based activation motif (ITAM) that promotes NK cell activation, cytokine production and release of cytotoxic granules (Gillis et al. 2014). Antibodies targeting VAR2CSA induce NK cell activation and degranulation (Damelang et al. 2021) and antibodies targeting the IE promote γδT cell activation (Farrington et al. 2020), but the association with protection from severe disease in children is unknown. FcγRIIIa is also expressed on intermediate monocytes that are expanded in severe malarial anaemia (Ogonda et al. 2010) where activation is thought to contribute to ADCP (Zhou et al. 2015). FcγRIIIb is predominantly expressed on neutrophils with a glycosylphosphatidylinositol (GPI) anchor that does not contain an intracellular domain (Gillis et al. 2014). Some studies have hypothesized that it acts as a decoy receptor to limit activation, however others have proposed it couples membrane bound CR3 to active granulocytes (Stöckl et al. 1995) or cooperates with FcγRIIa to promote ADNP (Garcia-Senosiain et al. 2021) (but not ROS production (Kapelski et al. 2014)). FcγRIIIb responses were generally associated with uncomplicated malaria at both the acute phase and convalescence. In line with our results, FcγRIIIb binding antibodies targeting DBLβ domains were also associated with acute uncomplicated malaria compared to cerebral malaria in the Malawian cohort. Antibodies enhance ADNP of ICAM-1 binding IE (Zelter et al. 2022). ADNP of VAR2CSA expressing IE has been associated with protection from placental malaria (Aitken et al. 2021) and ADNP of merozoites has been associated with protection from uncomplicated malaria (Garcia-Senosiain et al. 2021). Interestingly, activated neutrophils were found to be associated with pathology in retinopathy positive cerebral malaria compared to retinopathy negative malaria (Feintuch et al. 2016), suggesting that different facets of neutrophil function may have deleterious consequences and regulation of neutrophil activity may be important. One regulatory factor, FcγRIIb, is found on neutrophils and monocytes and contains an immunoreceptor tyrosine-based inhibition motif (ITIM) that engages with ITAM Fc receptors to dampen the activation response (Gillis et al. 2014). FcγRIIb was highly correlated to FcγRIIIb and FcγRIIIa and was associated with both severe and uncomplicated malaria in the selected features at acute and convalescence.

In addition to Fc receptor binding to antigen specific antibodies, IgG4 was frequently selected by machine learning as one of the best classifiers of disease severity. IgG4 was generally expressed at low levels, similar to the Melbourne controls, such that few people were considered seropositive. It is possible that IgG4 binds with higher affinity to some antigens, such as CIDRα1.1, than the other subclasses. IgG4 responses were associated with both severe and uncomplicated malaria, suggesting it may be both protective and detrimental. This has been mirrored in studies of merozoite antigens, where IgG4 has been associated with reduced risk of uncomplicated malaria (Nebie et al. 2008) and susceptibility to cerebral malaria compared to uncomplicated malaria (Mbengue et al. 2019b). IgG4 binds with low affinity to Fc receptors and C1q (Gillis et al. 2014) and may dampen the immune response by blocking binding sites for cytophilic subclasses IgG1 and IgG3. A dampened response to some antigens (DBLβ5, CIDRα2.1, CIDRγ12) may be beneficial to prevent excessive inflammation but may also prevent important immune clearance mechanisms, such as phagocytosis, of other PfEMP1 variants (CIDRα1.1). This may explain why IgG4 targeting CIDRα1.1 was associated with increased odds of severe malaria at both the acute and convalescent time points. IgG4 was also amongst the top features selected to classify cerebral and uncomplicated malaria in the Malawian children, where we hypothesized it was a marker of repeated exposure in Part IV. Class-switching towards IgG4 with repeat exposure has been classically described in beekeepers who are repeatedly exposed to bee venom antigen (Allen 2022), and more recently described as a result of repeat exposure to mRNA vaccination against SARS-CoV2 (Pillai 2023). Class switching towards IgG4 with repeat exposure would explain why FcγRIIb and FcγRIIIb binding antibodies to DBLβ5 were associated with severe malaria at the acute time point, possibly representing a first-time exposure, but IgG4 targeting DBLβ5 was associated with uncomplicated malaria at convalescence, possibly following a repeat exposure. However, there were no detectable changes in antigen specific IgG4 from acute to convalescence, suggesting that IgG4 responses were antigen driven rather than time dependent. IgG4 is favoured in asymptomatic helminth infections, such as schistosomiasis or lymphatic filariasis (Jassim et al. 1987; Mishra et al. 2019) and may skew the antibody response to other antigens towards IgG4 (Mishra et al. 2019). However, mass drug administration of diethylcarbamazine plus albendazole was conducted around the time of sample collection and dramatically reduced the incidence of filariasis in the Madang region (Weil et al. 2008).

In summary, Fc features were more useful correlates of disease severity than IgG titers. FcγRIIIa, FcγRIIIb, FcγRIIb and IgG4 responses were the top selected features used to identify children with severe and uncomplicated malaria. FcγRIIIb was generally associated with uncomplicated malaria whereas FcγRIIIa, FcγRIIb and IgG4 did not favour severe or uncomplicated malaria. FcγRIIIb responses were prevalent at the acute phase and decayed to convalescence, whilst FcγRIIIa responses were boosted from acute to convalescence. This could promote activation of neutrophils at the acute phase to remove infecting variants via ADNP, followed by activation of NK cells and intermediate monocytes towards the resolution of infection. The role of IgG4 in malaria in children is unclear but it appears to be a useful marker that should not be ignored in studies of blood stage immunity to malaria.

10.5 Study strengths and limitations.

A strength of this study was the use of machine learning analysis as an unbiased method to select the top correlates of disease presentation. Overall, antibody features to PfEMP1 were effective markers of disease severity, such that even eight randomly selected features could classify children as having severe or uncomplicated malaria with above 50% accuracy (57% accuracy at the acute phase and 67% accuracy at convalescence). Eight features were chosen to assess the classification performance of the most frequently selected features by Elastic Net regularized Logistic regression. However, the overall PLSDA model performance did not noticeably improve with the addition of each of the eight features and it may be possible to further optimize the model to reduce the number of selected features with only a small cost to the classification accuracy. Furthermore, the addition of many of the features improved the classification accuracy for the uncomplicated malaria group but non severe malaria group (particularly at convalescence). Were this model to be developed into a predictive tool for clinical diagnosis, it may be a priority to classify susceptibility to severe malaria alone and therefore a smaller number of features could be selected. Additionally, the features were added to the PLSDA model in order of frequency of selection, however there were 22-23 features that appeared in greater than 70% of the Elastic Net regularized regression modeling and other combinations of features that have not been assessed here may have similar classification performance.

Another strength of this study is the use of a broad selection of proteins associated with severe and uncomplicated malaria and the inclusion of multiple examples of proteins with the same binding phenotype, which increased the chances of covering isolates circulating in the region at the time of sample collection. The limitations of recombinant proteins include the possibility of incorrect protein folding and excluding epitopes that may span multiple domains.

Another strength is the inclusion of follow up convalescent samples which allowed us to identify changes in antibody features over a single infection and to identify targets for which antibodies were boosted during infection. Changes of antibodies over time were identified by univariate logistic regression, however more advanced modelling such as linear mixed effects modelling may be more appropriate to account for random variation in the dataset (Hasdemir et al. 2020). Antibodies to many antigens decayed rapidly, and it is possible that children with uncomplicated malaria had a severe episode following the study and were therefore not immunologically protected. In our case-control study design, we were unable to determine long term associations of antibodies and protection.

There were additional limitations in the samples included in this study. The original cohort was well matched for age and sex, however for this study we had limited sample availability that meant there were important differences in the groups. Differences in age and ethnicity may have been emphasized when down selecting samples for the multivariate regression analysis. Whilst we controlled for age in univariate regression analysis and PLSDA, future analysis could consider better ways to control for exposure, such as including the MSP2/AMA1 responses as co-variates. Other studies have controlled for exposure by classifying children based on the number of new parasite clone infections over a period of time (molecular Force of Infection) (Mueller et al. 2012; Tessema et al. 2019), but this requires longitudinal follow-up samples and PCR analysis. Finally, the children with severe malaria had a broad range of pathologies but time constraints and study power prevented further investigation of individual malaria subsets. A previous study in the same cohort of antibodies to CIDRα domains did not find differences in uncomplicated malaria compared to subsets of severe malaria syndromes (e.g. cerebral malaria, anaemia or acidosis) (Rambhatla et al. 2019). However in this study we have considered a broad range of antibody responses and PfEMP1 targets and stratification by severe pathology may reveal more specific antibody signatures.

In conclusion, this study has laid the groundwork for further development of a predictive tool to identify children with severe and uncomplicated malaria that may be useful for diagnostics and surveillance of severe malaria in PNG, or may inform the development of therapeutic interventions such as multivalent vaccines and monoclonal antibody cocktails.

Part VI

Antibody dependant monocyte and neutrophil phagocytosis of ICAM-1 binding IE and protection from severe malaria

11 Background

Cytoadherence is key to the pathogenesis of malaria and is mediated by PfEMP1 on the surface of IE binding to various endothelial cell receptors. ICAM-1 is one such adhesion receptor that is widely expressed on the brain endothelium of individuals with fatal cerebral malaria (Turner et al. 1994). There are two types of ICAM-1 binding PfEMP1: dual ICAM-1+ECPR binders and dual ICAM-1+CD36 binders. EPCR binding IE have been associated with cerebral malaria and severe malaria (Jespersen et al. 2016; Lavstsen et al. 2012; Mkumbaye et al. 2017b), as has the subset of dual ICAM-1+EPCR binders (Kessler et al. 2017; Lennartz et al. 2017; Tuikue Ndam et al. 2017). Dual ICAM-1+EPCR binding IE are thought to be particularly important in the pathogenesis of cerebral malaria, by strengthening adhesion of IE to brain endothelial cells and possibly disrupting the protein-C pathway required for maintenance of the blood brain barrier (Jensen et al. 2020). CD36 binding IE are more common but are not associated with severe malaria and CD36 is not ubiquitously expressed on brain endothelial cells (Mkumbaye et al. 2017b; Rogerson et al. 1999; Turner et al. 1994). The subset of dual ICAM-1+CD36 binding IE are more common in cases of uncomplicated malaria than severe malaria (Tuikue Ndam et al. 2017).

Antibodies targeting the recombinant domains of ICAM-1+EPCR dual binders have been associated with protection from uncomplicated malaria, severe malaria (Kanoi et al. 2018; Oleinikov et al. 2012; Tessema et al. 2018) and cerebral malaria in some studies (Travassos et al. 2018) but not others (Joste et al. 2020). There is a lack of studies that have measured antibodies to the whole PfEMP1 on the surface of IE that bind both ICAM-1+EPCR (Badaut et al. 2021), which may be more representative of the antibody response *in vivo*.

To better understand the relationship between antibodies targeting ICAM-1 binding IE and disease manifestations we need reliable assays that can best detect a protective antibody response. In malaria in pregnancy, opsonic phagocytosis of the IE was a better correlate of protection that levels of IgG binding to the IE (Aitken et al. 2021). Both monocytes and neutrophils phagocytose opsonized IE by recognition of the antibody Fc region by cell surface Fc receptors. Only a few published studies have considered the association of antibody dependant cell phagocytosis (ADCP) or antibody dependant neutrophil phagocytosis (ADNP) of IE and specific malaria pathologies (Aitken et al. 2021; Ataíde et al. 2011; Chan et al. 2019a; Quintana et al. 2018). ADCP and ADNP of VAR2CSA expressing IE has been associated with protection from placental malaria (Aitken et al. 2021) and ADCP has been associated with increased birthweight following malaria in pregnancy (Ataíde et al. 2011). ADCP of IT4VAR19 IE is elevated in uncomplicated compared to severe malaria (Chan et al. 2019a) but there are no differences for the rosetting parasite line FCR3S1.2 (Quintana et al. 2018). No published studies have considered the association of ADCP or ADNP of ICAM-1 binding IE and severe malaria.

To address these knowledge gaps, we investigated whether ADCP and ADNP of ICAM-1 binding IE are associated with protection from severe malaria in children living in malaria endemic areas. We used IE that were selected to express IT4VAR13 that binds to ICAM-1+CD36 and 3D7VAR04 that binds to ICAM-1+EPCR. IE were opsonized with serum samples from two case-control studies – Malawian children with cerebral malaria or uncomplicated malaria, and PNG children with severe malaria (mixed etiology) or uncomplicated malaria, with samples taken at the acute phase of infection and at convalescence. A well established assay using the monocytic THP-1 cell line was used to measure ADCP (Kassa et al. 2022), and a more recently developed assay using freshly isolated neutrophils from healthy Melbourne donors was used to measure ADNP (Aitken et al. 2021).

11.1 Hypotheses

11.1.1 One

Among children living in malaria endemic areas, levels of antibody that target ICAM-1+EPCR binding infected erythrocytes and induce ADCP by monocytes are higher in children with uncomplicated malaria compared to severe malaria. There are no differences in antibodies that target ICAM-1+CD36 binding infected erythrocytes and induce ADCP by monocytes.

11.1.2 Two

Among children living in malaria endemic areas, levels of antibody that target ICAM-1+EPCR binding infected erythrocytes and induce ADNP are higher in children with uncomplicated malaria compared to severe malaria. There are no differences in antibodies that target ICAM-1+CD36 binding infected erythrocytes and induce ADNP.

11.2 Aims

(i) Select for parasites expressing PfEMP1 variants that are predicted to bind to ICAM-1+EPCR and ICAM-1+CD36

(ii) Measure THP-1 ADCP of ICAM-1+EPCR binding IE and ICAM-1+CD36 binding IE opsonised with sera from Malawian children and determine associations with cerebral malaria and uncomplicated malaria

(iii) Measure THP-1 ADCP of ICAM-1+EPCR binding IE and ICAM-1+CD36 binding IE opsonised with sera from PNG children and determine associations with severe malaria and uncomplicated malaria

(iv) Measure ADNP of ICAM-1+EPCR binding IE and ICAM-1+CD36 binding IE opsonised with sera from Malawian children and determine associations with cerebral malaria and uncomplicated malaria

(v) Measure ADNP of ICAM-1+EPCR binding IE and ICAM-1+CD36 binding IE opsonised with sera from PNG children and determine associations with severe malaria and uncomplicated malaria

(vi) Evaluate donor variability within the ADNP assay and correlations between the ADNP, THP-1 assay and multiplex immunoassays.

12 Results

12.1 Clinical samples used in ADCP and ADNP assays

A detailed description of sample collection and clinical characteristics are provided in Materials Methods Section 7.1.

Malawian children were classified as having cerebral or uncomplicated malaria and the median age across the two groups and two time points ranged from 48 - 56 months old. Acute cerebral and uncomplicated malaria samples were matched for age, precise location of residency and urban/rural setting. Of the convalescent samples, 18 had acute samples available (paired). Convalescent samples were matched by age and urban and rural setting but not by precise location of residency (Table 13.

PNG children were classified as having severe or uncomplicated malaria and the median age across the two groups and two time points ranged from 39 - 45 months old. Children classified as having severe malaria had a range of manifestations: 38% had severe anaemia, 13% had cerebral malaria (BCS 0-2) and 12% had respiratory acidosis. Out of all the convalescent samples, 118 had acute samples available. Children were mostly from the Madang region (86%) but were not matched for precise location of residency (Table 14.

A different number of samples was used for each assay because of low sample availability and exclusions based on poor experimental replicates, as listed in Table 13 and 14.

Table 13: **Summary of Malawian children cohort categorized by disease severity**

Characteristic	**Uncomp. act.**	**Uncomp. con.**	**Cerebral act.**	**Cerebral con.**
n in final assay				
ADCP IT4VAR13	45	31	40	43
ADNP IT4VAR13	44	32	4	42
ADCP 3D7VAR04	53	50	51	47
ADNP 3D7VAR04	37	28	38	43
Age, median [IQR], months				
	48 [26 - 84]	49 [26 - 72]	56 [35 - 83]	55 [24 - 84]
Age group, n %				
0 – 48 months	28 (53%)	23 (46%)	22 (43%)	23 (49%)
49 - 90 months	25 (47%)	27 (54%)	29 (57%)	24 (51%)
Sex, n %				
Female	19 (36%)	15 (30%)	18 (35%)	20 (43%)
Male	34 (64%)	35 (70%)	33 (65%)	27 (57%)
Location, n %				
Urban	26 (49%)		28 (55%)	23 (49%)
Rural	27 (51%)		23 (45%)	24 (51%)
Blantyre Coma Score, median [IQR]				
	5 [5 - 5]		2 [1 – 2]^{b}	2 [1 – 2]^{c}
Retinopathy positive, n (%)				
	0 (0%)		43 (83%)	39 (83%)
	0 (0%)	0 (0%)	1 (2%)^{d}	1(2%)^{e}
Temperature, median [IQR], degrees Celsius				
	38.7 [38-39.1]	36.3 [36.1-36.6]		
Parasitaemia, median [IQR], per μL)				
			3678 [402–28720]	2885 [380–59020]
Haemoglobin, median [IQR], (g/dL)				
	10.4 [8.7-11.7]^{a}	11.2 [10.2-12.1]	8.1 [7.0-9.2]	8 [6.9-9.1]
Severe anaemia (<50 g/dl), n (%)				

Table 13: *Uncomp. and Cerebral*: Uncomplicated malaria and cerebral malaria. *act. and conv.*: acute and convalescent. *n*: number of samples. IQR: Inter Quartile Range. (^{a}) 52 samples with clinical information available for haemoglobin. (^{b}) 24 samples with clinical information available for Blantyre Coma Score (BSC). (^{c}) 24 samples with clinical information available for Blantyre Coma Score (BSC). (^{d}) 48 samples with clinical information available for haemoglobin. (^{e}) 44 samples with clinical information available for haemoglobin.

Table 14: **Summary of PNG children cohort categorized by disease severity**

Characteristic	**Uncomp. act.**	**Uncomp. con.**	**Severe act.**	**Severe con.**
n in final analaysis				
ADCP IT4VAR13	73	88	204	90
ADNP IT4VAR13	73	100	204	86
ADCP 3D7VAR04	73	38	204	87
ADNP 3D7VAR04	73	46	204	70
Age, median [IQR], months				
	45 [30-56]	43[31-58]	40 [29-55]	39 [30-51]
Age group, n (%)				
0 – 48 months	37 (54%)	58 (58%)	138 (68%)	62 (70%)
49 - 90 months	31 (46%)	42 (42%)	66 (32%)	27 (30%)
Sex, n (%) [a]				
Female	32 (47%)	42 (43%)	86 (42%)	42 (47%)
Male	36 (52%)	56 (57%)	118 (58%)	48 (53%)
Ethnicity, n (%) [b]				
Madang	57 (86%)	89 (89%)	138 (71%)	68 (76%)
Other	9 (14%)	11 (11%)	55 (29%)	22 (24%)
Blantyre Coma Score, n (%) [c]				
0-2			25 (13%)	
3			13 (6%)	
4			31 (15%)	
5	73 (100%)	73 (100%)	133 (66%)	
Haemoglobin (g/dL) [d]				
	8.4 [7.6-9.7]	10.6 [9.6-11.5]	7.9 [5.5-9.4]	11.0 [9.6-11.5]
Severe anaemia (<50 g/dl), n (%)				
	0 (0%)	0 (0%)	44 (22%)	0 (0%)
Acidosis, n (%)				
			17 (12%)	
Species, n (%) [e]				
P*f*	35 (78%)		173 (85%)	73 (83%)
P*fv*	10 (22%)		31 (15%)	15 (17%)
Parasitaemia, median [IQR], per μL				
P*f*	20230 [3370-84773]	0 [0-563]	70606 [13935-156711]	0 [0–0]
P*fv*	0 [0–0]	0 [0–69]	0 [0–0]	0 [0–0]

Uncomp. and Severe: Uncomplicated malaria and cerebral malaria. *act. and conv.*: acute and convalescent. *n*: number of samples. IQR: Inter Quartile Range. (a) 98 uncomplicated convalescent samples with clinical information available for Sex. (b) 70 uncomplicated acute and 193 severe acute samples with clinical information available for haemoglobin. (c) 203 severe acute samples with clinical information available for Blantyre Coma Score (BCS). (d) 57 uncomplicated acute, 91 uncomplicated convalescent and 81 severe convalescent samples with clinical information available for Haemoglobin. (e) *Plasmodium* species was determined by PCR and microscopy as *P. falciparum* (*Pf*) or mixed *P. falciparum* and *P. vivax* infection. 40 uncomplicated acute, 175 severe acute and 88 severe convalescent samples with clinical information available for Species. (f) 27 uncomplicated acute, 97 uncomplicated convalescent, 204 severe acute and 87 severe convalescent samples with clinical information available for Parasitaemia.

12.2 Selection of infected erythrocytes that express *var04*

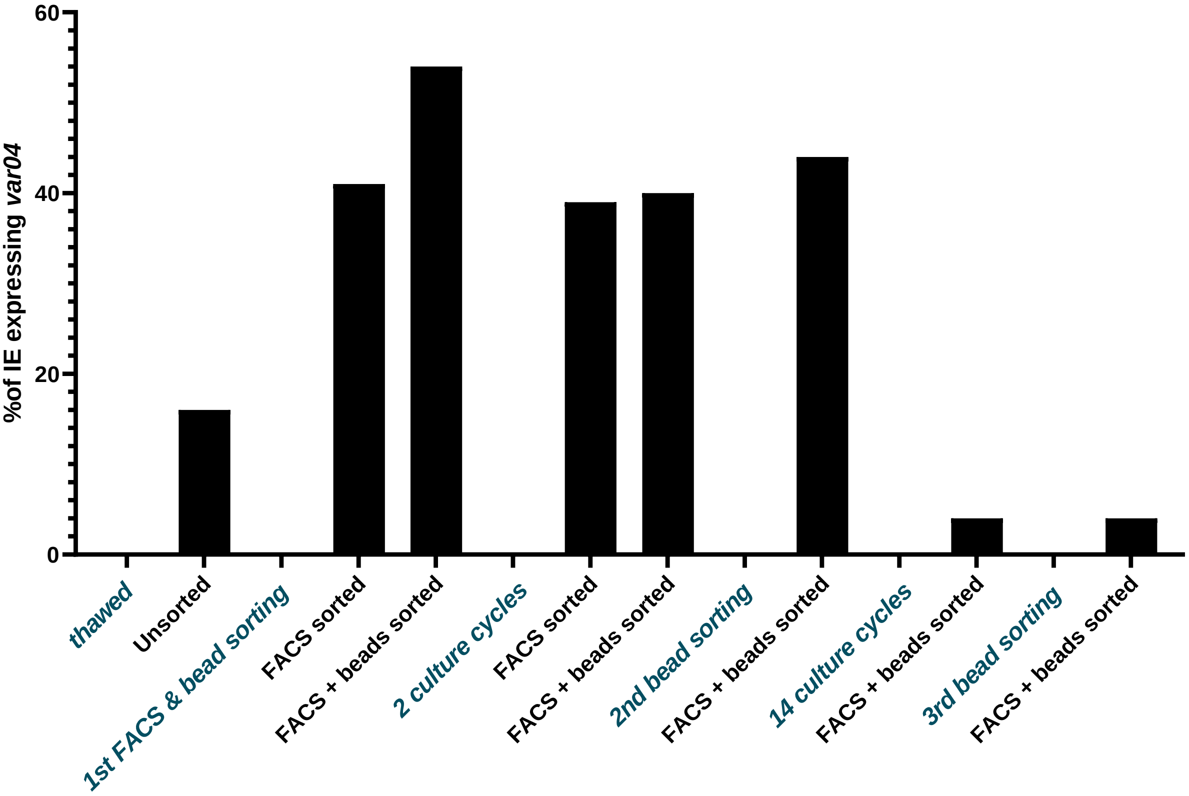


Figure 19: **Percentage of infected erythrocytes expressing *var04* following selection by FACS and antibody coated beads.** Figure shows chronological events from left to right.

Infected erythrocytes that express *var04* were selected by FACS using a monoclonal antibody targeting DBLβ domain of *var04*. Newly thawed 3D7 parasites had 10-16% expression of *var04*. Selection by FACS increased the expression of *var04* by 25 – 32% (Figure 19, 'FACS sorted'). Selection with antibody coated beads further increased the expression of *var04* in parasites that had been pre-selected by FACS, by 4-13% (Figure 19, 'FACS + beads sorted' following '1st bead sorting' and '2nd bead sorting'). Bead selection did not increase *var04* when expression was low to begin with (Figure 19, FACS + beads sorted, following 3rd bead sorting). When left in culture, 3D7VAR04 switched expression of PfEMP1 at an estimated rate of 5.5% per cycle (mean of three experiments, see example in Figure 19, 'FACS + bead sorted' after '14 culture cycles'). The estimated expression of *var04* prior to final experiments using clinical samples ranged from 44 – 55%. IT4 parasites had 76% expression of *var13* when thawed and were not further selected. The estimated expression of *var13* ranged from 36 – 52% prior to experiments.

12.3 Antibody Dependent Cell Phagocytosis (ADCP) of ICAM-1 binding IE opsonized with sera from Malawian children

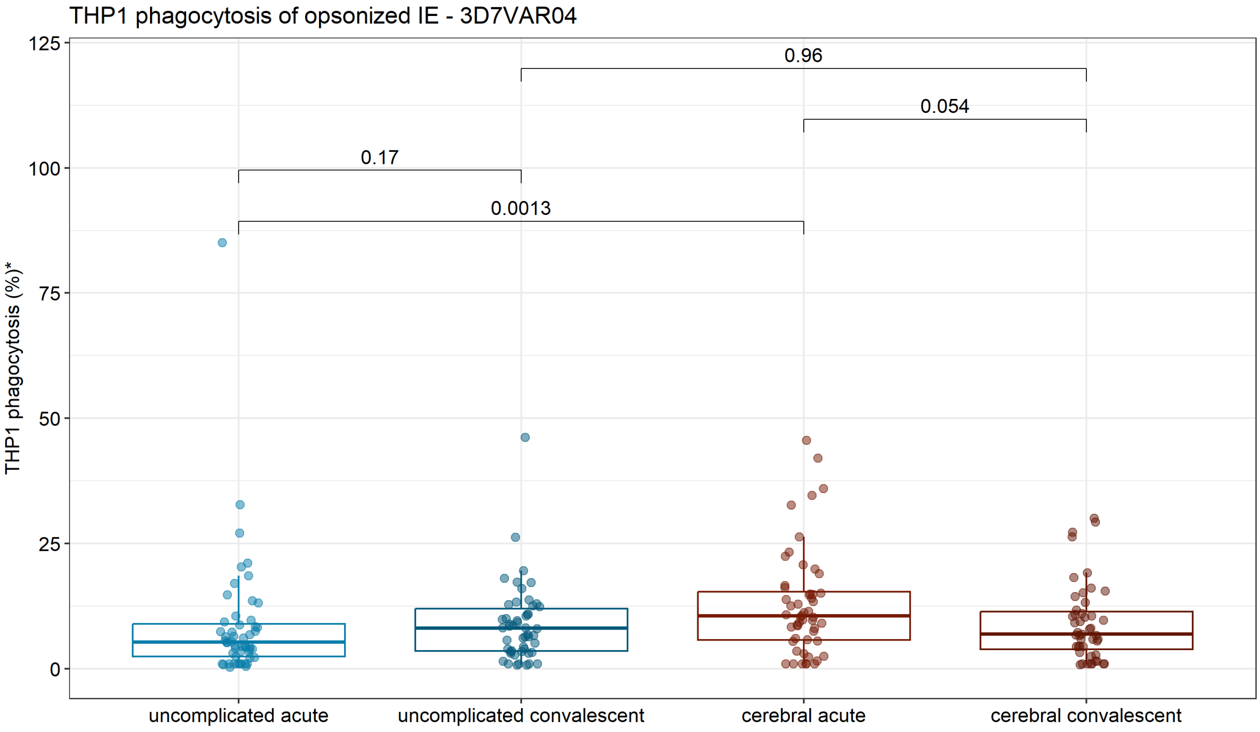


Figure 20: **ADCP of ICAM-1+EPCR binding 3D7VAR04 IE opsonized with sera from Malawian children.** 3D7VAR04 infected erythrocytes were opsonized with sera from Malawian children with cerebral malaria (red) or uncomplicated malaria (blue), collected at the acute phase of infection or at convalescence. Y-axis represents the percentage of DHE stained THP-1 cells, relative to the positive control (* % phagocytosis relative to positive control). Boxes represent median and inter-quartile range (IQR) from Q1-Q3, and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR). Median ADCP levels were compared by Wilcoxon Rank Sum test, indicated by horizontal bars, and associated P-values are shown. There were no corrections for multiple comparisons.

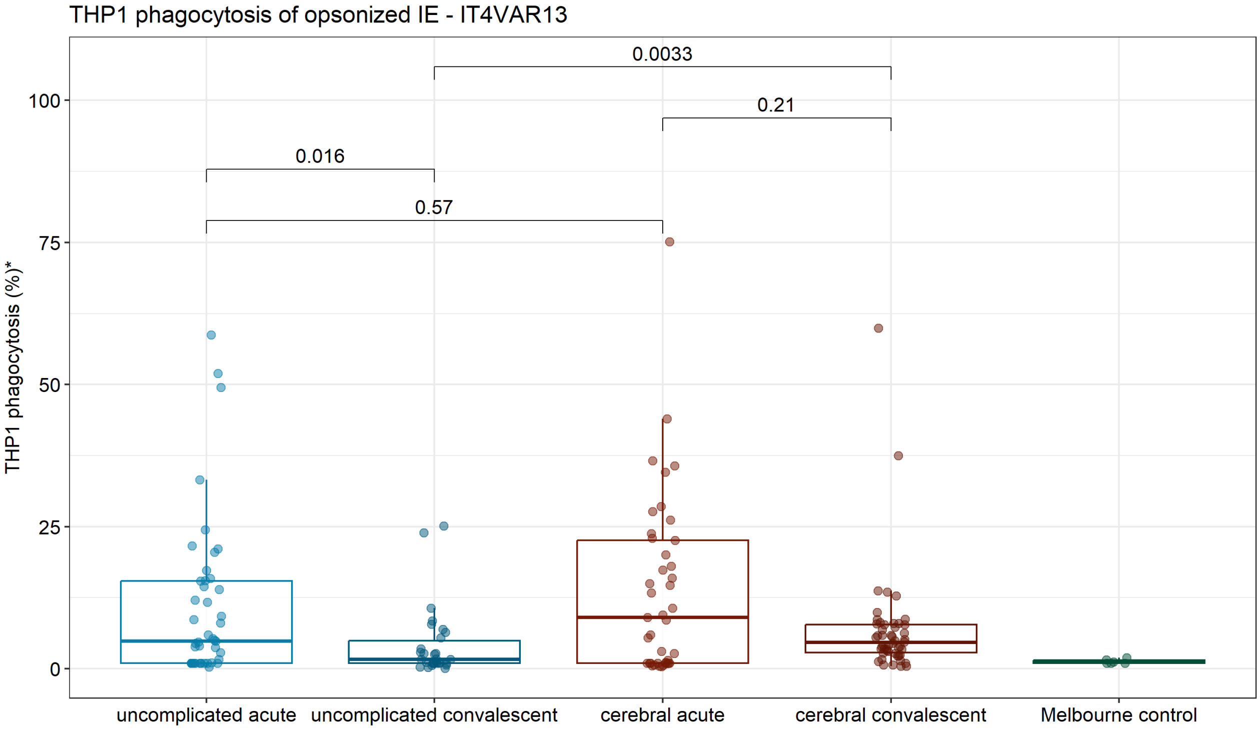


Figure 21: **ADCP of ICAM-1+CD36 binding IT4VAR13 IE opsonized with sera from Malawian children.** IT4VAR13 infected erythrocytes were opsonized with sera from Malawian children with cerebral malaria (red) or uncomplicated malaria (blue), collected at the acute phase of infection or at convalescence. Y-axis represents the percentage of DHE stained THP-1 cells, relative to the positive control (* % phagocytosis relative to positive control). Boxes represent median and inter-quartile range (IQR) from Q1-Q3, and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR). Median ADCP levels were compared by Wilcoxon Rank Sum test, indicated by horizontal bars, and associated P-values are shown. Uncomplicated acute and cerebral convalescent groups were statistically different from the Melbourne control (P-values 0.041 and 0.0024, respectively, not shown on graph) and uncomplicated convalescent and cerebral acute were not significantly different from the Melbourne control (P-values 0.34 and 0.087, respectively, not shown on graph). There were no corrections for multiple comparisons.

We measured ADCP by THP-1 cells of ICAM-1+EPCR binding 3D7VAR04 and ICAM-1+CD36 binding IT4VAR13 selected IE opsonized with sera from Malawian children. Malawian children with acute cerebral malaria had higher ADCP of 3D7VAR04 IE than children with acute uncomplicated malaria (Figure 20). There were no differences in ADCP of ICAM-1+EPCR binding 3D7VAR04 IE in the acute and convalescent samples, and no differences between cerebral and uncomplicated malaria at convalescence. There were no differences in ADCP of IT4VAR13 IE between children with acute cerebral and acute uncomplicated malaria (Figure 21). Children in convalescence for cerebral malaria had higher ADCP of ICAM-1+CD36 binding IT4VAR13 IE than children with uncomplicated malaria. Children with uncomplicated malaria had significantly higher ADCP of IT4VAR13 IE at the acute phase than in convalescence.

12.4 Antibody Dependent Neutrophil Phagocytosis (ADNP) of ICAM-1 binding IE opsonized with sera from Malawian children

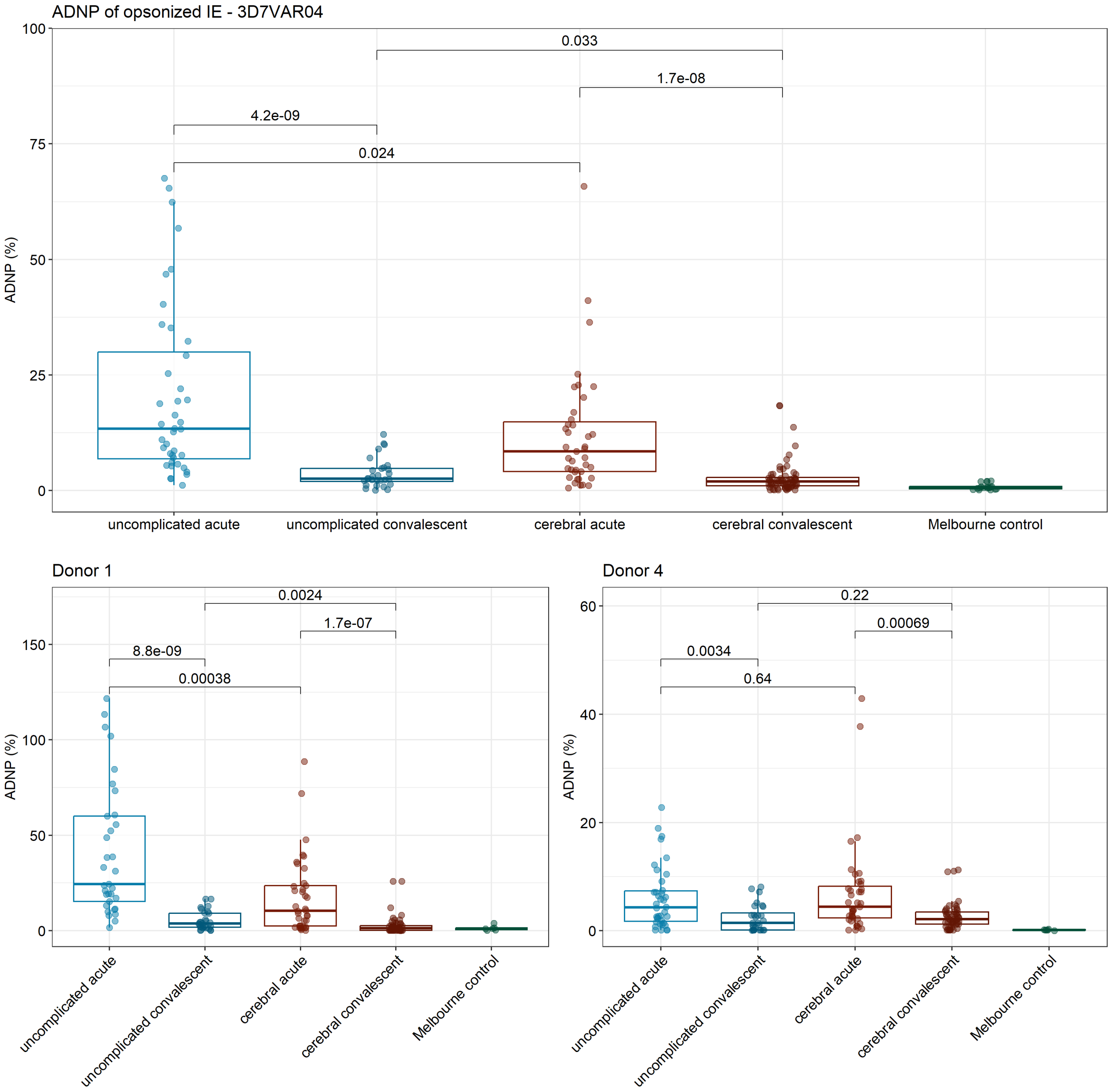


Figure 22: **ADNP of ICAM-1+EPCR binding 3D7VAR04 IE opsonized with sera from Malawian children.** 3D7VAR04 infected erythrocytes were opsonized with sera from Malawian children with cerebral malaria (red) or uncomplicated malaria (blue), collected at the acute phase of infection or at convalescence. Y-axis represents the percentage of DHE and CD66b positive neutrophils, relative to the positive control (% phagocytosis). Top plot represents mean responses from two neutrophil donors, that are shown individually in bottom plots (Donor 1 and Donor 4). Boxes represent median and inter-quartile range (IQR) from Q1-Q3, and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR). Median ADNP levels were compared by Wilcoxon Rank Sum test, indicated by horizontal bars, and associated P-values are shown. There were no corrections for multiple comparisons. All groups were significantly different to the Melbourne Control group (P-values <0.001, not shown on graph).

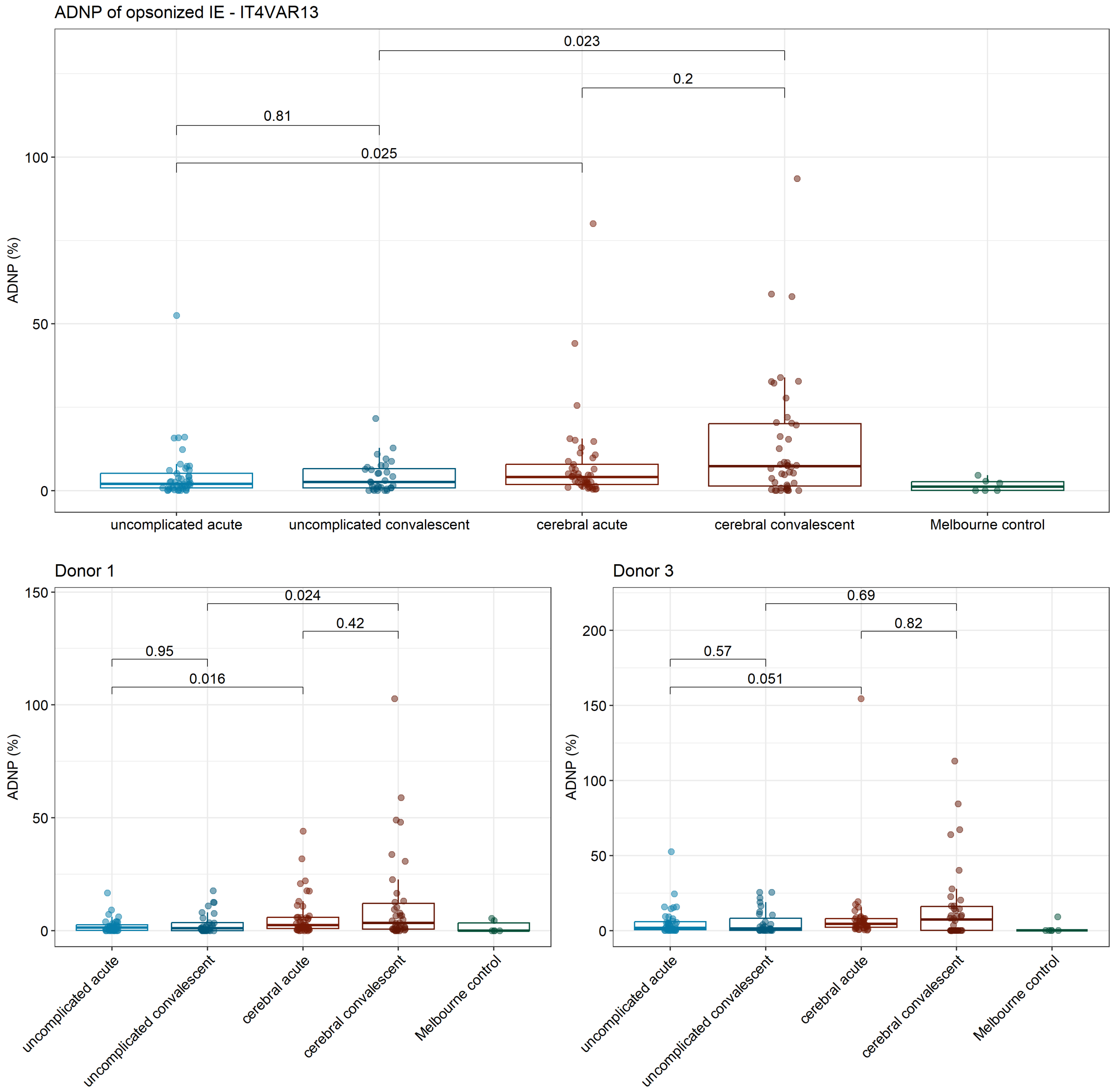


Figure 23: **ADNP of ICAM-1+CD36 binding IT4VAR13 infected erythrocytes opsonized with sera from Malawian children.** ADNP of IT4VAR13 IE opsonized with sera from Malawian children. IT4VAR13 infected erythrocytes were opsonized with sera from Malawian children with cerebral malaria (red) or uncomplicated malaria (blue), collected at the acute phase of infection or at convalescence. Y-axis represents the percentage of DHE and CD66b positive neutrophils relative to the positive control (% phagocytosis). Top plot represents mean responses from two neutrophil donors, that are shown individually in bottom plots (Donor 1 and Donor 3). Boxes represent median and inter-quartile range (IQR) from Q1-Q3, and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR). Median ADNP levels were compared by Wilcoxon Rank Sum test, indicated by horizontal bars, and associated P-values are shown. There were no corrections for multiple comparisons. Uncomplicated acute and convalescent groups were not statistically different from the Melbourne control (P-values 0.2 and 0.14, respectively, not shown on graph) and cerebral acute and convalescent groups were significantly different from the Melbourne control (P-values 0.023 and 0.02, respectively, not shown on graph).

We measured ADNP of ICAM-1+EPCR binding 3D7VAR04 and ICAM-1+CD36 binding IT4VAR13 selected IE opsonized with sera from Malawian children. Children with acute uncomplicated malaria had higher ADNP of ICAM-1+EPCR binding 3D7VAR04 IE compared to children with acute cerebral malaria (Figure 22). Children with cerebral and uncomplicated malaria had higher ADNP of 3D7VAR04 IE at the acute phase compared to convalescence. At convalescence, children with uncomplicated malaria had higher ADNP of 3D7VAR04 IE compared to children with cerebral malaria. All children with malaria had higher ADNP of 3D7VAR04 IE compared to malaria naïve Melbourne donors, at the acute phase and at convalescence.

Children with cerebral malaria had higher ADNP to ICAM-1+CD36 binding IT4VAR13 IE compared to children with uncomplicated malaria, at the acute phase and at convalescence (Figure 12.4). There were no differences in ADNP between the acute and convalescent samples. Children with cerebral malaria had higher ADNP to IT4VAR13 IE than malaria naïve Melbourne donors at both time points. There were no differences in children with uncomplicated malaria and Melbourne controls at either time point.

12.5 Antibody Dependent Cell Phagocytosis (ADCP) of ICAM-1 binding IE opsonized with sera from Papua New Guinean (PNG) children

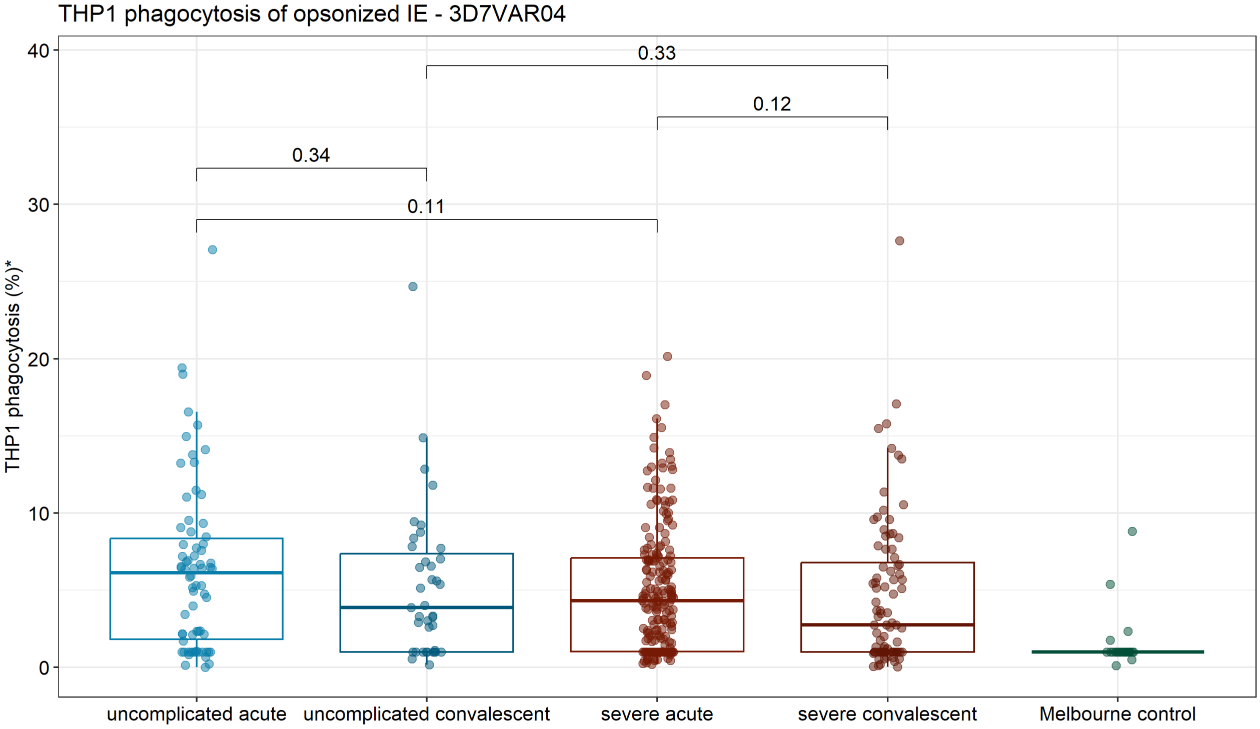


Figure 24: **ADCP of ICAM-1+EPCR binding 3D7VAR04 IE opsonized with sera from PNG children.** 3D7VAR04 infected erythrocytes were opsonized with sera from PNG children with severe malaria (red) or uncomplicated malaria (blue), collected at the acute phase of infection or at convalescence. Y-axis represents the percentage of DHE stained THP-1 cells, relative to the positive control (* % phagocytosis relative to positive control). Boxes represent median and inter-quartile range (IQR) from Q1-Q3, and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR). Median ADCP levels were compared by Wilcoxon Rank Sum test, indicated by horizontal bars, and associated P-values are shown. There were no corrections for multiple comparisons. All groups were significantly different to the Melbourne controls (P-values <= 0.001, not shown on graph)

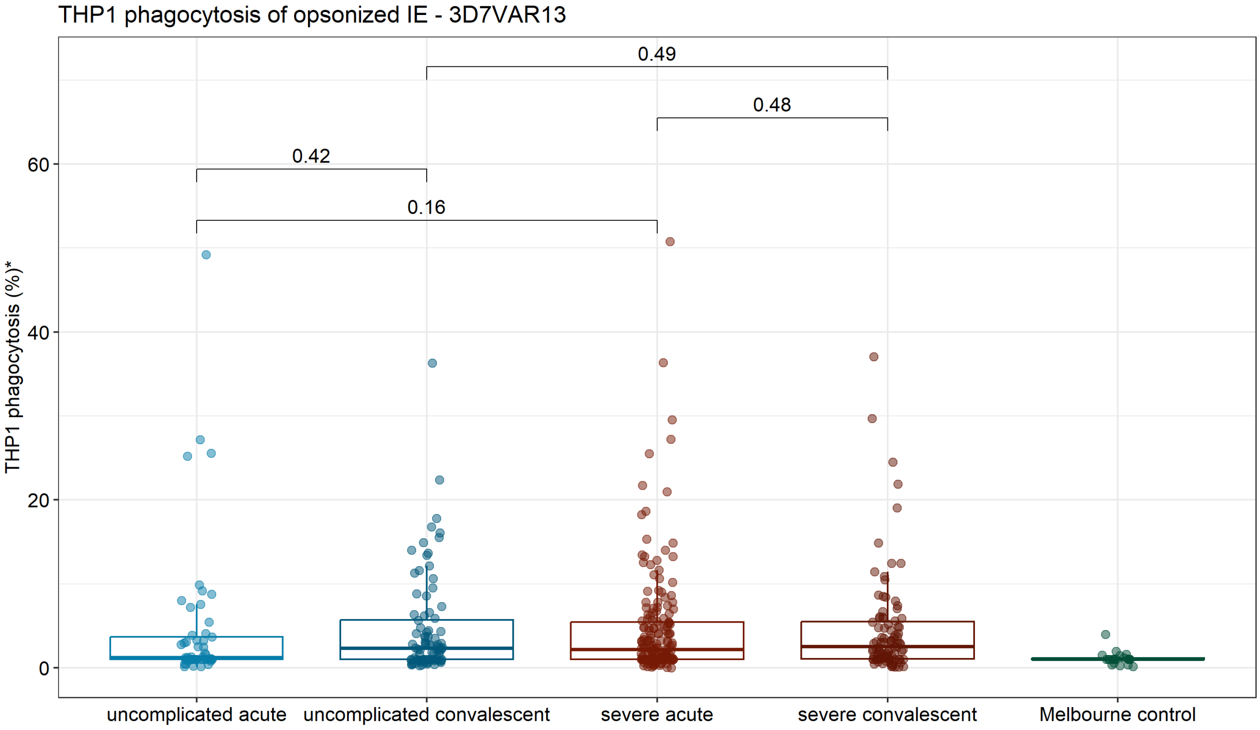


Figure 25: **ADCP of ICAM-1+CD36 binding IT4VAR13 IE opsonized with sera from PNG children.** IT4VAR13 infected erythrocytes were opsonized with sera from PNG children with severe malaria (red) or uncomplicated malaria (blue), collected at the acute phase of infection or at convalescence. Y-axis represents the percentage of DHE stained THP-1 cells, relative to the positive control (* % phagocytosis relative to positive control). Boxes represent median and inter-quartile range (IQR) from Q1-Q3, and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR). Median ADCP levels were compared by Wilcoxon Rank Sum test, indicated by horizontal bars, and associated P-values are shown. There were no corrections for multiple comparisons. The uncomplicated acute group was not statistically different from the Melbourne control (P-value 0.03, not shown on graph) and all other groups were significantly different from the Melbourne control (P-values <0.001, not shown on graph).

Next, we measured ADCP by THP-1 cells of ICAM-1+EPCR binding 3D7VAR04 and ICAM-1+CD36 binding IT4VAR13 selected IE opsonized with sera from PNG children with severe or uncomplicated malaria. There were no significant differences in ADCP of ICAM-1+EPCR binding 3D7VAR04 IE in children with severe malaria compared to uncomplicated malaria, for acute or convalescent samples (Figure 24). There were no differences in ADCP between the acute and convalescent samples.

There were no significant differences in ADCP of ICAM-1+CD36 binding IT4VAR13 IE in children with severe malaria compared to uncomplicated malaria, for acute or convalescent samples (Figure 25).

12.6 Antibody Dependent Neutrophil Phagocytosis (ADNP) of ICAM-1 binding IE opsonized with sera from Papua New Guinean (PNG) children

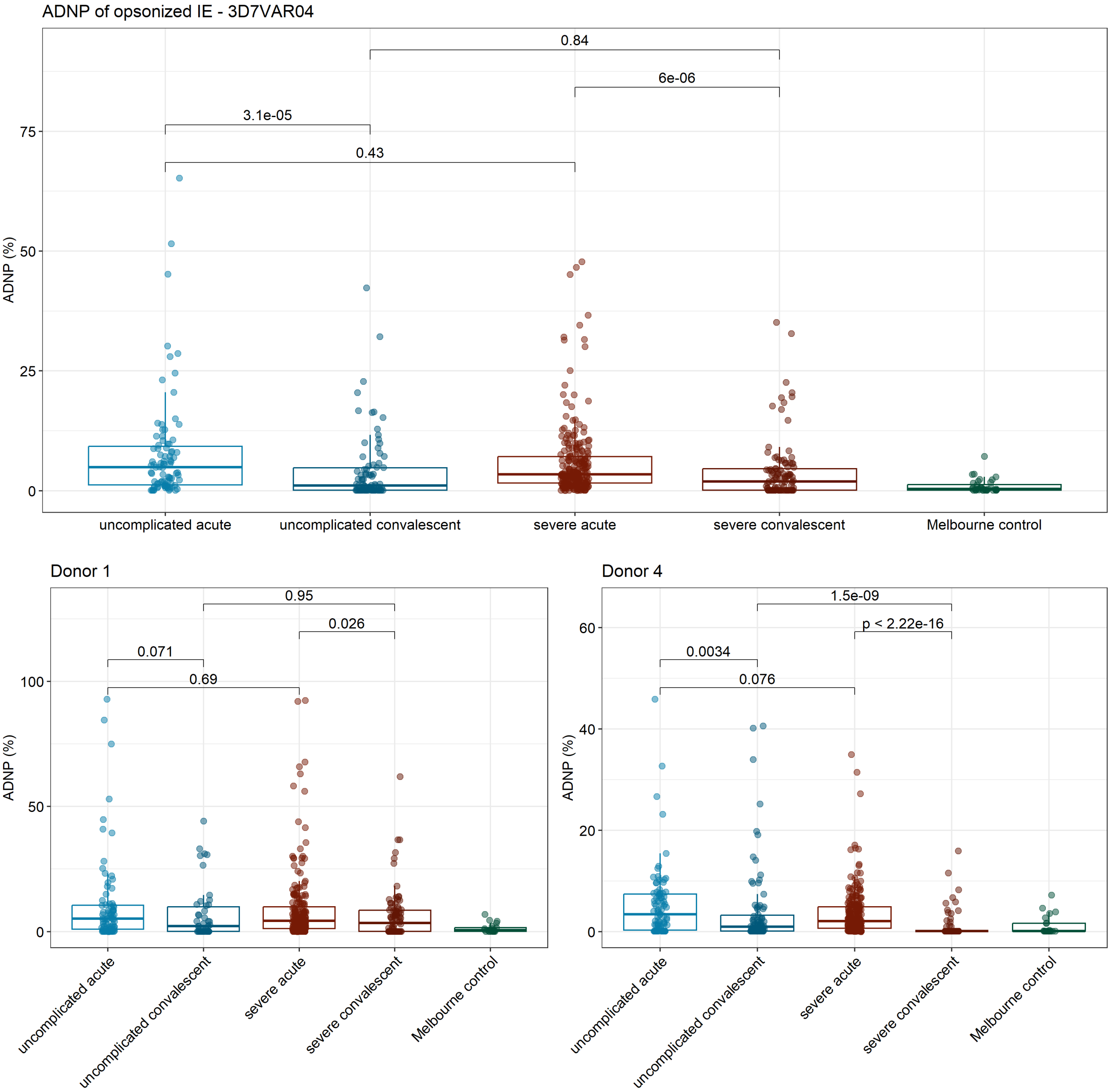


Figure 26: **ADNP of ICAM-1+EPCR binding 3D7VAR04 IE opsonized with sera from PNG children.** 3D7VAR04 infected erythrocytes were opsonized with sera from PNG children with severe malaria (red) or uncomplicated malaria (blue), collected at the acute phase of infection or at convalescence. Y-axis represents the percentage of DHE and CD66b positive neutrophils (% phagocytosis). Top plot represents mean responses from two neutrophil donors, that are shown individually in bottom plots (Donor 1 and Donor 4). Boxes represent median and inter-quartile range (IQR) from Q1-Q3, and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR). Median ADCP levels were compared by Wilcoxon Rank Sum test, indicated by horizontal bars, and associated P-values are shown. There were no corrections for multiple comparisons. Uncomplicated acute and severe acute groups were significantly different to the Melbourne Control group (P-values <0.001, not shown on graph). Uncomplicated convalescent and severe convalescent groups were not significantly different to the Melbourne Control group (P-values 0.14 and 0.071, respectively, not shown on graph).

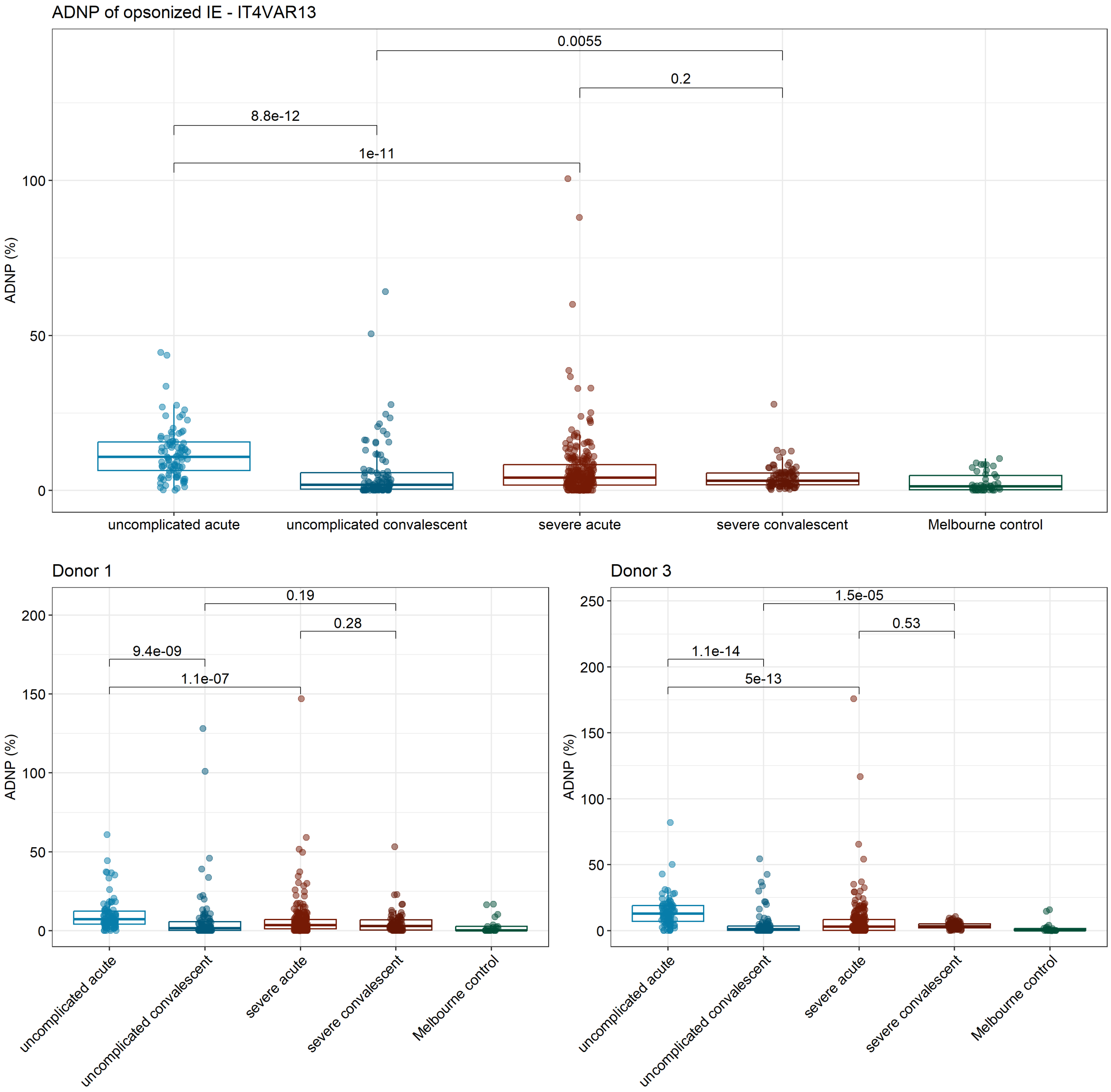


Figure 27: **ADNP of ICAM-1+CD36 binding IT4VAR13 infected erythrocytes opsonized with sera from PNG children.** IT4VAR13 infected erythrocytes were opsonized with sera from PNG children with cerebral malaria (red) or uncomplicated malaria (blue), collected at the acute phase of infection or at convalescence. Y-axis represents the percentage of DHE and CD66b positive neutrophils (% phagocytosis). Top plot represents mean responses from two neutrophil donors, that are shown individually in bottom plots (Donor 1 and Donor 3). Boxes represent median and inter-quartile range (IQR) from Q1-Q3, and whiskers range from (Q1-1.5*IQR) to (Q3+1.5*IQR). Median ADCP levels were compared by Wilcoxon Rank Sum test, indicated by horizontal bars, and associated P-values are shown. There were no corrections for multiple comparisons. The uncomplicated convalescent group was not statistically different from the Melbourne control (P-value 0.25, not shown on graph) and all other groups were significantly different from the Melbourne control (P-values <0.001, not shown on graph).

We measured ADNP of ICAM-1+EPCR binding 3D7VAR04 and ICAM-1+CD36 binding IT4VAR13 IE opsonized with sera from PNG children with severe or uncomplicated malaria. There were no differences in ADNP of ICAM-1+EPCR binding 3D7VAR04 IE in children with severe compared to uncomplicated malaria, in the acute or convalescent samples (Figure 26). Children with cerebral and uncomplicated malaria had higher ADNP of 3D7VAR04 IE at the acute phase compared to convalescence.

Children with uncomplicated malaria had higher ADNP to ICAM-1+CD36 binding IT4VAR13 IE than children with severe malaria at acute phase (Figure 27). Children with uncomplicated malaria had higher ADNP of IT4VAR13 IE at the acute phase compared to convalescence, and there were no differences in children with severe malaria. At convalescence, children with severe malaria had higher ADNP of IT4VAR13 IE than children with uncomplicated malaria.

Table 15: Summary of statistical comparisons for 3D7VAR04

ADCP	**ADNP**
Malawi - 3D7VAR04	
UM$_{act} < CM_{acute}$	UM$_{acute} > CM_{acute}$
UM$_{conv} = CM_{conv}$	UM$_{conv} > CM_{conv}$
UM$_{acute} = UM_{conv}$	UM$_{acute} > UM_{conv}$
CM$_{acute} = CM_{conv}$	CM$_{acute} > CM_{conv}$
Malawi - IT4VAR13	
UM$_{act} = CM_{acute}$	UM$_{acute} < CM_{acute}$
UM$_{conv} < CM_{conv}$	UM$_{conv} < CM_{conv}$
UM$_{acute} > UM_{conv}$	UM$_{acute} = UM_{conv}$
CM$_{acute} = CM_{conv}$	CM$_{acute} = CM_{conv}$
PNG - 3D7VAR04	
UM$_{acute} = SM_{acute.}$	UM$_{acute} = SM_{acute}$
UM$_{conv.} = SM_{conv.}$	UM$_{conv.} = SM_{conv.}$
UM$_{acute} = UM_{conv.}$	UM$_{acute} > UM_{conv.}$
SM$_{acute} = SM_{conv.}$	SM$_{acute} > SM_{conv.}$
PNG - IT4VAR13	
UM$_{acute} = SM_{acute.}$	UM$_{acute} > SM_{acute}$
UM$_{conv.} = SM_{conv.}$	UM$_{conv.} < SM_{conv.}$
UM$_{acute} = UM_{conv.}$	UM$_{acute} > UM_{conv.}$
SM$_{acute} = SM_{conv.}$	SM$_{acute} = SM_{conv.}$

Table 15: $>$ *and* $<$: indicates mean antibody response is greater than or less than, and meets the threshold of statistical significance determined by Mann Whitney U test p-value of 0.05. $=$: indicates no statistically significant difference in mean antibody response between groups. *UM and CM*: uncomplicated malaria and cerebral malaria. *ADCP* : Antibody dependant cell phagocytosis. *ADNP* : Antibody dependant neutrophil phagocytosis. *conv*: convalescent.

12.7 Inter-assay variation

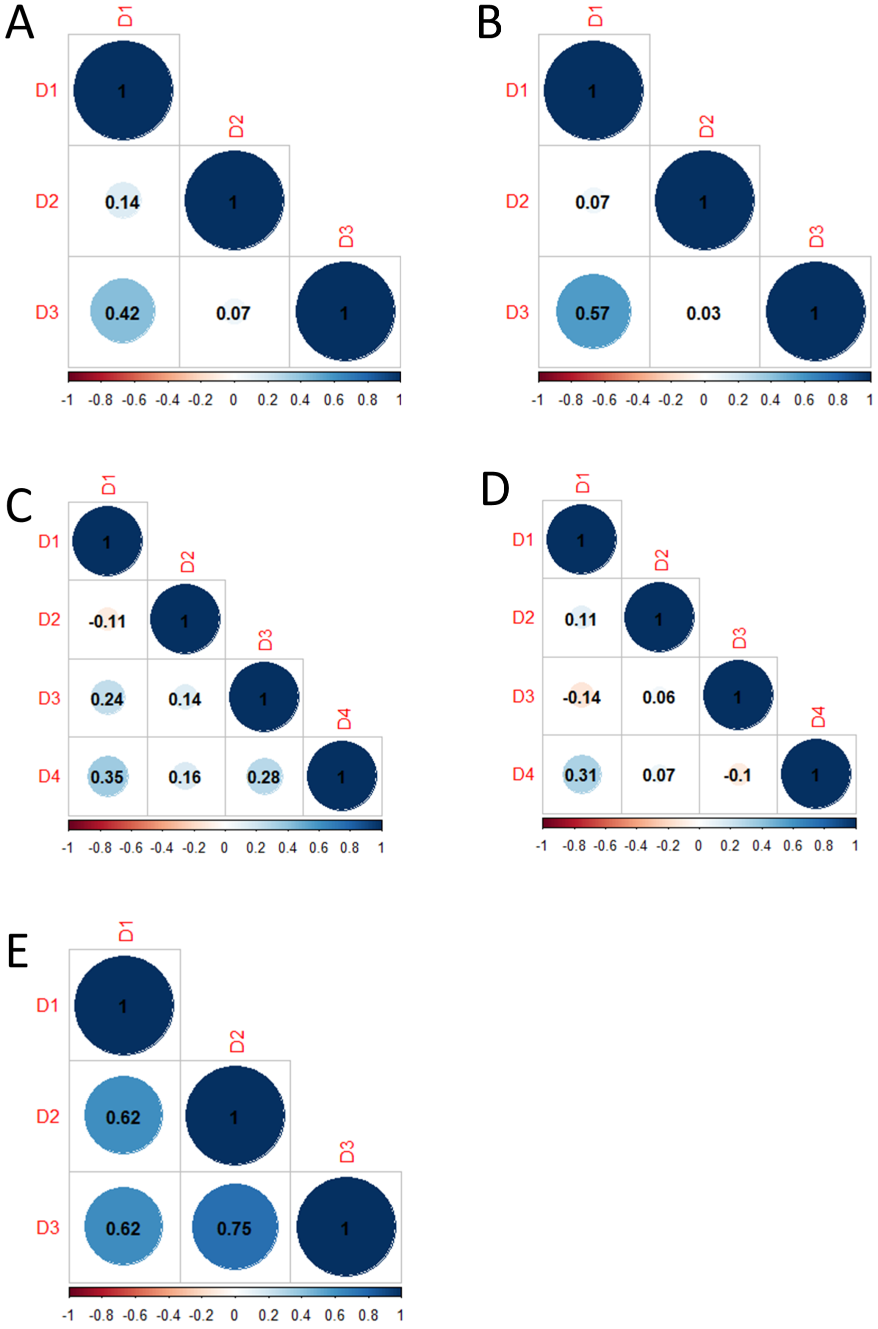


Figure 28: **ADNP inter-donor correlations.** Correlations between three independent neutrophil donors for: ADNP of IT4VAR13 IE opsonized with sera from A) PNG children and B) Malawian children; ADNP of 3D7VAR04 IE opsonized with sera from C) PNG children D) Malawian children and E) ADNP of CS2 IE opsonized with sera from Malawian adults. For A and B, ADNP using donors 1 and 3 (D1 and D3) were performed on the same day. For C and D, ADNP using donors 1 and 4 (D1 and D4) were performed on the same day. Circle size represents strength of correlation based on Spearman's correlation coefficient, r, shown on circles. Blue represents positive association and red represents negative association. ADNP: Antibody dependent neutrophil phagocytosis. D1, D2, D3, D4: four independent neutrophil donors.

ADNP assays were repeated with three independent neutrophil donors and PNG and Malawi samples were tested simultaneously for each donor.

For IT4VAR13 IE, ADNP assays with Donor 1 and Donor 3 were performed simultaneously. There was a moderate correlation between Donor 1 and Donor 3 ADNP induced by sera from PNG children and Malawian children (r = 0.42 and 0.57, respectively, Figure 28A and B). There was no correlation between Donor 2 and Donor 1 or Donor 3 in either cohort.

For 3D7VAR04 IE, ADNP assays with Donor 1 and Donor 4 were performed simultaneously (on the same day). For ADNP of 3D7VAR04 IE induced by sera from PNG children, there was a weak correlation between Donor 1 and Donor 4 (r = 0.35), Donor 1 and Donor 3 (r = 0.24) and Donor 4 and Donor 3 (r = 0.28), (Figure 28C). For ADNP induced by sera from Malawian children, there was a weak correlation between Donor 1 and Donor 4 (r = 0.31, Figure 28D) and no correlation between other donors.

To determine donor-donor variation of IE with more stable PfEMP1 expression, we performed ADNP of 3D7CS2 IE opsonised with sera from Malawian pregnant women (Figure 28E, experiment performed by Wina Hasang, University of Melbourne). There were moderate – strong correlations of ADNP of 3D7CS2 IE between 3 donors (r range from 0.62 – 0.75, Figure 28E).

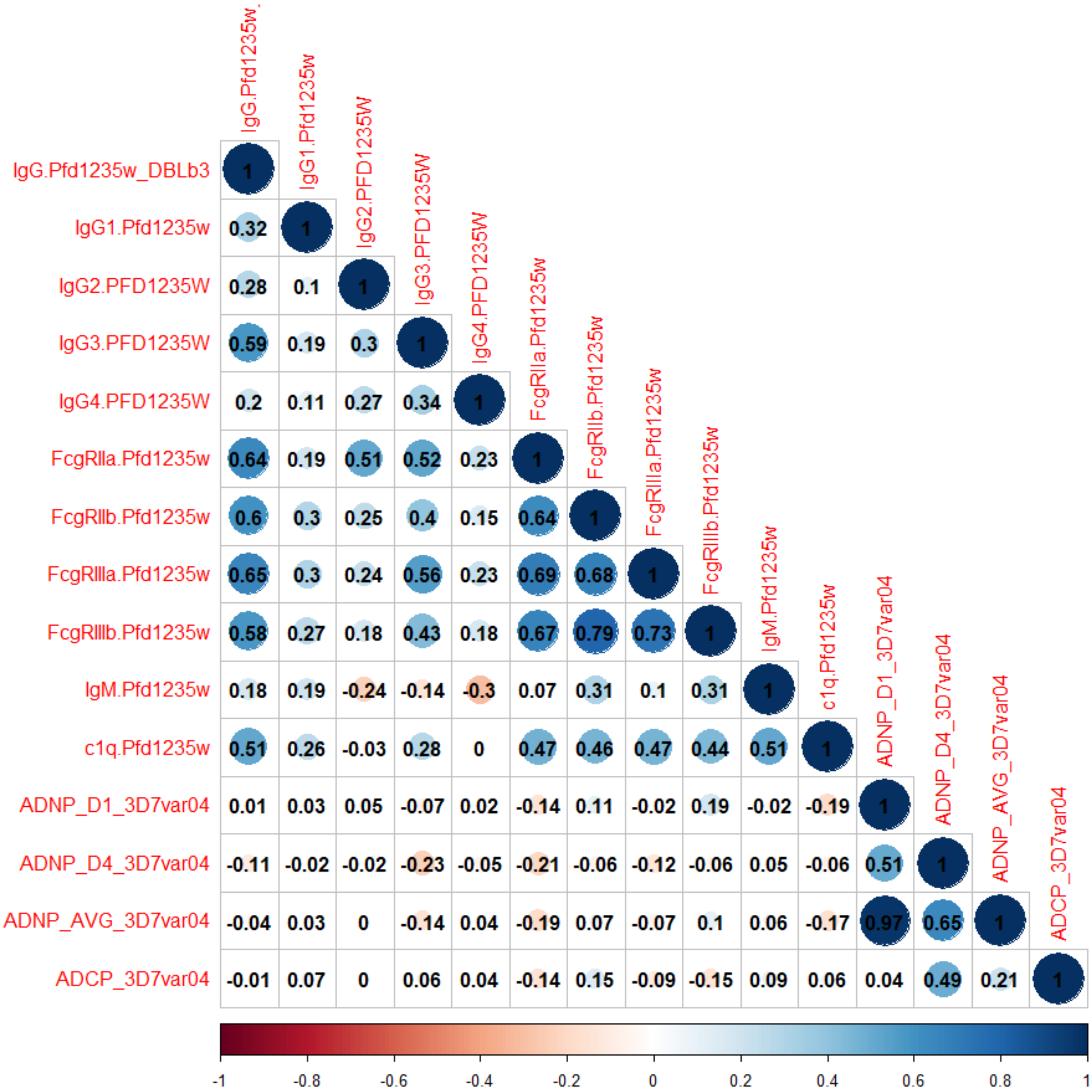


Figure 29: **Correlations between ADNP of 3D7VAR04, ADCP of 3D7VAR04 and antibody Fc features targeting the DBLβ3 domain of 3D7VAR04 (Pfd1235w), when IE/beads were opsonized with sera from Malawian children.** Circle size represents strength of correlation based on Spearman's correlation coefficient, r, shown on circles. Blue represents positive association and red represents negative association. ADNP: Antibody dependent neutrophil phagocytosis. ADCP: Antibody dependent cell phagocytosis. D1 and D3: Donor 1 and 3.

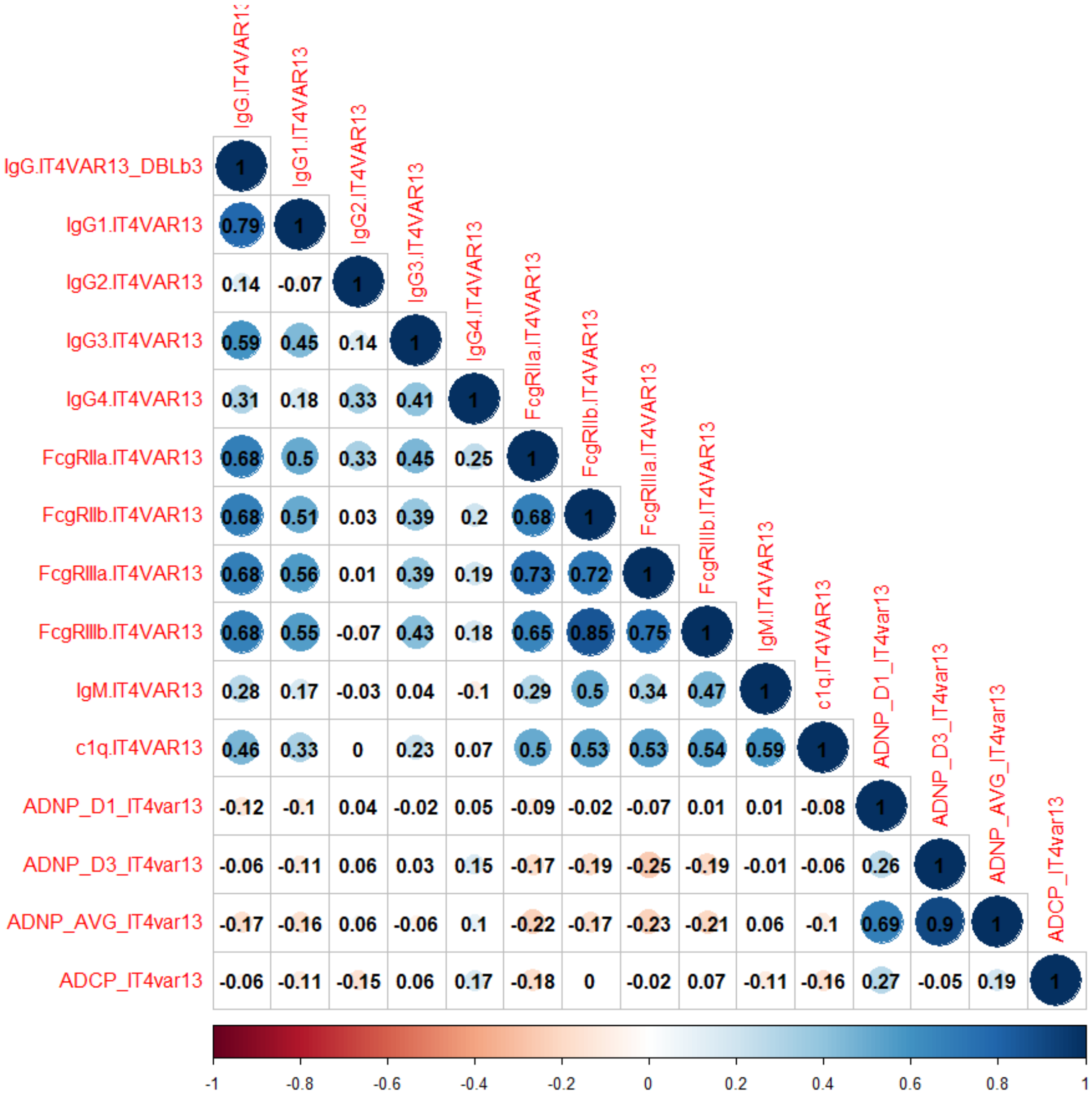


Figure 30: **Correlations between ADNP of IT4VAR13, ADCP of IT4VAR13 and antibody Fc features targeting the DBLβ3 domain of IT4VAR13 (IT4VAR13), when opsonized with sera from Malawian children.** Circle size represents strength of correlation based on Spearman's correlation coefficient, r, shown on circles. Blue represents positive association and red represents negative association. ADNP: Antibody dependent neutrophil phagocytosis. ADCP: Antibody dependent cell phagocytosis. D1 and D3: Donor 1 and 3.

We compared correlations between ADCP, ADNP and Fc features of antibodies targeting the respective DBLβ3 domains of 3D7VAR04 and IT4VAR13, when opsonised with sera from Malawian children (Materials and Methods Table 9, protein M6/Pfd1235w and protein N27, respectively).

There were no correlations between antibody Fc features targeting recombinant DBLβ3 of 3D7VAR04 and ADNP or ADCP of 3D7VAR04 IE opsonized with Malawian children's sera (Figure 29). There was a weak correlation between ADCP and ADNP of 3D7VAR04 (Figure 29, ADCP with average ADNP r = 0.21, and ADCP with Donor 4 ADNP, r = 0.49).

There were no correlations between antibody responses to recombinant DBLβ3 of IT4VAR13 and ADNP or ADCP of IT4VAR13 IE opsonized with Malawian children's sera (Figure 30). There was a weak correlation between ADCP and ADNP (Figure 30, ADCP with average ADNP, $r = 0.19$ and ADCP with ADNP of donor 1, $r = 0.27$).

13 Discussion

13.1 Overview

Both monocytes and neutrophils phagocytose antibody opsonized IE but few studies have considered the association of ADCP or ADNP of opsonized IE with protection from severe malaria. The PfEMP1 variants involved in the pathogenesis of severe malaria in children are not entirely clear but ICAM-1 binding IE appear to play an important role. In particular, expression of dual ICAM-1 + EPCR binding PfEMP1 has been associated with cerebral malaria, whereas ICAM-1 + CD36 binding IE have been associated with uncomplicated malaria. The aim of this study was to evaluate if ADCP or ADNP of ICAM-1 binding IE are associated with protection from cerebral malaria or severe malaria in children. We measured ADNP and ADCP of IT4VAR13 (ICAM+CD36 binding) IE and 3D7VAR04 (ICAM-1+EPCR binding) IE opsonized with sera from Malawian children with cerebral and uncomplicated malaria and PNG children with severe and uncomplicated malaria. We found that the measures of ADCP were not associated with protection in cerebral malaria or severe malaria, and ADNP of ICAM-1+EPCR binding IE was associated with protection from cerebral malaria.

13.2 ADNP and ADCP in Malawian children with cerebral malaria and uncomplicated malaria

We hypothesized that ADCP of 3D7VAR04 (ICAM+EPCR binding) would be associated with protection from cerebral malaria, and ADCP to IT4VAR13 (ICAM+CD36 binding) would not. Contrary to our hypothesis, our results suggest an association with ADCP of ICAM-1 binding parasites and cerebral malaria, rather than protection. No published studies have considered ADCP of IE in cerebral malaria compared to uncomplicated malaria. Cerebral malaria is a complex manifestation of malaria that is likely influenced by multiple non-independent pathways, including IE sequestration, severe inflammation and excessive activation of coagulation pathways (Ramachandran et al. 2022). One plausible explanation of the association of ADCP of ICAM-1 IE and cerebral malaria, is that ADCP enhances severe inflammation and contributes to cerebral malaria pathology. Monocytes containing malaria pigment are found in blood vessels of the brain in fatal cerebral malaria patients (Dorovini-Zis et al. 2011; Hochman et al. 2015), as well as in blood vessel haemorrhages observed in the retina of cerebral malaria patients (MacCormick et al. 2022). An imbalance of pro-inflammatory and anti-inflammatory cytokines (such as TNF, IL-6 and IL-10) in favour of inflammation is associated with cerebral malaria and pro-inflammatory cytokines may contribute to endothelial activation, neuroinflammation and blood brain barrier disruption (Dunst et al. 2017). Monocytes are the predominant source (*in* *vitro*) of proinflammatory cytokines associated with severe malaria (Stanisic et al. 2014) and cytokine secretion can be induced by opsonic phagocytosis (Ludlow et al. 2012). It is possible that antibodies produced by children with cerebral malaria enhance the inflammatory response of monocytes and that this offsets any benefit of removing ICAM-1 binding IE. Children with uncomplicated malaria may have produced antibodies that better induce other functions, such as adhesion inhibition, complement activation or ADNP.

ADNP of IT4VAR13 IE (ICAM+CD36 binding) was elevated in cerebral malaria compared to uncomplicated malaria at the acute and convalescent time points, in line with ADCP. In contrast to ADCP, ADNP of 3D7VAR04 IE (ICAM+EPCR binding) was elevated in uncomplicated malaria at both acute and convalescent time points. This is the first study to consider ADNP of ICAM-1 binding IE opsonized with clinical sera from individuals with cerebral malaria and is in line with our hypothesis that ADNP of ICAM-1+EPCR binding IE, but not ICAM+CD36 binding IE, is associated with protection from cerebral malaria. An alternative explanation is that children with uncomplicated malaria are more exposed to ICAM-1 binding parasite lines than children with cerebral malaria. However, given the opposite trend was observed for ADCP, this is unlikely.

Ideally, protective antibodies would show evidence of a sustained response, rather than a waning response at convalescence. The median ADNP response to 3D7VAR04 IE (ICAM+EPCR binding) was higher in malaria exposed individuals than the Melbourne control group, at both time points, suggesting some acquisition of antibody mediated immunity. However, we observed lower median ADNP of 3D7VAR04 IE in acute compared to convalescent samples, in both cerebral malaria and uncomplicated malaria groups. Weak evidence for this trend was also observed in ADCP of 3D7VAR04 (non-statistically significant). The transient nature of antibodies targeting the IE surface has been observed in previous studies (Kinyanjui et al. 2003; Olsen et al. 2019; Aitken et al. 2010) and may be variant dependent (Kinyanjui et al. 2003). Dynamics of antibodies targeting recombinant DBLβ domains are highly variable between Ghanaian children, but median titers peak at 2 weeks and decay by 6 weeks, in line with our results (Olsen et al. 2019). Given the short follow up time of 4 weeks in our study, the lower antibody response at convalescence could suggest a short half-life of antibodies produced in the acute phase. IgG3 has a half-life of 2 - 6 days, compared to 21 days for IgG1, IgG2 and IgG4 (Saxena et al. 2016), and may be the dominant subclass induced by ICAM-1+EPCR binding IE. Alternatively, a higher antibody response in the acute phase may indicate the presence of low affinity cross reactive antibodies that recognize the tested parasite isolates but are predominantly directed against different infecting variants and become undetectable at convalescence. In line with this, Ofori *et al.* demonstrated that children predominantly develop an antibody response to the infecting parasite isolates but also develop a weak and transient response to other isolates from the community (Ofori et al. 2002). The half-life of circulating IgG is longer in older children and previously exposed children (at least with respect to merozoite antigens), due to the development of long term antibody secreting cells (memory B cells) (Akpogheneta et al. 2008; Rogier et al. 2022). We carefully matched children for location and urban/rural setting of residency in the acute samples, to reduce this potential confounding effect of prior exposure when comparing the groups. Matching for location may also reduce variability in distance to hospital and therefore duration of infection between the groups. Children were also well matched for age between the acute and convalescent time points; therefore age (which can also be linked to exposure) is unlikely to influence the differences observed between time points.

13.3 ADNP and ACDP in PNG children with severe malaria and uncomplicated malaria.

ADCP of 3D7VAR04 or IT4VAR13 opsonized with sera from PNG children were not associated with protection from severe malaria. There were no differences in acute compared to convalescent ADCP of IT4VAR13 or 3D7VAR04. In a previous study of the children from the same sample collection, ADCP of E8B IE (also predicted to be ICAM-1+CD36 binders) was higher in severe malaria compared to uncomplicated malaria and elevated in convalescence compared to the acute timepoint amongst children with uncomplicated malaria (Rambhatla 2018). Differences compared to our study are likely to be due to the samples used - due to limited sample availability, our study had approximately half the power of this previous study. However, both studies align with the conclusion that ADCP to ICAM-1+CD36 binding IE is not associated with protection from severe malaria in PNG children. This was the first study to consider ADCP of ICAM-1+EPCR binding IE in individuals with severe malaria. ADCP of EPCR binding IE (IT4VAR19) has been associated with protection from severe malaria (Chan et al. 2019a; Rambhatla 2018; Rathnayake 2022) in this cohort of children from PNG. Other PfEMP1 variants, such as group A EPCR binding IE that express specific CIDR domains (non-*var04*), may be more relevant to the pathogenesis of severe malaria in this region.

There were no differences in ADNP of 3D7VAR04 IE (ICAM+EPCR binding) opsonized with sera from children with severe malaria compared to uncomplicated malaria. ADNP of IT4VAR13 IE (ICAM+CD36 binding) was elevated in acute uncomplicated malaria compared to severe malaria but was higher in severe malaria compared to uncomplicated malaria at convalescence. It is difficult to form a unifying hypothesis about these inconsistent differences and this raises concerns about the reliability of the ADNP assay, as will be discussed in the following section. Similarly to the Malawian cohort we observed higher ADNP of 3D7VAR04 and IT4VAR13 in acute uncomplicated malaria compared to convalescence, as well as in acute severe malaria compared to convalescence for 3D7VAR04. Strikingly (and unlike in the Malawi cohort), in these groups that had lower ADNP at convalescence than the acute time point, there were no differences between convalescent samples and the Melbourne controls. This suggests rapid decay of antibodies and a lack of seroconversion at convalescence. As discussed, a rapid decay of antibodies has been previously observed and may be due to induction of IgG3 or low affinity cross reactive antibodies to other variants. The longer follow up time of 8 weeks may have also contributed to the more dramatic trend compared to the Malawian children.

In summary, ADCP of ICAM-1 binding IE was not associated with protection from severe malaria or cerebral malaria. ADNP of 3D7VAR04 IE (ICAM+EPCR binding) was associated with protection from cerebral malaria in Malawian children but not severe malaria in PNG children. This is in line with the hypothesis that ICAM-1+EPCR binding IE are associated with the pathogenesis of cerebral malaria and not severe malaria (Jensen et al. 2020). Children from PNG had a mixture of severe manifestations and future studies could compare ADNP in PNG amongst children with cerebral malaria only. Surprisingly, ADNP of IT4VAR13 IE (ICAM+CD36 binding) was associated with protection from acute severe malaria but associated with severe malaria in convalescence in PNG children.

13.4 ADCP and ADNP assay value and limitations.

There was a weak correlation between ADNP and ADCP assays. One interpretation is that the assays are representative of different immune mechanisms and that it's necessary to consider both assays when characterizing the immune response. Neutrophils and THP-1 cells differ in the expression of Fc receptors, and THP-1 ADCP is mediated by FcRI and FcγRIIa (Tebo et al. 2002) whereas ADNP is mediated by FcγRIIa and possibly FcγRIIIb (Garcia-Senosiain et al. 2021). Differences in ADCP and ADNP may therefore be influenced by differences in antibody subclass, since FcγRI has higher affinity to IgG1 and IgG3 than FcγRIIIb, and FcγRIIIb has the highest affinity for IgG3 (Gillis et al. 2014). Additionally, a recent malaria study showed that afucosylated IgG1 to VAR2CSA PfEMP1 IE enhances FcγRIIIa interaction and NK cell activation (Larsen et al. 2021), and it is possible that afucosylation also influences FcγRIIIb mediated activity on neutrophils but not THP-1 (which lack FcγRIIIa/b (Gil Gonzalez et al. 2022). However, a whole blood assay measuring ADNP and ADCP of IT4VAR19 IE (EPCR binding) revealed consistent trends in monocytes and neutrophils (Rathnayake 2022) and the lack of correlation between ADNP and ADCP may be reflective of limitations in the respective assays.

Neutrophils are the dominant immune cell in the blood yet little is known about their role in the immune response to malaria (Aitken et al. 2018). This is likely due to the difficulty isolating neutrophils from whole blood without activation and the inability to culture or successfully freeze and thaw isolated neutrophils (Blanter et al. 2022). Using a recently published ADNP assay (Aitken et al. 2021) we found moderate inter-donor correlations for ADNP assays performed on the same day but low inter-donor correlations for assays performed on different days. The poor correlation likely reflects variation in the PfEMP1 expression between days, as *var04/var13* expression ranged from 36 – 55%. In line with this, ADNP of CS2 IE showed stronger inter-donor correlations than IT4VAR13 or 3D7VAR04 and the CS2 parasite line is known to have stable expression of VAR2CSA. In addition to performing experiments with two donors on the same day, we reduced the risk of technical variation by running all serum samples on the same day, in technical duplicates. However, we have not investigated activation of neutrophils during the isolation procedure, which may also contribute to inter-assay and intra-assay variation. Neutrophils isolated from whole blood by various methods have altered activation markers including CD16 and CD32 Fc receptor expression, although the negative selection based isolation method (as used in our study) is thought to cause the least activation (Blanter et al. 2022). Future studies should consider neutrophil activation, viability and Fc receptor expression following isolation in our hands. Polymorphisms in FcγRIIa and FcγRIIIb receptors can also influence the affinity of antibodies to different IgG subclasses (Bruhns et al. 2009) and thereby sensitization for ADNP (Salmon et al. 1990), and may vary between neutrophil donors. Polymorphisms in FcγRIIa and FcγRIIIb have been associated with malaria severity in previous studies (Adu et al. 2014; Fall et al. 2022; Omi et al. 2002), therefore it would be interesting to know the contribution of Fc receptor polymorphisms to donor-donor variability in ADNP. Additionally, ICAM-1 can interact directly with PfEMP1 to induce ADNP (Zelter et al. 2022) and ICAM-1 expression on the neutrophil cell surface can vary with disease (Dabrowski et al. 2014). Although healthy donors were used in these experiments, future studies could consider variation in neutrophil ICAM-1 expression in healthy and malaria exposed individuals, and whether this alters ADNP.

The ADCP assay using the THP-1 monocytic cell line is well established (Kassa et al. 2022) and has been used by multiple published studies to characterize the immune response to malaria in pregnancy (Aitken et al. 2021; Ataíde et al. 2011) and severe malaria (Chan et al. 2019a; Quintana et al. 2018). THP-1 cells can be easily cultured, retain uniform characteristics and don't require a blood donor. THP-1 cells are representative of classical monocytes (CD14+CD16-), and phagocytose opsonized IE via FγRI and FcγRIIa working in synergy (Tebo et al. 2002). However, monocytes expressing FcγRIIIa are likely an important component of the immune response to malaria. Intermediate monocytes (CD14+16+) are the most efficient subset for ADCP in the presence of complement (Zhou et al. 2015) and expand during blood stage infection (Dobbs et al. 2017). Additionally, a lower frequency of non-classical monocytes was associated with death in Beninese children (Royo et al. 2019). Therefore the ideal *in vitro* model would enable characterization of ADCP by the various monocytic subsets. Indeed, ADCP with isolated monocytes and THP-1 cells correlated weakly (Aitken et al. 2021). The lack of FcγRIIIb on THP-1 cells may partially explain the poor correlation of ADNP and ADCP in this study.

Antibody features to the ICAM-1 binding DBLβ3 domains of IT4VAR13 and 3D7VAR04 were characterized in the same population of Malawian children and PNG children in Part IV and V. We expected that antibody subclasses or FcγR interactions measured by multiplex would correlate with ADCP or ADNP of IE expressing the corresponding *var* gene. However, there were no correlations between ADCP/ADNP and antibody features measured by multiplex immunoassay. This may reflect the differences in recombinant proteins compared to native proteins, such as incorrect protein folding, or reflect antibody interactions that span multiple domains of the whole PfEMP1. The lack of correlation may also reflect a combined effect of antibodies targeting multiple epitopes contributing to ADNP/ADCP, whereas only single PfEMP1 fragments were probed by multiplex immunoassay. IT4VAR19 IE that lack PfEMP1 showed significantly reduced ADCP when opsonized with PNG children sera (Chan et al. 2019a), suggesting PfEMP1 is the major target of antibodies on the cell surface and other non-PfEMP1 targets are unlikely to have a major influence on our results. The lack of correlation may also be influenced by the low purity of the selected parasites. We did not perform sequencing and therefore don't know which PfEMP1 types were expressed by the rest of the population and may have influenced ADNP/ADCP. However, Aitken, Damelang, Ortega-Pajares *et al.* reported low correlation between ADNP and ADCP of VAR2CSA expressing IE that are thought to have stable PfEMP1 variant expression, suggesting the consistency of the selected PfEMP1 is not the dominant contributing factor to the lack of correlation (Aitken et al. 2021).

In summary, although we expected some differences in ADCP and ADNP due to the differences in expression of Fc receptors, there are several limitations in these assays that might explain the lack of correlation between ADNP and ADCP. The high inter-assay variation for ADNP suggests that further optimization is required. Future studies should prioritize characterizing neutrophils following isolation from whole blood and characterizing inter-donor variation of cell surface receptors. Future studies could consider the use of the HL-60 neutrophil like cell line to overcome challenges of isolating fresh neutrophils from whole blood, as has been trialled in HIV studies (Worley et al. 2018). The THP-1 assay is more reliable but is not necessarily a perfect model of *in vivo* biology due to the lack of monocyte subclasses. Future studies could consider the use of a transgenic THP-1 line that expresses FcγRIIIa (Gil Gonzalez et al. 2022) and may enable the study of intermediate monocytes whilst retaining the benefits of using a cell line. We have not considered the contribution of non-opsonic phagocytosis in this study. Interactions with EPCR, ICAM-1 and CD36 on monocytes or neutrophils may contribute to phagocytosis and influence clinical outcomes (McGilvray et al. 2000; Zelter et al. 2022). Further optimization of these assays is required in order to validate the antibody responses measured with recombinant proteins by multiplex immunoassay, or provide an alternative and reliable way to characterize the immune response to ICAM-1 binding IE.

Part VII

Final discussion, Future directions & Concluding remarks

14 Final Discussion

14.1 Field of study and thesis objectives

Malaria is a major public health burden in tropical and sub-tropical regions of the world. Children under five years old lack protective immunity that is acquired from multiple exposures to malaria, and account for the majority of severe cases of malaria. In the most severe form of malaria, cerebral malaria, children enter a coma, experience brain swelling and can die of respiratory arrest, and it's estimated that malaria causes 500,000 annual deaths in children under five (World Health Organization 2021c). Antibodies are a key component of protective immunity to malaria however the target antigens of protective antibodies are unclear. The first and only WHO endorsed vaccine for malaria, RTS,S, provides modest and short-lived protection from severe disease, therefore identifying novel therapeutic target antigens would be useful for the development of novel therapeutics. PfEMP1 is an important candidate target antigen expressed on the surface of infected erythrocytes that mediates sequestration of parasites in the microvasculature, leading to severe disease. Although PfEMP1 is a highly variable protein, antibodies to key PfEMP1 sub-domains may confer protection from severe disease. Additionally, understanding the functions of antigen specific antibodies is important for understanding the efficacy of an intervention, such as a vaccine. There is currently a lack of understanding of the mechanisms by which antibodies confer protection and the best way to measure protective immunity. There are many functions of antibodies that are mediated by the antibody Fc region, and measuring the biophysical properties and interactions of the antibody Fc region can provide valuable insight into the mechanism of protective immunity.

The objective of this thesis was to understand the PfEMP1 targets and functions of antibodies that are associated with protection from severe malaria in children living in endemic regions. Specifically, this thesis aimed to: 1) characterize the key PfEMP1 targets and Fc features of antibodies that correlate with protection from the most severe manifestation of malaria, cerebral malaria, in a cohort of Malawian children; 2) characterize the key PfEMP1 targets and Fc features of antibodies that correlate with protection from severe malaria, including multiple severe manifestations, in a cohort of Papua New Guinean children; 3) assess the association of disease severity with antibodies that induce neutrophil or monocyte phagocytosis of infected erythrocytes expressing ICAM-1 binding variants of PfEMP1.

To achieve these aims, we studied two case-control cohorts of children living in malaria endemic areas: Malawian children who had cerebral and uncomplicated (non-severe) malaria, and Papua New Guinean children who had severe or uncomplicated malaria. For aims 1) and 2), we used a series of multiplex immunoassays designed for measuring antibody responses to large numbers of antigens simultaneously, to characterize a range of antigen-specific antibody Fc features, including antibody class, subclass and ability to bind to Fc receptors and fix complement. To analyze the large dataset, we applied traditional univariate analysis as well as more advanced machine learning methods coupled with logistic regression, to identify combinations of antibody responses that correlated with disease severity. For aim 3), we selected parasite-infected erythrocytes expressing two PfEMP1 variants of interest that bind to ICAM-1 receptors on endothelial cells and measured ADCP and ADNP of opsonized infected erythrocytes by flow cytometry.

14.2 New insights and translational potential

14.2.1 Targets of antibodies in Malawi vs. Papua New Guinea: DBLβ domains are vaccine candidates.

The ideal therapeutic or prognostic tool would be transferable to multiple geographical settings. This has been a major challenge for the development of a vaccine based on the pregnancy-specific PfEMP1 variant, VAR2CSA, and a combination of multiple VAR2CSA vaccines may be required to achieve recognition of heterologous variants (Sirima et al. 2020; Doritchamou et al. 2019). In Part IV and V, we measured antibody responses to recombinant PfEMP1 domains in children from Malawi and Papua New Guinea. We included domains that were differentially transcribed in Papuan individuals with severe and uncomplicated malaria as well as a range of DBLβ and CIDRα from multiple parasite lines that have previously been associated with severe or uncomplicated malaria. There are limited studies of PfEMP1 expression outside of Africa, however there could be some differences between the variants circulating in Papua New Guinea and Malawi, as well as differences between the variants causing severe malaria (that encompasses multiple pathologies) compared to cerebral malaria specifically. To improve our chances of including variants that were circulating in the population at the time of sample collection, we included multiple examples of domains with the same binding phenotype. Encouragingly, there were similarities in the domains selected by machine learning as targets of antibodies associated with uncomplicated malaria in Malawi and PNG. DBLβ domains were amongst the antibody targets associated with uncomplicated in Malawi and at both the acute and convalescent time points in PNG. In particular, a group A ICAM-1 binding DBLβ3_M6 (Pfd1235w) was selected by machine learning in both cohorts. Therefore, our study supports the development of a vaccine or therapeutic based on DBLβ domains as an intervention targeting both severe and cerebral malaria, in multiple geographic settings.

The expression of DBLβ domains that have an upstream EPCR binding CIDRα1 domain has been specifically associated with cerebral malaria, whereas DBLβ domains with adjacent CD36 binding domains have been associated with uncomplicated malaria (Lennartz et al. 2017). Interestingly, both types of DBLβ domains were amongst the selected targets of antibodies associated with uncomplicated malaria, in both cohorts. A role of antibodies targeting CD36 and EPCR binding variants in protection from severe malaria has been mirrored in two previous studies (Tessema et al. 2019; Travassos et al. 2018), including a longitudinal study in PNG (Tessema et al. 2019). Naturally acquired and vaccine-induced antibodies targeting ICAM-1 binding DBLβ domains are broadly cross reactive and impair binding to ICAM-1 receptors (Bengtsson et al. 2013; Olsen et al. 2018), therefore it may be possible to induce immunity targeting both CD36 and EPCR binding variants using the DBLβ domain as the basis of a vaccine. The cross-reactive nature of DBLβ domains may also explain why they were selected by machine learning as correlates of protective immunity in preference to CIDRα1 domains in PNG. Despite multiple univariable differences in antibody responses to CIDRα1 domains between severe and uncomplicated malaria, immunization with CIDRα does not induce cross reactive antibodies in rats (Turner et al. 2018) and therefore a broader range of variants may be required to explain variation in the groups, compared to DBLβ domains.

A combination of 7 features was required to maximize the accuracy of the model to differentiate between the groups for the Malawian cohort and a combination of 8 features was used for the PNG cohort. Therefore, the use of multiple antigens may maximize the efficacy of a vaccine or prognostic tool. Other than DBLβ domains, other novel targets of antibodies that were associated with uncomplicated malaria in both Malawi and PNG included a CIDRγ12 domain, that has no known function but is upregulated in Papuan adults with severe malaria (Tonkin-Hill et al. 2018). Antibodies targeting DBLδ1 domains were also common to the features selected for both cohorts, however they were associated with both uncomplicated and severe malaria. The other targets of antibodies that were selected by machine learning differed in Malawi and PNG. Differences in the selected targets may reflect the differences in severe pathologies (cerebral malaria in Malawi compared to a broad range of pathologies in PNG) that are likely to be caused by a broad range of mechanisms involving different PfEMP1 variants.

It is also important to note that there were differences in the protein arrays probed between the two cohorts. For the PNG cohort study, a wide variety of CIDRα1 domains were probed and multiple univariate differences were detected between severe and uncomplicated malaria. In contrast, there were no univariable differences detected amongst the Malawian children, where only two CIDRα domains were probed. Further, the proteins that were identified from Papuan individuals (Table 8, (Tonkin-Hill et al. 2018)) were more frequently represented amongst the target antigens selected in the PNG study, compared to the Malawi study, possibly because these variants were identified in parasites with a closer geographic proximity and possibly closer genetic resemblance to PNG isolates. Differences in the target antigens causing severe malaria between the two cohorts may be driven by host genetic polymorphisms that differ in Africa and PNG. For example, CR1 deficiency has a high prevalence in PNG (Cockburn et al. 2004) and may explain a lack of association of rosetting variants and severe disease PNG (Al-Yaman et al. 1995) compared to Africa (Albrecht et al. 2014) in previous studies. Therefore, it is reasonable to suggest there may be other host genetic differences that influence the *var* gene repertoire in the two populations studied here.

14.2.2 Fc features of antibodies in Malawi and PNG: PfEMP1 variants induce a range of Fc interactions and multiple pathways to protection

In both systems serology studies (in Malawian and PNG children) we observed few differences in IgG titers between children with severe (or cerebral) and uncomplicated malaria, however we identified a number of differences in antigen specific antibody Fc features. An important finding of this thesis is that we confirm that measuring the biophysical properties and interactions of the antibody Fc region can provide useful correlates of malarial disease severity. Based on the findings in thesis, future studies that aim to characterize the antibody response following an intervention, such as a vaccine, should consider a systems serology approach.

A broad range of Fc features were selected by machine learning and associated with uncomplicated malaria across the two cohorts, including FcγRIIIb, FcγRIIb, FcγRIIIa, and C1q binding antibodies, as well as the non-cytophilic subclasses IgG2 and IgG4, whereas IgG and IgG1 were associated with severe disease. Whilst it is possible that some of these features work in concert, such as FcγRIIIa and FcγRIIb responses that were highly correlated in the PNG study, it is likely that they represent many antigen specific pathways to protection. One hypothesis that can be drawn from these antibody profiles is that both stimulatory and modulatory activity can be associated with protection. FcγRIIIb, FcγRIIb and IgG4 may have immune modulatory roles and were associated with protection when targeting specific antigens. FcγRIIb inhibits innate immune cell activity via the ITIM region and FcγRIIIb and IgG4 may have an immune modulatory role by acting as a decoy for pro-inflammatory subclasses and receptors. Additionally, ADCP of ICAM-1+EPCR binding infected erythrocytes was associated with susceptibility to cerebral malaria in Malawian children. Previous studies also support a role of excessive inflammation in severe pathology. Cerebral malaria can result in monocyte infiltrates to the cerebral vasculature (Hochman et al. 2015) and an imbalance of pro-inflammatory and anti-inflammatory cytokines that are secreted by monocytes is associated with pathology (Dunst et al. 2017). Plasma levels of neutrophil elastase, a marker of NETosis, are elevated in retinopathy positive compared to retinopathy negative cerebral malaria and can contribute to endothelial cell degradation (Feintuch et al. 2016). Therefore, immune modulatory functions may balance inflammation in cerebral malaria and play a role in protection. However, further cell based functional assays are required to understand the immune responses that the Fc interactions we have measured induce and their contribution to pathology.

The differences in Fc features that appeared between the groups may reflect the differences in target antigens between the two cohorts, as has already been discussed, but there may also be variation in biophysical properties of the antibody Fc region between individuals living in Malawi and PNG. Allotypes are polymorphisms in the antibody Fc region heavy and light chains (C_H2 and C_H3) that vary between different ethnic groups (Damelang et al. 2019). Allotypes can affect the antibody hinge length and half-life (Damelang et al. 2019) and can have consequences for C1q affinity (Rispens et al. 2014) and phagocytosis activity (Chu et al. 2020). Particular allotypes at the GM locus on the IgG1, IgG2 and IgG3 heavy chains have been associated with the likelihood of an episode of uncomplicated malaria or cerebral malaria (Giha et al. 2009; Kyei-Baafour et al. 2021; Migot-Nabias et al. 2008) and affect the association of age with antibody titers to PfEMP1 (Migot-Nabias et al. 2011). Therefore differences in antibody allotypes between the two populations may contribute to differences in the Fc features selected by machine learning.

An unexpected finding of this thesis is that antibody Fc features change from the acute to convalescent phase of infection. Broadly, we observed a loss in FcγRIIIb and FcγRIIb responses and a gain in FcγRIIIa responses. This was unexpected considering the high similarities between FcγRIIIa and FcγRIIIb (Roberts et al. 2018) and validation in other cohorts will be important. The implications may be that the functional antibody responses changes over the course of infection in children, as these receptors are abundant on different types of immune cells (Gillis et al. 2014). At this point it is unclear whether these changes represent long-term, exposure dependent changes that occur during childhood, such as with fucosylation changes to the antibody Fc region that can influence Fc receptor affinity (Oosterhoff et al. 2022), or changes that occur over the course of each new infection. Based on these findings, it is possible that differences in age and exposure between the PNG and Malawian children may contribute to differences in the Fc features selected as important correlates of protection.

14.2.3 Identifying a reliable laboratory assay to detect protective immunity to malaria

Systems serology is a well-suited approach to study the immune response to PfEMP1 in that it allows broad characterization of a range of PfEMP1 antigens and Fc features without the need for cell culture. Although we could accurately distinguish between children who differed in disease severity using antibody features measured by multiplex immunoassays, it is important to validate whether the antibody responses measured by multiplex immunoassay represent functional activity of whole cell systems and are a good correlate of *in vitro* antibody activity. Previous studies have found that Fc receptor binding to antibodies can be a surrogate of more complex *in vitro* assays. For example, FcγRIIa binding to antigen specific antibodies correlates moderately to strongly with NK cell line ADCC in the context of HIV and influenza (Kristensen et al. 2016; McLean et al. 2017). In context of malaria, FcγRIIa and FcγRIII binding to antibodies targeting recombinant CSP correlate moderately with ADCP of CSP coated beads (which in turn correlates with ADCP of sporozoites). However, neutrophil and THP-1 phagocytosis of infected erythrocytes expressing VAR2CSA correlate weakly with antibody and Fc receptor responses measured by multiplex immunoassay (Aitken et al. 2021). Similarly, in our study we observed no correlation between antibody Fc features measured by multiplex immunoassays and ADNP or ADCP. This suggest that Fc features of antibodies measured by multiplex immunoassay cannot currently be used as surrogates for interactions between immune cells and the infected erythrocytes.

There are several possible points of variation that may contribute to the lack of correlation between the multiplex immunoassays and ADNP/ADCP. First, a significant limitation in the ADNP and ADCP assays was the inability to select infected erythrocytes that display a stable and homologous PfEMP1 variant. Inter-donor correlations improved when ADNP assays were performed on the same day and inter-donor correlations were higher for CS2 parasites that have a stable expression of VAR2CSA. With repeated selection of infected erythrocytes by two methods, we achieved a maximum of 60% purity of *var04* expression. Therefore the lack of correlations between the multiplex assays and phagocytosis assays may be due to differences in the PfEMP1 variants probed. Additionally, there may be other targets of antibodies on the infected erythrocyte surface that contribute to ADNP/ADCP. Knockdown of PfEMP1 export proteins showed that PfEMP1 accounts for 80% of antibody binding to the surface of the infected erythrocyte (Chan et al. 2012) however it is possible that RIFIN or STEVOR, which are also immunogenic (Raghavan et al. 2023; Travassos et al. 2018), may contribute to ADCP or ADNP. Additionally, individuals in endemic areas develop antibodies targeting band 3 protein on the erythrocyte surface (Winograd et al. 2005), possibly due to modification of band 3 by the intracellular parasite (Crandall et al. 1993), and antibodies targeting band 3 may contribute to opsonic phagocytosis (Ayi et al. 2004). Finally, recombinant proteins don't necessarily represent native PfEMP1 epitopes. Small differences in the border of the recombinant domains can significantly influence antibody recognition to VAR2CSA epitopes (Nielsen et al. 2015a). Further, antibodies depleted for IgG targeting recombinant VAR2CSA proteins could still recognize native VAR2CSA on the infected erythrocyte and inhibit adhesion to CSA receptor, suggesting that there are conformational epitopes of VAR2CSA that are not captured by recombinant proteins (Doritchamou et al. 2016b), and the same may be true for other PfEMP1 variants. In the case of ICAM-1 binding variants, immunization of rats with recombinant DBLβ induces antibodies that can inhibit infected erythrocyte binding to ICAM-1 (Olsen et al. 2018), however a combined effect of multiple epitopes may be involved in functional activity such as ADNP and ADCP.

Despite the lack of correlation between the assays, the broad screening by multiplex immunoassay highlighted the importance of the interaction of antibodies targeting DBLβ domains with FcγRIIIb, a receptor that is abundant on neutrophils. This prompted us to investigate the role of ADNP as a potential mechanism of protection and led to the important finding that ADNP of ICAM-1+EPCR binding IE is associated with protection from cerebral malaria in Malawian children but not from severe malaria in PNG children. Additionally, the decay in antibodies that bind FcγRIIIb and gain in antibodies that bind FcγRIIIa from acute presentation to convalescence documented by multiplex immunoassay may partially explain the decay in ADNP, but not ADCP, over this period that was observed in both cohorts, since neutrophils expresses high levels of FcγRIIIb and THP-1 cells do not. Given that these important findings were mirrored across the different assays, it would be worthwhile to conduct further studies to understand the contribution of the interactions measured by multiplex immunoassay to cell-cell interactions, as well as the points of variation between the multiplex immunoassay and cell-based immunoassays.

14.3 Limitations

A strength of this thesis was the use of clinical samples from case-control studies that were well classified for severe and uncomplicated malaria. Case-control studies are limited by a lack of information on the duration of infection prior to acute sample collection and treatment, which is likely to influence antibody levels and disease severity. We are unable to distinguish between antibody responses to the current infection and pre-existing responses from prior exposure. Additionally, the kinetics of disease progression and antibody development may differ between uncomplicated malaria, cerebral malaria and other severe malaria syndromes. Samples collected in convalescence revealed important insights into target antigens for which antibodies are acquired in severe disease, and changes in Fc features over the course of infection. However, the maintenance and protective association of these responses beyond the 4/8 week follow up periods is unknown. The ideal study would be a prospective design with additional samples collected pre-illness and post-illness, however prospective studies are far more difficult to coordinate than case-control studies.

A challenge with studies of naturally acquired immunity is controlling for the confounding effect of prior exposure. A strength of the Malawian study was that children were well matched for age and location of residency, to account for differences in exposure with age and transmission dynamics. Due to sample availability, there were differences in age between the PNG children with severe and uncomplicated malaria. We adjusted for age in the regression analysis, however there was no available information on residency location to account for differences in exposure due to transmission dynamics. In both cohorts, there was evidence for differences in antibody responses to merozoite antigens that can be surrogate markers of exposure in young children, suggesting exposure may still be contributing to differences in antibody responses to PfEMP1 antigens. Alternatively, antibody responses to merozoite antigens may be a correlate of protective immunity that has not been explored by this study.

Although this thesis assessed antibody responses to a broad range of PfEMP1 domains, this study may have missed other important variants circulating in Malawi and PNG, which could be identified by mRNA transcription analysis of variants in each region. Another limitation in the choice of recombinant proteins was the mixture of expression systems used, including WGCFS, *E. coli* and HEK293 systems. Cell based expression systems have limited capacity to express *P. falciparum* genes with A/T rich regions and repeated sequences, and slow expression rates in prokaryotic cell based systems (such as *E. coli*) can result in incorrect conformational folding (Tsuboi et al. 2010). WGCFS overcomes these challenges and produces high yields of correctly folded recombinant proteins (Tsuboi et al. 2010) and therefore may be the preferable expression system for PfEMP1 proteins. Encouragingly, differences between cerebral and uncomplicated malaria were observed for DBLβ domains expressed in both WGCFS and *E. coli* systems, suggesting some level of consistency in the antibody response across different expression systems, however consistency was not formally assessed. An additional limitation in the multiplex immunoassay studies included the mixture of recombinant Fc dimers and tetramers. Fc dimers have lower non-specific background binding and a greater dynamic range than tetrameric or monomeric Fc receptor reagents (McLean et al. 2017). Interestingly, Fc receptors that were in tetrameric form were selected by machine learning (FcγRIIIb) and not receptors in dimeric form (FcγRIIa), possibly suggesting a bias in the Malawi cohort analysis.

As has already been discussed, a strength of this study was the use of multiple neutrophil donors to study ADNP however the ADNP assays had limited reproducibility due to parasite PfEMP1 expression variation and inter-donor variation. Additionally, we did not perform reproducibility studies for the ADCP assays, although less variation is expected for a cell line compared to freshly isolated monocytes. Other limitations include the lack of monocyte subsets represented by the THP-1 cell lines and that we did not confirm that THP-1 cells or neutrophils positive for DHE stained parasites measured by flow cytometry corresponded with intracellular uptake of the parasites. We assume that the lysis prior to flow cytometry removes any erythrocytes that are outside of the immune cell however confocal microscopy could confirm this.

14.4 Future directions

- *Validation of features selected by machine learning in other cohorts*

Further analysis of our data could demonstrate whether the features selected by machine learning in the Malawi study can be used to identify the subset of children with cerebral malaria in the PNG children, and other cohorts, and whether features selected in the PNG study (against a broad range of severe syndromes) can be used to identify children with cerebral malaria in the Malawian children, and other cohorts. This was not performed for this thesis due to technical difficulties and time constraints on measuring C1q binding antibodies in the PNG cohort study (as C1q was a key protective Fc feature in the Malawian cohort). For the PNG children, it's likely that there are other combinations of domains that can be used in combination to predict disease severity with similarly high accuracy, since there were many features that were associated with uncomplicated malaria and even 8 randomly selected features performed with 67% accuracy. Therefore, it may be possible to identify a subset of features that can be used in combination across both cohorts. The number of features included in the final regression model may also be further optimized depending on the desired application. For example, a smaller number of features may be used to classify children with severe malaria only, rather than classifying children with both severe and uncomplicated malaria, if this modelling were to be developed into a prognostic tool.

- *Contribution of selected antigens to pathology*

It would be interesting to investigate whether the selected target antigens (or antibodies targeting these antigens) mechanistically contribute to severe pathology. One possibility would be to investigate whether some of the lesser well characterized antigens, such as CIDRγ12, bind to endothelial cell receptors, and may thereby contribute to sequestration or endothelial cell activation. The binding phenotype of different antigens could be assessed by multiplex immunoassay using recombinant receptors, as has been described for ICAM-1 (Oleinikov et al. 2009). Additionally, near to whole parasite genomes for multiple parasite isolates are available (Otto et al. 2018) and may be used to map whether some of the lesser well characterized domains identified in this study are commonly expressed alongside domains with known functional phenotypes, such as EPCR or ICAM-1 binding. It would be interesting to know if the antibody responses are linked to circulating factors that have been associated with cerebral malaria and proposed to impair the blood brain barrier, such as circulating inflammatory cytokines IL-1β, IL-6 and TNF, and angiopoietins Ang-1 and Ang-2 (Jensen et al. 2020). Although it is difficult to obtain post-mortem histological sections for cerebral malaria, it might be possible to link the antigen specific antibody responses identified in this study to brain swelling identified by neuroimaging, as has been done for transcription of *var* genes in a previous study (Kessler et al. 2017), or to blood flow to the brain detected by Transcranial Doppler Ultrasound (O'Brien et al. 2022).

- *Contribution of other, non-PfEMP1 antigens as measures of immunity*

Future studies could consider the use of antibodies to non-trophozoite stage antigens to model differences in disease severity, alone or in combination with PfEMP1 antigens. Antibody responses to merozoite antigens and CSP were included in this study as technical positive controls for multiplex immunoassays and indicators of prior exposure, however they may be valuable indicators of protective immunity. Multi-stage vaccines have previously been proposed as a potential way to maximize protection from severe disease or to induce both transmission blocking and protective immunity (Bathurst et al. 1993; Spiegel et al. 2015), although so far multi-stage vaccines have demonstrated limited immunogenicity in early clinical trials (Porter et al. 2011). Additionally, were this modelling to be developed into a prognostic tool to predict the risk of severe malaria, other epidemiological or clinical indicators could also be incorporated to improve the predictive accuracy.

- *Further development of cell-based immunoassays to assess immune functions induced by Fc interactions*

A major challenge of this thesis was the selection of parasites that express a stable and uniform PfEMP1 variant in culture and better methods to do so should be a priority of future studies. Multiple factors are thought to contribute to *var* gene switching, including epigenetic regulation. For example, a recent study found that long-noncoding RNAs, including the redox protein thioredoxin peroxidase I (PfPx-1), interact directly with *var* genes to modify expression and knock-down of PfPx-1 lead to slower *var* switch rate (Heinberg et al. 2022). It may be possible to epigenetically modify parasites to have stable expression of PfEMP1 variants or slower switching rates that don't require as frequent selection. Future studies could also confirm the *var* genes transcribed by the selected parasites by mRNA sequencing, in addition to the method used in this study involving recognition of PfEMP1 variants by polyclonal antibodies.

Establishing parasite lines with stable expression of ICAM-1 binding PfEMP1 would enable us to investigate the contribution of FcγRIIIb receptor binding antibodies targeting DBLβ domains to ADNP of ICAM-1 binding IE. One option would be to deplete serum samples of IgG specific for recombinant DBLβ domains and compare relative median levels of ADNP with non-depleted samples (similarly to the methods used by Doritchamou *et al.*, (Doritchamou et al. 2016a)). Additionally, FcγRIIIb receptors can be blocked (as in Tebo *et al.*, (Tebo et al. 2002)) to verify their contribution to ADNP of IE and DBLβ coated latex beads can be used instead of parasitized erythrocytes in the ADNP assay (as in Aitken, Damelang, Ortega-pajares *et al.*, (Aitken et al. 2021)).

For a deeper understanding of the ADNP assay, future studies could investigate other factors that may contribute to inter-donor variation in ADNP, including biological variation in neutrophil expression of Fc receptors, ICAM-1 receptors and neutrophil subsets. Several other antibody Fc functions have been implicated in protective immunity in this thesis, including complement mediated lysis and phagocytosis (directed by C1q fixation) and NK cell activation (directed by FcγRIIIa binding), and further studies should investigate these mechanisms using infected erythrocytes. Additionally, it would be interesting to know if IgG4 has a particular functional activity, such as adhesion blocking to endothelial receptors, or is a marker of repeat exposure to malaria. Although it is difficult to quantify prior exposure, age can be used as a surrogate in high transmission settings, therefore we would be able perform a more detailed analysis of the association of antibody responses with age (such as by PLS regression) to identify variables that may be associated with exposure, rather than protection.

- *Investigating changes in antibody Fc features from acute to convalescence*

By comparing antibody responses from the acute malaria presentation to convalescence, we observed a change in antibody Fc features over the course of infection. Changes included a decay in FcγRIIIb binding and an increase in FcγRIIIa binding, and this was reflected by the decay in neutrophil phagocytosis (that involves FcγRIIIb) but not THP-1 phagocytosis (that does not involve FcγRIIIb). As has been discussed in this thesis, antibody glycosylation patterns can contribute to Fc receptor affinity, therefore future studies could characterize the antibody glycosylation patterns of antigen specific antibodies in children over the course of infection, and determine the affinity of the identified patterns with Fc receptors. It would also be interesting to characterize the association of glycosylation patterns with age to determine if there are long term changes that occur with age and exposure, as is the case with fucosylation (Oosterhoff et al. 2022), that might have implications for changes in immune function.

14.5 Concluding remarks

This thesis highlighted a role for antibodies targeting PfEMP1 antigens, especially DBLβ domains, in protection from severe malaria; the importance of measuring the quality of PfEMP1 specific antibodies instead of only the quantity of IgG; and that antibodies targeting PfEMP1 likely promote multiple pathways to protection, including antibody dependent neutrophil phagocytosis of infected erythrocytes. These findings may support the development of a DBLβ based vaccine or monoclonal antibody therapy. The systems serology approach applied in this thesis may be used as a foundation for designing vaccine adjuvants and monoclonal antibodies that induce antigen specific Fc effector functions, or for a prognostic clinical tool to predict severe disease.

Whilst such advanced tools would be useful to reduce severe malaria, there are a multitude of contextual factors that contribute to the burden of malaria and success of interventions. It is not a coincidence that malaria predominantly affects children living in low-middle income countries, including Malawi and Papua New Guinea. Low income, lack of education and low quality or overcrowded housing are risk factors for malaria in children under 5 (Canelas et al. 2019; Klejnstrup et al. 2018; Sarfo et al. 2023). Delayed access to healthcare due to poor infrastructure and lack of essential malaria treatment supplies are risk factors for development of severe malaria (Ippolito et al. 2022; Sarfo et al. 2023). Whilst bed nets are effective and widely distributed, they are under often underused, damaged or repurposed (World Health Organization 2021c). In Malawi, fishing is a common use of bed nets due to food insecurity (Berthe et al. 2019). Taken together, investment into a multitude of socio-economic and environmental factors would likely improve the burden of severe malaria, as well as other infectious diseases. The WHO estimates an annual funding gap of 3.5 billion USD exists to provide adequate resources to eliminate malaria. Continued efforts to develop cost-effective, innovative tools and better purpose existing tools to reduce the burden of malaria within the context of low and middle income countries are needed.

Part VIII

Publications

The following peer-reviewed publications were products of this thesis:

Analysis of Antibody Reactivity to Malaria Antigens by Microsphere-Based Multiplex Immunoassay (Methods in Molecular Biology)

Isobel S Walker, Amy W Chung, Timon Damelang, and Stephen J Rogerson.

DOI: 0.1007/978-1-0716-2189-9_23.

Pathogenicity and virulence of malaria: Sticky problems and tricky solutions (Virulence)

Isobel S Walker, Stephen J Rogerson.

DOI: 10.1080/21505594.2022.2150456.

Part IX

R-Packages, citations

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. `http://www.R-project.org/.`

caret: Max Kuhn (2022). caret: Classification and Regression Training. R package version 6.0-93. `https://CRAN.R-project.org/package=caret`

Dyplyr: Hadley Wickham, Romain François, Lionel Henry and Kirill Müller (2020). dplyr: A Grammar of Data Manipulation. R package version 1.0.2. `https://CRAN.R-project.org/package=dplyr`

ggpubr: Alboukadel Kassambara (2020). ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0. `https://CRAN.R-project.org/package=ggpubr`

ggrepel: Kamil Slowikowski (2021). ggrepel: Automatically Position Non-Overlapping Text Labels with 'ggplot2'. R package version 0.9.1. `https://CRAN.R-project.org/package=ggrepel`

ggplot2: H. Wickham. ggplot2:Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

glmnet: Jerome Friedman and Robert Tibshirani and Trevor Hastie (2010). Regularization Paths for Generalized Linear Models via Coordinate Descent. Journal of Statistical Software. 33(1) 1-22. `doi:10.18637/jss.v033.i01`

J. Kenneth Tay and Balasubramanian Narasimhan and Trevor Hastie (2023). Elastic Net Regularization Paths for All Generalized Linear Models. Journal of Statistical Software. 106(1) 1-31. `doi:10.18637/jss.v106.i01`

mice: Stef van Buuren and Karin Groothuis-Oudshoorn (2011). MICE: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software. 45(3) 1-67 `doi:10.18637/jss.v045.i03`

mixOmics: Rohart F and Gautier B and Singh A and Le Cao K-A (2017). mixOmics: An R package for 'omics feature selection and multiple data integration. PLoS computational biology. 13(11), e1005752. `http://www.mixOmics.org`

RColorBrewer: Erich Neuwirth (2014). ColorBrewer Palettes. R package version 1.1-2. `https://CRAN.R-project.org/package=RColorBrewer`

tidyr: Hadley Wickham and Lionel Henry (2020). Tidy Messy Data. R package version 1.0.2. `https://CRAN.R-project.org/package=tidyr`

tidyverse: Hadley Wickham and Mara Averick and Jennifer Bryan and Winston Chang and Lucy D'Agostino McGowan and Romain François and Garrett Grolemund and Alex Hayes and Lionel Henry and Jim Hester and Max Kuhn and Thomas Lin Pedersen and Evan Miller and Stephan Milton Bache and Kirill Müller and Jeroen Ooms and David Robinson and Dana Paige Seidel and Vitalie Spinu and Kohske Takahashi and Davis Vaughan and Claus Wilke and Kara Woo and Hiroaki Yutani Welcome to tidyverse . Journal of Open Source Software, 4(43), 1686, `https://doi.org/10.21105/joss.01686`

Part X

Bibliography

References

A-Elgadir, T. M., M. I. Elbashir, K. Berzins, E. M. Masuadi, I. E. A-Elbasit, G. ElGhazali, and H. A. Giha (2008). “The profile of IgG-antibody response against merozoite surface proteins 1 and 2 in severe Plasmodium falciparum malaria in Eastern Sudan”. In: *Parasitology research* 102, pp. 401–409.

Acquah, F. K., J. Adjah, K. C. Williamson, and L. E. Amoah (June 2019). “Transmission-Blocking Vaccines: Old Friends and New Prospects.” In: *Infection and immunity* 87.6, e00775–18.

Adamou, R., C. Dechavanne, I. Sadissou, T. D’Almeida, A. Bouraima, P. Sonon, R. Amoussa, G. Cottrell, A. Le Port, M. Theisen, E. J. Remarque, S. Longacre, K. Moutairou, A. Massougbodji, A. J. F. Luty, G. Nuel, F. Migot-Nabias, A. Sanni, A. Garcia, J. Milet, and D. Courtin (June 2019). “Plasmodium falciparum merozoite surface antigen-specific cytophilic IgG and control of malaria infection in a Beninese birth cohort”. In: *Malaria journal* 18.1, p. 194.

Adams, Y., P. Khunrae, M. K. Higgins, A. Ghumra, and J. A. Rowe (Mar. 2014). “Rosetting Plasmodium falciparum-infected erythrocytes bind to human brain microvascular endothelial cells in vitro, demonstrating a dual adhesion phenotype mediated by distinct P. falciparum erythrocyte membrane protein 1 domains.” In: *Infection and immunity* 82.3, pp. 949–59.

Adams, Y., R. W. Olsen, A. Bengtsson, N. Dalgaard, M. Zdioruk, S. Satpathi, P. K. Behera, P. K. Sahu, S. E. Lawler, K. Qvortrup, S. C. Wassmer, and A. T. R. Jensen (Jan. 2021). “Plasmodium falciparum erythrocyte membrane protein 1 variants induce cell swelling and disrupt the blood–brain barrier in cerebral malaria”. In: *Journal of Experimental Medicine* 218.3, e20201266.

Adepoju, P. (Apr. 2019). “RTS,S malaria vaccine pilots in three African countries.” In: *Lancet* 393.10182, p. 1685.

Adu, B., M. P. G. Jepsen, T. A. Gerds, E. Kyei-Baafour, M. Christiansen, D. Dodoo, and M. Theisen (2014). “Fc gamma receptor 3B (FCGR3B-c. 233C> A-rs5030738) polymorphism modifies the protective effect of malaria specific antibodies in Ghanaian children”. In: *The Journal of infectious diseases* 209.2, pp. 285–289.

Ahmed Ismail, H., U. Ribacke, L. Reiling, J. Normark, T. Egwang, F. Kironde, J. G. Beeson, M. Wahlgren, and K. E. Persson (2013). “Acquired antibodies to merozoite antigens in children from Uganda with uncomplicated or severe Plasmodium falciparum malaria”. In: *Clinical and Vaccine Immunology* 20.8, pp. 1170–1180.

Aitken, E. H., S. Mahanty, and S. J. Rogerson (Apr. 2020). “Antibody effector functions in malaria and other parasitic diseases: a few needles and many haystacks.” In: *Immunology and cell biology* 98.4, pp. 264–275.

Aitken, E. H., B. Mbewe, M. Luntamo, K. Maleta, T. Kulmala, M.-J. Friso, F. J. Fowkes, J. G. Beeson, P. Ashorn, and S. J. Rogerson (2010). “Antibodies to chondroitin sulfate A-binding infected erythrocytes: dynamics and protection during pregnancy in women receiving intermittent preventive treatment”. In: *The Journal of infectious diseases* 201.9, pp. 1316–1325.

Aitken, E. H., A. Alemu, and S. J. Rogerson (2018). “Neutrophils and Malaria”. In: *Frontiers in immunology* 9.December, p. 3005.

Aitken, E. H., T. Damelang, A. Ortega-Pajares, A. Alemu, W. Hasang, S. Dini, H. W. Unger, M. Ome-Kaius, M. A. Nielsen, A. Salanti, J. Smith, S. Kent, P. M. Hogarth, B. D. Wines,

J. A. Simpson, A. Chung, and S. J. Rogerson (2021). "Developing a multivariate prediction model of antibody features associated with protection of malaria-infected pregnant women from placental malaria". In: *eLife* 10, pp. 1–30.

Akhouri, R. R., S. Goel, H. Furusho, U. Skoglund, and M. Wahlgren (Feb. 2016). "Architecture of Human IgM in Complex with P. falciparum Erythrocyte Membrane Protein 1." In: *Cell reports* 14.4, pp. 723–736.

Akpogheneta, O. J., N. O. Duah, K. K. A. Tetteh, S. Dunyo, D. E. Lanar, M. Pinder, and D. J. Conway (Apr. 2008). "Duration of naturally acquired antibody responses to blood-stage Plasmodium falciparum is age dependent and antigen specific." In: *Infection and immunity* 76.4, pp. 1748–1755.

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter (2002). "Molecular Biology of the Cell". In.

Albrecht, L., D. Angeletti, K. Moll, K. Blomqvist, D. Valentini, F. L. D'Alexandri, M. Maurer, and M. Wahlgren (Dec. 2014). "B-cell epitopes in NTS-DBL1alpha- of PfEMP1 recognized by human antibodies in rosetting Plasmodium falciparum". In: *PloS One* 9.12, e113248.

Albrecht, L., S. C. P. Lopes, A. B. I. E. da Silva, V. Barbosa, R. P. Almeida, A. M. Siqueira, J. A. Leite, N. C. Bittencourt, H. G. Dos Santos, C. Bourgard, L. F. C. Garcia, A. C. A. V. Kayano, I. S. Soares, B. Russell, L. Rénia, M. V. G. Lacerda, and F. T. M. Costa (Oct. 2020). "Rosettes integrity protects Plasmodium vivax of being phagocytized." In: *Scientific reports* 10.1, p. 16706.

Allen, C. D. C. (Jan. 2022). "Features of B Cell Responses Relevant to Allergic Disease." In: *Journal of immunology (Baltimore, Md. : 1950)* 208.2, pp. 257–266.

Allen, S. J., A. O'Donnell, N. D. Alexander, C. S. Mgone, T. E. Peto, J. B. Clegg, M. P. Alpers, and D. J. Weatherall (June 1999). "Prevention of cerebral malaria in children in Papua New Guinea by southeast Asian ovalocytosis band 3." In: *The American journal of tropical medicine and hygiene* 60.6, pp. 1056–1060.

Alonso, P. L., J. Sacarlal, J. J. Aponte, A. Leach, E. Macete, J. Milman, I. Mandomando, B. Spiessens, C. Guinovart, M. Espasa, Q. Bassat, P. Aide, O. Ofori-Anyinam, M. M. Navia, S. Corachan, M. Ceuppens, M.-C. Dubois, M.-A. Demoitié, F. Dubovsky, C. Menéndez, N. Tornieporth, W. R. Ballou, R. Thompson, and J. Cohen (Oct. 2004). "Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children: randomised controlled trial." In: *Lancet (London, England)* 364.9443, pp. 1411–1420.

Amanna, I. J., N. E. Carlson, and M. K. Slifka (Nov. 2007). "Duration of humoral immunity to common viral and vaccine antigens." In: *The New England journal of medicine* 357.19, pp. 1903–1915.

Angeletti, D., L. Albrecht, M. Wahlgren, and K. Moll (2013). "Analysis of antibody induction upon immunization with distinct NTS-DBL1alpha–domains of PfEMP1 from rosetting Plasmodium falciparum parasites." In: *Malaria journal* 12, p. 32.

Aniweh, Y., P. B. Nyarko, E. Charles-Chess, F. Ansah, F. H. A. Osier, E. Quansah, L. G. Thiam, G. Kamuyu, K. Marsh, D. J. Conway, K. K. A. Tetteh, and G. A. Awandare (2020). "Plasmodium falciparum Merozoite Associated Armadillo Protein (PfMAAP) Is Apically Localized in Free Merozoites and Antibodies Are Associated With Reduced Risk of Malaria." In: *Frontiers in immunology* 11, p. 505.

Araj, B. N., B. Swihart, R. Morrison, P. G. Hurtado, A. Teo, A. Mahamar, O. Attaher, B. S. Diarra, S. Gaoussou, D. Issiaka, A. Dicko, P. E. Duffy, and M. Frieda (2021). "Antibody

Levels to Plasmodium falciparum Erythrocyte Membrane Protein 1-DBLgamma11 and DBLδ-1 Predict Reduction in Parasite Density". In: *mSystems* 6.3, pp. 1–10.

Arakawa, C., C. Gunnarsson, C. Howard, M. Bernabeu, K. Phong, E. Yang, C. A. DeForest, J. D. Smith, and Y. Zheng (Jan. 2020). "Biophysical and biomolecular interactions of malaria-infected erythrocytes in engineered human capillaries." In: *Science advances* 6.3, eaay7243.

Arman, M., A. Raza, L. J. Tempest, K. E. Lyke, M. A. Thera, A. Koné, C. V. Plowe, O. K. Doumbo, and J. A. Rowe (Nov. 2007). "Platelet-mediated clumping of Plasmodium falciparum infected erythrocytes is associated with high parasitemia but not severe clinical manifestations of malaria in African children." In: *The American journal of tropical medicine and hygiene* 77.5, pp. 943–6.

Arman, M. and J. A. Rowe (Dec. 2008). "Experimental conditions affect the outcome of Plasmodium falciparum platelet-mediated clumping assays". In: *Malaria Journal* 7.1, p. 243.

Arnold, K. B. and A. W. Chung (2018). "Prospects from systems serology research". In: *Immunology* 153.3, pp. 279–289.

Arora, G., G. T. Hart, J. Manzella-Lapeira, J. Y. Doritchamou, D. L. Narum, L. M. Thomas, J. Brzostowski, S. Rajagopalan, O. K. Doumbo, B. Traore, L. H. Miller, S. K. Pierce, P. E. Duffy, P. D. Crompton, S. A. Desai, and E. O. Long (June 2018). "NK cells inhibit Plasmodium falciparum growth in red blood cells via antibody-dependent cellular cytotoxicity." In: *eLife* 7, pp. 1–20.

Ataíde, R., V. Mwapasa, M. E. Molyneux, S. R. Meshnick, and S. J. Rogerson (2011). "Antibodies that induce phagocytosis of malaria infected erythrocytes: Effect of HIV infection and correlation with clinical outcomes". In: *PLoS ONE* 6.7, pp. 1–7.

Aucan, C., Y. Traoré, F. Tall, B. Nacro, T. Traoré-Leroux, F. Fumoux, and P. Rihet (Mar. 2000). "High immunoglobulin G2 (IgG2) and low IgG4 levels are associated with human resistance to Plasmodium falciparum malaria." In: *Infection and immunity* 68.3, pp. 1252–1258.

Avril, M., A. K. Tripathi, A. J. Brazier, C. Andisi, J. H. Janes, V. L. Soma, D. J. Sullivan, P. C. Bull, M. F. Stins, and J. D. Smith (2012). "A restricted subset of var genes mediates adherence of Plasmodium falciparum-infected erythrocytes to brain endothelial cells". In: *Proceedings of the National Academy of Sciences* 109.26, e1782–90.

Avril, M., M. Bernabeu, M. Benjamin, A. J. Brazier, and J. D. Smith (2016). "Interaction between endothelial protein C receptor and intercellular adhesion molecule 1 to mediate binding of plasmodium falciparum-infected erythrocytes to endothelial cells". In: *mBio* 7.4, pp. 1–10.

Ayi, K., F. Turrini, A. Piga, and P. Arese (2004). "Enhanced phagocytosis of ring-parasitized mutant erythrocytes: a common mechanism that may explain protection against falciparum malaria in sickle trait and beta-thalassemia trait". In: *Blood* 104.10, pp. 3364–3371.

Azasi, Y., G. Lindergard, A. Ghumra, J. Mu, L. H. Miller, and J. A. Rowe (2018). "Infected erythrocytes expressing DC13 PfEMP1 differ from recombinant proteins in EPCR-binding function". In: *Proceedings of the National Academy of Sciences*.

Azasi, Y., L. M. Low, A. N. Just, S. S. R. Raghavan, C. W. Wang, P. Valenzuela-Leon, J. A. Rowe, J. D. Smith, T. Lavstsen, L. Turner, E. Calvo, and L. H. Miller (June 2021). "Complement C1s cleaves PfEMP1 at interdomain conserved sites inhibiting Plasmodium falciparum cytoadherence." In: *Proceedings of the National Academy of Sciences of the United States of America* 118.22.

Bachmann, A., E. Bruske, R. Krumkamp, L. Turner, J. S. Wichers, M. Petter, J. Held, M. F. Duffy, B. K. L. Sim, S. L. Hoffman, P. G. Kremsner, B. Lell, T. Lavstsen, M. Frank, B. Mordmüller, and E. Tannich (July 2019). "Controlled human malaria infection with Plasmodium falciparum demonstrates impact of naturally acquired immunity on virulence gene expression". In: *PLOS Pathogens* 15.7, e1007906.

Bachmann, A., C. Esser, M. Petter, S. Predehl, V. von Kalckreuth, S. Schmiedel, I. Bruchhaus, and E. Tannich (Oct. 2009). "Absence of erythrocyte sequestration and lack of multicopy gene family expression in Plasmodium falciparum from a splenectomized malaria patient". In: *PLoS ONE* 4.10, e7459.

Bachmann, A., S. Predehl, J. May, S. Harder, G. D. Burchard, T.-W. Gilberger, E. Tannich, and I. Bruchhaus (Sept. 2011). "Highly co-ordinated var gene expression and switching in clinical Plasmodium falciparum isolates from non-immune malaria patients." In: *Cellular microbiology* 13.9, pp. 1397–409.

Badaut, C., P. Visitdesotrakul, A. Chabry, P. Bigey, B. Tornyigah, J. Roman, J. A. Maroufou, A. Amoussou, B. S. Ayivi, G. Sagbo, N. T. Ndam, A. V. Oleinikov, and R. Tahar (2021). "IgG acquisition against PfEMP1 PF110521 domain cassette DC13, DBLβ3D4 domain, and peptides located within these constructs in children with cerebral malaria". In: *Scientific Reports* 11.1, pp. 1–12.

Baker, D., O. Daramola, M. McCrossan, J. Harmer, and G. Targett (1994). "Subcellular localization of Pfs16, a Plasmodium falciparum gametocyte antigen". In: *Parasitology* 108.2, pp. 129–137.

Baratin, M., S. Roetynck, B. Pouvelle, C. Lemmers, N. K. Viebig, S. Johansson, P. Bierling, A. Scherf, J. Gysin, E. Vivier, and S. Ugolini (Feb. 2007). "Dissection of the role of PfEMP1 and ICAM-1 in the sensing of Plasmodium-falciparum-infected erythrocytes by natural killer cells." In: *PloS one* 2.2, e228.

Barfod, L., M. B. Dalgaard, S. T. Pleman, M. F. Ofori, R. J. Pleass, and L. Hviid (July 2011). "Evasion of immunity to Plasmodium falciparum malaria by IgM masking of protective IgG epitopes in infected erythrocyte surface-exposed PfEMP1." In: *Proceedings of the National Academy of Sciences of the United States of America* 108.30, pp. 12485–90.

Barfod, L., T. Dobrilovic, P. Magistrado, P. Khunrae, F. Viwami, J. Bruun, M. Dahlbäck, N. L. Bernasconi, M. Fried, and D. John (2010). "Chondroitin sulfate A-adhering Plasmodium falciparum-infected erythrocytes express functionally important antibody epitopes shared by multiple variants". In: *The Journal of Immunology* 185.12, pp. 7553–7561.

Barry, A. E., A. Trieu, F. J. I. Fowkes, J. Pablo, M. Kalantari-Dehaghi, A. Jasinskas, X. Tan, M. A. Kayala, L. Tavul, P. M. Siba, K. P. Day, P. Baldi, P. L. Felgner, and D. L. Doolan (Nov. 2011). "The stability and complexity of antibody responses to the major surface antigen of Plasmodium falciparum are associated with age in a malaria endemic area." In: *Molecular cellular proteomics : MCP* 10.11, p. M111.008326.

Baruch, D. I., X. C. Ma, H. B. Singh, X. Bi, B. L. Pasloske, and R. J. Howard (Nov. 1997). "Identification of a region of PfEMP1 that mediates adherence of Plasmodium falciparum infected erythrocytes to CD36: conserved function with variant sequence." In: *Blood* 90.9, pp. 3766–75.

Bathurst, I. C., H. L. Gibson, J. Kansopon, B. K. Hahm, K. M. Green, S. P. Chang, G. S. Hui, W. A. Siddiqui, J. Inselburg, P. Millet, et al. (1993). "An experimental vaccine cocktail for Plasmodium falciparum malaria". In: *Vaccine* 11.4, pp. 449–456.

Baum, E., K. Badu, D. M. Molina, X. Liang, P. L. Felgner, and G. Yan (Dec. 2013). "Protein microarray analysis of antibody responses to Plasmodium falciparum in western Kenyan highland sites with differing transmission levels". In: *PloS one* 8.12, e82246–e82246.

Beare, N. A. V., T. E. Taylor, S. P. Harding, S. Lewallen, and M. E. Molyneux (Nov. 2006). "Malarial retinopathy: a newly established diagnostic sign in severe malaria." In: *The American journal of tropical medicine and hygiene* 75.5, pp. 790–7.

Beeson, J. G., D. R. Drew, M. J. Boyle, G. Feng, F. J. Fowkes, and J. S. Richards (2016a). "Merozoite surface proteins in red blood cell invasion, immunity and vaccines against malaria". In: *FEMS microbiology reviews* 40.3, pp. 343–372.

— (2016b). "Merozoite surface proteins in red blood cell invasion, immunity and vaccines against malaria". In: *FEMS Microbiology Reviews* 40.3, pp. 343–372.

Bengtsson, A., L. Joergensen, T. S. Rask, R. W. Olsen, M. A. Andersen, L. Turner, T. G. Theander, L. Hviid, M. K. Higgins, A. Craig, A. Brown, and A. T. R. Jensen (2013). "A Novel Domain Cassette Identifies Plasmodium falciparum PfEMP1 Proteins Binding ICAM-1 and Is a Target of Cross-Reactive, Adhesion-Inhibitory Antibodies". In: *The Journal of Immunology* 190.1, pp. 240–249.

Berendt, A. R., D. L. Simmons, J. Tansey, C. I. Newbold, and K. Marsh (1989). "Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for Plasmodium falciparum." In: *Nature* 341.6237, pp. 57–59.

Berger, S. S., L. Turner, C. W. Wang, J. E. V. Petersen, M. Kraft, J. P. A. Lusingu, B. Mmbando, A. M. Marquard, D. B. A. C. Bengtsson, L. Hviid, M. A. Nielsen, T. G. Theander, and T. Lavstsen (July 2013). "Plasmodium falciparum Expressing Domain Cassette 5 Type PfEMP1 (DC5-PfEMP1) Bind PECAM1". In: *PLoS ONE* 8.7. Ed. by T. Spielmann, e69117.

Bernabeu, M., S. A. Danziger, M. Avril, M. Vaz, P. H. Babar, A. J. Brazier, T. Herricks, J. N. Maki, L. Pereira, A. Mascarenhas, E. Gomes, L. Chery, J. D. Aitchison, P. K. Rathod, and J. D. Smith (June 2016). "Severe adult malaria is associated with specific PfEMP1 adhesion types and high parasite biomass." In: *Proceedings of the National Academy of Sciences of the United States of America* 113.23, E3270–9.

Berthe, S., S. A. Harvey, M. Lynch, H. Koenker, V. Jumbe, B. Kaunda-Khangamwa, and D. P. Mathanga (2019). "Poverty and food security: drivers of insecticide-treated mosquito net misuse in Malawi". In: *Malaria Journal* 18, pp. 1–11.

Bertin, G. I., M. J. Alao, N. T. Ndam, A. Massougbodji, J. S. Jespersen, F. Guillonneau, S. Ezimegnon, C. W. Wang, F. Lalya, N. Fievet, T. Lavstsen, T. G. Theander, P. Deloron, and J. Doritchamou (2013). "Expression of the Domain Cassette 8 Plasmodium falciparum Erythrocyte Membrane Protein 1 Is Associated with Cerebral Malaria in Benin". In: *PLoS ONE* 8.7, e68368.

Blank, A., K. Fürle, A. Jäschke, G. Mikus, M. Lehmann, J. Hüsing, K. Heiss, T. Giese, D. Carter, E. Böhnlein, M. Lanzer, W. E. Haefeli, and H. Bujard (2020). "Immunization with full-length Plasmodium falciparum merozoite surface protein 1 is safe and elicits functional cytophilic antibodies in a randomized first-in-human trial." In: *NPJ vaccines* 5.1, p. 10.

Blanter, M., S. Cambier, M. De Bondt, L. Vanbrabant, N. Pörtner, S. Abouelasrar Salama, M. Metzemaekers, P. E. Marques, S. Struyf, P. Proost, and M. Gouwy (2022). "Method Matters: Effect of Purification Technology on Neutrophil Phenotype and Function." In: *Frontiers in immunology* 13, p. 820058.

Bournazos, S. and J. V. Ravetch (July 2017). "Diversification of IgG effector functions". In: *International Immunology* 29.7, pp. 303–310.

Bousema, T., A. Barry, M. C. Behet, I. Nébié, K. Lanke, L. Grignard, A. Ouedraogo, I. Soulama, C. Drakeley, R. Sauerwein, J. M. Bolscher, K. J. Dechering, A. B. Tiono, and B. P. Gonçalves (2019). "Functional antibodies against Plasmodium falciparum sporozoites are associated with a longer time to qPCR-detected infection among schoolchildren in Burkina Faso". In: *Wellcome Open Research* 3.May, pp. 1–40.

Bousema, T., C. J. Sutherland, T. S. Churcher, B. Mulder, L. C. Gouagna, E. M. Riley, G. A. T. Targett, and C. J. Drakeley (Mar. 2011). "Human immune responses that reduce the transmission of Plasmodium falciparum in African populations". In: *International journal for parasitology* 41.3-4, pp. 293–300.

Boyle, M. J., J. A. Chan, I. Handayuni, L. Reiling, G. Feng, A. Hilton, L. Kurtovic, D. Oyong, K. A. Piera, B. E. Barber, T. William, D. P. Eisen, G. Minigo, C. Langer, D. R. Drew, F. de Labastida Rivera, F. H. Amante, T. N. Williams, S. Kinyanjui, K. Marsh, D. L. Doolan, C. Engwerda, F. J. I. Fowkes, M. J. Grigg, I. Mueller, J. S. McCarthy, N. M. Anstey, and J. G. Beeson (Sept. 2019a). "IgM in human immunity to Plasmodium falciparum malaria". In: *Science Advances* 5.9, eaax4489.

— (2019b). "IgM in human immunity to Plasmodium falciparum malaria". In: *Science Advances* 5.9.

Boyle, M. J., L. Reiling, G. Feng, C. Langer, F. H. Osier, H. Aspeling-Jones, Y. S. Cheng, J. Stubbs, K. K. A. Tetteh, D. J. Conway, J. S. McCarthy, I. Muller, K. Marsh, R. F. Anders, and J. G. Beeson (Mar. 2015). "Human antibodies fix complement to inhibit Plasmodium falciparum invasion of erythrocytes and are associated with protection against malaria". In: *Immunity* 42.3, pp. 580–590.

Brown, J. and M. E. Smalley (1981). "Inhibition of the in vitro growth of Plasmodium falciparum by human polymorphonuclear neutrophil leucocytes". In: *Clinical and Experimental Immunology* 46.1, pp. 106–109.

Bruhns, P., B. Iannascoli, P. England, D. A. Mancardi, N. Fernandez, S. Jorieux, and M. Daëron (2009). "Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses". In: *Blood* 113.16, pp. 3716–3725.

Bull, P. C., B. S. Lowe, M. Kortok, C. S. Molyneux, C. I. Newbold, and K. Marsh (1998). "Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria". In: *Nature Medicine* 4.3, pp. 358–60.

Cabrera, G., M. Cot, F. Migot-Nabias, P. G. Kremsner, P. Deloron, and A. J. F. Luty (May 2005). "The sickle cell trait is associated with enhanced immunoglobulin G antibody responses to Plasmodium falciparum variant surface antigens." In: *The Journal of infectious diseases* 191.10, pp. 1631–1638.

Cabrera, G., C. Yone, A. E. Tebo, J. van Aaken, B. Lell, P. G. Kremsner, and A. J. F. Luty (Jan. 2004). "Immunoglobulin G isotype responses to variant surface antigens of Plasmodium falciparum in healthy Gabonese adults and children during and after successive malaria attacks". In: *Infection and immunity* 72.1, pp. 284–294.

Calis, J. C., K. S. Phiri, E. B. Faragher, B. J. Brabin, I. Bates, L. E. Cuevas, R. J. de Haan, A. I. Phiri, P. Malange, M. Khoka, P. J. Hulshof, L. van Lieshout, M. G. Beld, Y. Y. Teo, K. A. Rockett, A. Richardson, D. P. Kwiatkowski, M. E. Molyneux, and M. B. van Hensbroek (2016). "Severe anemia in Malawian children". In: *Malawi Medical Journal* 28.3, pp. 99–107.

Canelas, T., C. Castillo-Salgado, O. S. Baquero, and H. Ribeiro (2019). "Environmental and socioeconomic analysis of malaria transmission in the Brazilian Amazon, 2010–2015". In: *Revista de saude publica* 53, p. 49.

Carlson, J., H. Helmby, A. V. Hill, D. Brewster, B. M. Greenwood, and M. Wahlgren (Dec. 1990). “Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies.” In: *Lancet* 336.8729, pp. 1457–60.

Carter, R., P. M. Graves, D. B. Keister, and I. A. Quakyi (1990). “Properties of epitopes of Pfs 48/45, a target of transmission blocking monoclonal antibodies, on gametes of different isolates of Plasmodium falciparum”. In: *Parasite immunology* 12.6, pp. 587–603.

Celada, A., A. Cruchaud, and L. H. Perrin (1983a). “Phagocytosis of Plasmodium falciparum-parasitized erythrocytes by human polymorphonuclear leukocytes”. In: *Journal of Parasitology* 69.1, pp. 49–53.

Celada, A., A. Cruchaud, and L. H. Perrin (1983b). “Assessment of immune phagocytosis of Plasmodium falciparum infected red blood cells by human monocytes and polymorphonuclear leukocytes. A method for visualizing infected red blood cells ingested by phagocytes”. In: *Journal of immunological methods* 63.2, pp. 263–271.

Cham, G. K. K., L. Turner, J. Lusingu, L. Vestergaard, B. P. Mmbando, J. D. Kurtis, A. T. R. Jensen, A. Salanti, T. Lavstsen, and T. G. Theander (Sept. 2009). “Sequential, ordered acquisition of antibodies to Plasmodium falciparum erythrocyte membrane protein 1 domains.” In: *Journal of immunology (Baltimore, Md. : 1950)* 183.5, pp. 3356–3363.

Chan, J.-A., M. J. Boyle, K. A. Moore, L. Reiling, Z. Lin, W. Hasang, M. Avril, L. Manning, I. Mueller, M. Laman, T. Davis, J. D. Smith, S. J. Rogerson, J. A. Simpson, F. J. I. Fowkes, and J. G. Beeson (2019a). “Antibody Targets on the Surface of Plasmodium falciparum-Infected Erythrocytes That Are Associated With Immunity to Severe Malaria in Young Children”. In: *Journal of Infectious Diseases* 219.5, pp. 819–828.

— (2019b). “Antibody Targets on the Surface of Plasmodium falciparum-Infected Erythrocytes That Are Associated With Immunity to Severe Malaria in Young Children”. In: *J Infect Dis* 219.5, pp. 819–828.

Chan, J.-A., F. J. I. Fowkes, and J. G. Beeson (Oct. 2014). “Surface antigens of Plasmodium falciparum-infected erythrocytes as immune targets and malaria vaccine candidates.” In: *Cellular and molecular life sciences : CMLS* 71.19, pp. 3633–3657.

Chan, J.-A., K. B. Howell, L. Reiling, R. Ataide, C. L. Mackintosh, F. J. I. Fowkes, M. Petter, J. M. Chesson, C. Langer, G. M. Warimwe, M. F. Duffy, S. J. Rogerson, P. C. Bull, A. F. Cowman, K. Marsh, and J. G. Beeson (Sept. 2012). “Targets of antibodies against Plasmodium falciparum-infected erythrocytes in malaria immunity.” In: *The Journal of clinical investigation* 122.9, pp. 3227–38.

Chan, J. A., D. R. Drew, L. Reiling, A. Lisboa-Pinto, B. Dinko, C. J. Sutherland, A. E. Dent, K. Chelimo, J. W. Kazura, M. J. Boyle, and J. G. Beeson (2019c). “Low levels of human antibodies to gametocyte-infected erythrocytes contrasts the PfEMP1-dominant response to asexual stages in P. falciparum malaria”. In: *Frontiers in Immunology* 10.JAN, pp. 1–8.

Chan, J. A., D. I. Stanisic, M. F. Duffy, L. J. Robinson, E. Lin, J. W. Kazura, C. L. King, P. M. Siba, F. J. Fowkes, I. Mueller, and J. G. Beeson (2017). “Patterns of protective associations differ for antibodies to P. falciparum-infected erythrocytes and merozoites in immunity against malaria in children”. In: *European Journal of Immunology* 47.12, pp. 2124–2136.

Chandramohan, D., I. Zongo, I. Sagara, M. Cairns, R.-S. Yerbanga, M. Diarra, F. Nikièma, A. Tapily, F. Sompougdou, D. Issiaka, C. Zoungrana, K. Sanogo, A. Haro, M. Kaya, A.-A. Sienou, S. Traore, A. Mahamar, I. Thera, K. Diarra, A. Dolo, I. Kuepfer, P. Snell, P. Milligan, C. Ockenhouse, O. Ofori-Anyinam, H. Tinto, A. Djimde, J.-B. Ouédraogo, A.

Dicko, and B. Greenwood (2021). “Seasonal Malaria Vaccination with or without Seasonal Malaria Chemoprevention”. In: *New England Journal of Medicine* 385.11, pp. 1005–1017.

Chandrasiri, U. P., F. J. I. Fowkes, J. G. Beeson, J. S. Richards, S. Kamiza, K. Maleta, P. Ashorn, and S. J. Rogerson (2016). “Association between malaria immunity and pregnancy outcomes among Malawian pregnant women receiving nutrient supplementation”. In: *Malaria Journal* 15.1, p. 547.

Chatterjee, D. and I. A. Cockburn (2021). “The challenges of a circumsporozoite protein-based malaria vaccine”. In: *Expert Review of Vaccines* 20.2, pp. 113–125.

Chelimo, K., A. V. Ofulla, D. L. Narum, J. W. Kazura, D. E. Lanar, and C. C. John (Aug. 2005). “Antibodies to Plasmodium falciparum antigens vary by age and antigen in children in a malaria-holoendemic area of Kenya.” In: *The Pediatric infectious disease journal* 24.8, pp. 680–684.

Chen, L., Y. Xu, W. Wong, J. K. Thompson, J. Healer, E. D. Goddard-Borger, M. C. Lawrence, and A. F. Cowman (Feb. 2017). “Structural basis for inhibition of erythrocyte invasion by antibodies to Plasmodium falciparum protein CyRPA.” In: *eLife* 6, e21347.

Chen, Q., A. Barragan, V. Fernandez, A. Sundström, M. Schlichtherle, A. Sahlén, J. Carlson, S. Datta, and M. Wahlgren (Jan. 1998). “Identification of Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) as the rosetting ligand of the malaria parasite P. falciparum.” In: *The Journal of experimental medicine* 187.1, pp. 15–23.

Chen, Q., A. Heddini, A. Barragan, V. Fernandez, S. F. Pearce, and M. Wahlgren (July 2000). “The semiconserved head structure of Plasmodium falciparum erythrocyte membrane protein 1 mediates binding to multiple independent host receptors.” In: *The Journal of experimental medicine* 192.1, pp. 1–10.

Chesnokov, O., J. Merritt, S. O. Tcherniuk, N. Milman, and A. V. Oleinikov (Dec. 2018). “Plasmodium falciparum infected erythrocytes can bind to host receptors integrins alpha-Vβ3 and alpha-Vβ6 through DBLδ1D4 domain of PFL2665c PfEMP1 protein.” In: *Scientific reports* 8.1, p. 17871.

Chu, T. H., A. R. Crowley, I. Backes, C. Chang, M. Tay, T. Broge, M. Tuyishime, G. Ferrari, M. S. Seaman, S. I. Richardson, et al. (2020). “Hinge length contributes to the phagocytic activity of HIV-specific IgG1 and IgG3 antibodies”. In: *PLoS pathogens* 16.2, e1008083.

Chua, C. L. L., I. M. J. Ng, B. J. M. Yap, and A. Teo (2021). “Factors influencing phagocytosis of malaria parasites: the story so far”. In: *Malaria Journal* 20.1, p. 319.

Chung, A. W. and G. Alter (2017). “Systems serology: Profiling vaccine induced humoral immunity against HIV”. In: *Retrovirology* 14.1, pp. 1–11.

Claessens, A., Y. Adams, A. Ghumra, G. Lindergard, C. C. Buchan, C. Andisi, P. C. Bull, S. Mok, A. P. Gupta, C. W. Wang, L. Turner, M. Arman, A. Raza, Z. Bozdech, and J. A. Rowe (2012). “A subset of group A-like var genes encodes the malaria parasite ligands for binding to human brain endothelial cells”. In: *Proceedings of the National Academy of Sciences* 109.26, e1772–81.

Clatworthy, M. R., L. Willcocks, B. Urban, J. Langhorne, T. N. Williams, N. Peshu, N. A. Watkins, R. A. Floto, and K. G. C. Smith (Apr. 2007a). “Systemic lupus erythematosus-associated defects in the inhibitory receptor FcgammaRIIb reduce susceptibility to malaria.” In: *Proceedings of the National Academy of Sciences of the United States of America* 104.17, pp. 7169–74.

— (2007b). “Systemic lupus erythematosus-associated defects in the inhibitory receptor FcγRIIb reduce susceptibility to malaria”. In: *Proceedings of the National Academy of Sciences* 104.17, pp. 7169–7174.

Cleary, E., M. W. Hetzel, and A. C. A. Clements (2022). *A review of malaria epidemiology and control in Papua New Guinea 1900 to 2021: Progress made and future directions.*

Cockburn, I. A., R. Amino, R. K. Kelemen, S. C. Kuo, S.-W. Tse, A. Radtke, L. Mac-Daniel, V. V. Ganusov, F. Zavala, and R. Ménard (May 2013). "In vivo imaging of CD8+ T cell-mediated elimination of malaria liver stages". In: *Proceedings of the National Academy of Sciences of the United States of America* 110.22, pp. 9090–9095.

Cockburn, I. A., M. J. Mackinnon, A. O'Donnell, S. J. Allen, J. M. Moulds, M. Baisor, M. Bockarie, J. C. Reeder, and J. A. Rowe (Jan. 2004). "A human complement receptor 1 polymorphism that reduces Plasmodium falciparum rosetting confers protection against severe malaria." In: *Proceedings of the National Academy of Sciences of the United States of America* 101.1, pp. 272–7.

Cohen, S., I. A. McGregor, and S. Carrington (1961). "Gamma-globulin and acquired immunity to human malaria". In: *Nature* 192, pp. 733–737.

Cooke, G. S., C. Aucan, A. J. Walley, S. Segal, B. M. Greenwood, D. P. Kwiatkowski, and A. V. S. Hill (Dec. 2003). "Association of Fcgamma receptor IIa (CD32) polymorphism with severe malaria in West Africa." In: *The American journal of tropical medicine and hygiene* 69.6, pp. 565–8.

Cowman, A. F. and B. S. Crabb (Feb. 2006). "Invasion of red blood cells by malaria parasites." In: *Cell* 124.4, pp. 755–766.

Cowman, A. F., D. Berry, and J. Baum (2012). "The cellular and molecular basis for malaria parasite invasion of the human red blood cell". In: *Journal of Cell Biology* 198.6, pp. 961–971.

Crandall, I., W. E. Collins, J. GYsIN, and I. W. Sherman (1993). "Synthetic peptides based on motifs present in human band 3 protein inhibit cytoadherence/sequestration of the malaria parasite Plasmodium falciparum." In: *Proceedings of the National Academy of Sciences* 90.10, pp. 4703–4707.

Crompton, P. D., M. A. Kayala, B. Traore, K. Kayentao, A. Ongoiba, G. E. Weiss, D. M. Molina, C. R. Burk, M. Waisberg, A. Jasinskas, X. Tan, S. Doumbo, D. Doumtabe, Y. Kone, D. L. Narum, X. Liang, O. K. Doumbo, L. H. Miller, D. L. Doolan, P. Baldi, P. L. Felgner, and S. K. Pierce (Apr. 2010). "A prospective analysis of the Ab response to Plasmodium falciparum before and after a malaria season by protein microarray". In: *Proceedings of the National Academy of Sciences of the United States of America* 107.15, pp. 6958–6963.

Cummings, J. F., M. D. Spring, R. J. Schwenk, C. F. Ockenhouse, K. E. Kester, M. E. Polhemus, D. S. Walsh, I.-K. Yoon, C. Prosperi, L. Y. Juompan, et al. (2010). "Recombinant Liver Stage Antigen-1 (LSA-1) formulated with AS01 or AS02 is safe, elicits high titer antibody and induces IFN-γ/IL-2 CD4+ T cells but does not protect against experimental Plasmodium falciparum infection". In: *Vaccine* 28.31, pp. 5135–5144.

Cutts, J. C., P. A. Agius, Zaw Lin, R. Powell, K. Moore, B. Draper, J. A. Simpson, and F. J. Fowkes (2020). "Pregnancy-specific malarial immunity and risk of malaria in pregnancy and adverse birth outcomes: A systematic review". In: *BMC Medicine* 18.1, pp. 1–21.

D'Ombrain, M. C., T. S. Voss, A. G. Maier, J. A. Pearce, D. S. Hansen, A. F. Cowman, and L. Schofield (Aug. 2007). "Plasmodium falciparum erythrocyte membrane protein-1 specifically suppresses early production of host interferon-gamma." In: *Cell host microbe* 2.2, pp. 130–138.

Dabrowski, A., J. Osada, M. I. Dabrowska, U. Wereszczynska-Siemiatkowska, and A. Siemiatkowski (2014). "Increased expression of the intercellular adhesion molecule-1 (ICAM-1) on peripheral blood neutrophils in acute pancreatitis". In: *Advances in Medical Sciences* 59.1, pp. 102–107.

Damelang, T., E. H. Aitken, W. Hasang, E. Lopez, M. Killian, H. W. Unger, A. Salanti, A. Shub, E. McCarthy, K. Kedzierska, M. Lappas, S. J. Kent, S. J. Rogerson, and A. W. Chung (2021). "Antibody mediated activation of natural killer cells in malaria exposed pregnant women". In: *Scientific Reports* 11.1, p. 4130.

Damelang, T., S. J. Rogerson, S. J. Kent, and A. W. Chung (2019). "Role of IgG3 in infectious diseases". In: *Trends in immunology* 40.3, pp. 197–211.

Daou, M., B. Kouriba, N. Ouédraogo, I. Diarra, C. Arama, Y. Keita, S. Sissoko, B. Ouologuem, S. Arama, T. Bousema, O. K. Doumbo, R. W. Sauerwein, and A. Scholzen (2015). "Protection of Malian children from clinical malaria is associated with recognition of multiple antigens". In: *Malaria Journal* 14.1, p. 56.

Dasari, P., A. Fries, S. D. Heber, A. Salama, I.-W. Blau, K. Lingelbach, S. C. Bhakdi, R. Udomsangpetch, M. Torzewski, K. Reiss, and S. Bhakdi (Dec. 2014). "Malarial anemia: digestive vacuole of Plasmodium falciparum mediates complement deposition on bystander cells to provoke hemophagocytosis." In: *Medical microbiology and immunology* 203.6, pp. 383–393.

Datoo, M. S., M. H. Natama, A. Some, O. Traore, T. Rouamba, D. Bellamy, P. Yameogo, D. Valia, M. Tegneri, F. Ouedraogo, R. Soma, S. Sawadogo, F. Sorgho, K. Derra, E. Rouamba, B. Orindi, F. Ramos Lopez, A. Flaxman, F. Cappuccini, R. Kailath, S. Elias, E. Mukhopadhyay, A. Noe, M. Cairns, A. Lawrie, R. Roberts, I. Valea, H. Sorgho, N. Williams, G. Glenn, L. Fries, J. Reimer, K. J. Ewer, U. Shaligram, A. V. S. Hill, and H. Tinto (May 2021). "Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso: a randomised controlled trial." In: *Lancet* 397.10287, pp. 1809–1818.

Dekkers, G., L. Treffers, R. Plomp, A. E. H. Bentlage, M. de Boer, C. A. M. Koeleman, S. N. Lissenberg-Thunnissen, R. Visser, M. Brouwer, J. Y. Mok, H. Matlung, T. K. van den Berg, W. J. E. van Esch, T. W. Kuijpers, D. Wouters, T. Rispens, M. Wuhrer, and G. Vidarsson (2017). "Decoding the Human Immunoglobulin G-Glycan Repertoire Reveals a Spectrum of Fc-Receptor- and Complement-Mediated-Effector Activities." In: *Frontiers in immunology* 8, p. 877.

Dent, A. E., R. Nakajima, L. Liang, E. Baum, A. M. Moormann, P. O. Sumba, J. Vulule, D. Babineau, A. Randall, D. H. Davies, P. L. Felgner, and J. W. Kazura (2015). "Plasmodium falciparum Protein Microarray Antibody Profiles Correlate With Protection From Symptomatic Malaria in Kenya". In: *Journal of Infectious Diseases* 212.9, pp. 1429–38.

Dobaño, C., S. J. Rogerson, M. J. Mackinnon, D. R. Cavanagh, T. E. Taylor, M. E. Molyneux, and J. S. McBride (2008). "Differential antibody responses to Plasmodium falciparum merozoite proteins in Malawian children with severe malaria". In: *The Journal of infectious diseases* 197.5, pp. 766–774.

Dobaño, C., R. Santano, M. Vidal, A. Jiménez, C. Jairoce, I. Ubillos, D. Dosoo, R. Aguilar, N. A. Williams, N. Díez-Padrisa, A. Ayestaran, C. Valim, K. P. Asante, S. Owusu-Agyei, D. Lanar, V. Chauhan, C. Chitnis, S. Dutta, E. Angov, B. Gamain, R. L. Coppel, J. G. Beeson, L. Reiling, D. Gaur, D. Cavanagh, B. Gyan, A. J. Nhabomba, J. J. Campo, and G. Moncunill (2019). "Differential patterns of IgG subclass responses to plasmodium falciparum antigens in relation to malaria protection and RTS,S vaccination". In: *Frontiers in Immunology* 10.MAR, p. 439.

Dobbs, K. R., P. Embury, J. Vulule, P. S. Odada, B. A. Rosa, M. Mitreva, J. W. Kazura, and A. E. Dent (Sept. 2017). "Monocyte dysregulation and systemic inflammation during pediatric falciparum malaria." In: *JCI insight* 2.18.

Dodoo, D., T. G. Theander, J. A. Kurtzhals, K. Koram, E. Riley, B. D. Akanmori, F. K. Nkrumah, and L. Hviid (1999). "Levels of antibody to conserved parts of Plasmodium falciparum merozoite surface protein 1 in Ghanaian children are not associated with protection from clinical malaria". In: *Infection and immunity* 67.5, pp. 2131–2137.

Dondorp, A. M., B. J. Angus, K. Chotivanich, K. Silamut, R. Ruangveerayuth, M. R. Hardeman, P. A. Kager, J. Vreeken, and N. J. White (May 1999). "Red blood cell deformability as a predictor of anemia in severe falciparum malaria." In: *The American journal of tropical medicine and hygiene* 60.5, pp. 733–737.

Dondorp, A. M., C. I. Fanello, I. C. Hendriksen, E. Gomes, A. Seni, K. D. Chhaganlal, K. Bojang, R. Olaosebikan, N. Anunobi, K. Maitland, E. Kivaya, T. Agbenyega, S. B. Nguah, J. Evans, S. Gesase, C. Kahabuka, G. Mtove, B. Nadjm, J. Deen, J. Mwanga-Amumpaire, M. Nansumba, C. Karema, N. Umulisa, A. Uwimana, O. A. Mokuolu, O. T. Adedoyin, W. B. Johnson, A. K. Tshefu, M. A. Onyamboko, T. Sakulthaew, W. P. Ngum, K. Silamut, K. Stepniewska, C. J. Woodrow, D. Bethell, B. Wills, M. Oneko, T. E. Peto, L. von Seidlein, N. P. Day, N. J. White, and A. group (Nov. 2010). "Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial". In: *The Lancet* 376.9753, pp. 1647–1657.

Doolan, D. L., C. Dobaño, and J. K. Baird (Jan. 2009). "Acquired immunity to malaria." In: *Clinical microbiology reviews* 22.1, pp. 13–36.

Doritchamou, J., P. Bigey, M. A. Nielsen, S. Gnidehou, S. Ezinmegnon, A. Burgain, A. Massougbodji, P. Deloron, A. Salanti, and N. T. Ndam (2013). "Differential adhesion-inhibitory patterns of antibodies raised against two major variants of the NTS-DBL2X region of VAR2CSA". In: *Vaccine* 31.41, pp. 4516–4522.

Doritchamou, J., A. Teo, R. Morrison, G. Arora, J. Kwan, J. Manzella-Lapeira, S. Medina-Maldonado, J. Langhorne, L. Hviid, D. L. Narum, A. Dicko, M. Fried, and P. E. Duffy (Apr. 2019). "Functional Antibodies against Placental Malaria Parasites Are Variant Dependent and Differ by Geographic Region". In: *Infection and Immunity* 87.7. Ed. by J. H. Adams.

Doritchamou, J. Y. A., R. Herrera, J. A. Aebig, R. Morrison, V. Nguyen, K. Reiter, R. L. Shimp, N. J. MacDonald, D. L. Narum, M. Fried, et al. (2016a). "VAR2CSA domain-specific analysis of naturally acquired functional antibodies to Plasmodium falciparum placental malaria". In: *The Journal of infectious diseases* 214.4, pp. 577–586.

Doritchamou, J. Y. A., R. Herrera, J. A. Aebig, R. Morrison, V. Nguyen, K. Reiter, R. L. Shimp, N. J. MacDonald, D. L. Narum, M. Fried, and P. E. Duffy (Aug. 2016b). "VAR2CSA Domain-Specific Analysis of Naturally Acquired Functional Antibodies to Plasmodium falciparum Placental Malaria". In: *Journal of Infectious Diseases* 214.4, pp. 577–586.

Dorovini-Zis, K., K. Schmidt, H. Huynh, W. Fu, R. O. Whitten, D. Milner, S. Kamiza, M. Molyneux, and T. E. Taylor (May 2011). "The neuropathology of fatal cerebral malaria in malawian children." In: *The American journal of pathology* 178.5, pp. 2146–2158.

Doumbo, O. K., M. A. Thera, A. K. Koné, A. Raza, L. J. Tempest, K. E. Lyke, C. V. Plowe, and J. A. Rowe (Dec. 2009). "High levels of Plasmodium falciparum rosetting in all clinical forms of severe malaria in African children". In: *American Journal of Tropical Medicine and Hygiene* 81.6, pp. 987–993.

Drakeley, C. J., P. H. Corran, P. G. Coleman, J. E. Tongren, S. L. R. McDonald, I. Carneiro, R. Malima, J. Lusingu, A. Manjurano, W. M. M. Nkya, M. M. Lemnge, J. Cox, H. Reyburn, and E. M. Riley (Apr. 2005). “Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure”. In: *Proceedings of the National Academy of Sciences* 102.14, pp. 5108–5113.

Drakeley, C., L. Mulder, T. Tchuinkam, S. Gupta, R. Sauerwein, and G. Targett (1998). “Transmission-blocking effects of sera from malaria-exposed individuals on Plasmodium falciparum isolates from gametocyte carriers”. In: *Parasitology* 116.5, pp. 417–423.

Duffy, F., M. Bernabeu, P. A. Babar, A. Kessle, C. W. Wang, M. Vaz, L. Cherry, W. L. Mandala, S. J. Rogerson, T. E. Taylor, K. B. Seydel, T. Lavstsen, E. Gomes, K. Kim, J. Lusingu, P. K. Rathod, J. D. Aitchison, and J. D. Smith (2019). “Meta-analysis of Plasmodium falciparum var Signatures Contributing to Severe Malaria in African Children and Indian Adults”. In: *mBio* 10.2, e00217–19.

Duffy, M. F., A. Caragounis, R. Noviyanti, H. M. Kyriacou, E. K. Choong, K. Boysen, J. Healer, J. A. Rowe, M. E. Molyneux, G. V. Brown, and S. J. Rogerson (Aug. 2006). “Transcribed var Genes Associated with Placental Malaria in Malawian Women”. In: *Infection and Immunity* 74.8, pp. 4875–4883.

Duffy, M. F., T. J. Byrne, C. Carret, A. Ivens, and G. V. Brown (2009). “Ectopic Recombination of a Malaria var Gene during Mitosis Associated with an Altered var Switch Rate”. In: *Journal of Molecular Biology* 389.3, pp. 453–469.

Duffy, M. F., R. Noviyanti, T. Tsuboi, Z. P. Feng, L. Trianty, B. F. Sebayang, E. Takashima, F. Sumardy, D. A. Lampah, L. Turner, T. Lavstsen, F. J. Fowkes, P. Siba, S. J. Rogerson, T. G. Theander, J. Marfurt, R. N. Price, N. M. Anstey, G. V. Brown, and A. T. Papenfuss (2016). “Differences in PfEMP1s recognized by antibodies from patients with uncomplicated or severe malaria”. In: *Malaria Journal* 15.1, p. 258.

Duffy, P. E. and D. C. Kaslow (Mar. 1997). “A novel malaria protein, Pfs28, and Pfs25 are genetically linked and synergistic as falciparum malaria transmission-blocking vaccines.” In: *Infection and immunity* 65.3, pp. 1109–1113.

Duffy, P. E. and M. Fried (Nov. 2003). “Antibodies that inhibit Plasmodium falciparum adhesion to chondroitin sulfate A are associated with increased birth weight and the gestational age of newborns.” In: *Infection and immunity* 71.11, pp. 6620–3.

Dunst, J., F. Kamena, and K. Matuschewski (July 2017). “Cytokines and Chemokines in Cerebral Malaria Pathogenesis”. In: *Frontiers in cellular and infection microbiology* 7, p. 324.

Elliott, S. R., A. K. Brennan, J. G. Beeson, E. Tadesse, M. E. Molyneux, G. V. Brown, and S. J. Rogerson (2005). “Placental malaria induces variant-specific antibodies of the cytophilic subtypes immunoglobulin G1 (IgG1) and IgG3 that correlate with adhesion inhibitory activity”. In: *Infection and immunity* 73.9, pp. 5903–5907.

Elliott, S. R., F. J. I. Fowkes, J. S. Richards, L. Reiling, D. R. Drew, and J. G. Beeson (2014). “Research priorities for the development and implementation of serological tools for malaria surveillance.” In: *F1000prime reports* 6, p. 100.

Fabra-Garcıa, A., A. S. Yang, M. C. Behet, Z. Yap, Y. van Waardenburg, S. Kaviraj, K. Lanke, G.-J. Van Gemert, M. M. Jore, T. Bousema, et al. (2022). “Human antibodies against noncircumsporozoite proteins block Plasmodium falciparum parasite development in hepatocytes”. In: *JCI insight* 7.6.

Facer, C. A. (July 1980). “Direct antiglobulin reactions in Gambian children with P. falciparum malaria. III. Expression of IgG subclass determinants and genetic markers and association with anaemia.” In: *Clinical and experimental immunology* 41.1, pp. 81–90.

Falk, N., M. Kaestli, W. Qi, M. Ott, K. Baea, A. Cortés, and H.-P. Beck (Aug. 2009). "Analysis of Plasmodium falciparum var genes expressed in children from Papua New Guinea." In: *The Journal of infectious diseases* 200.3, pp. 347–356.

Fall, A. K. D. J., D. Courtin, R. Adamou, S. Edslev, A. Hansen, N. Domingo, M. Christiansen, B. Adu, J. Milet, A. Garcia, et al. (2022). "Fc gamma receptor IIIB NA1/NA2/SH polymorphisms are associated with malaria susceptibility and antibody levels to P. falciparum merozoite antigens in Beninese children". In: *International Journal of Molecular Sciences* 23.23, p. 14882.

Farrington, L. A., P. C. Callaway, H. M. Vance, K. Baskevitch, E. Lutz, L. Warrier, T. I. McIntyre, R. Budker, P. Jagannathan, F. Nankya, K. Musinguzi, M. Nalubega, E. Sikyomu, K. Naluwu, E. Arinaitwe, G. Dorsey, M. R. Kamya, and M. E. Feeney (Oct. 2020). "Opsonized antigen activates Vδ2+ T cells via CD16/FCgammaRIIIa in individuals with chronic malaria exposure." In: *PLoS pathogens* 16.10, e1008997.

Feachem, R. G. A., I. Chen, O. Akbari, A. Bertozzi-Villa, S. Bhatt, F. Binka, M. F. Boni, C. Buckee, J. Dieleman, A. Dondorp, A. Eapen, N. Sekhri Feachem, S. Filler, P. Gething, R. Gosling, A. Haakenstad, K. Harvard, A. Hatefi, D. Jamison, K. E. Jones, C. Karema, R. N. Kamwi, A. Lal, E. Larson, M. Lees, N. F. Lobo, A. E. Micah, B. Moonen, G. Newby, X. Ning, M. Pate, M. Quiñones, M. Roh, B. Rolfe, D. Shanks, B. Singh, K. Staley, J. Tulloch, J. Wegbreit, H. J. Woo, and W. Mpanju-Shumbusho (2019). "Malaria eradication within a generation: ambitious, achievable, and necessary." In: *Lancet (London, England)* 394.

Feintuch, C. M., A. Saidi, K. Seydel, G. Chen, A. Goldman-Yassen, N. K. Mita-Mendoza, R. S. Kim, P. S. Frenette, T. Taylor, and J. P. Daily (Feb. 2016). "Activated Neutrophils Are Associated with Pediatric Cerebral Malaria Vasculopathy in Malawian Children." In: *mBio* 7.1, e01300–15.

Feng, G., E. Aitken, F. Yosaatmadja, L. Kalilani, S. R. Meshnick, A. Jaworowski, J. A. Simpson, and S. J. Rogerson (2009). "Antibodies to variant surface antigens of Plasmodium falciparum–infected erythrocytes are associated with protection from treatment failure and the development of anemia in pregnancy". In: *The Journal of infectious diseases* 200.2, pp. 299–306.

Feng, G., M. J. Boyle, N. Cross, J.-A. Chan, L. Reiling, F. Osier, D. I. Stanisic, I. Mueller, R. F. Anders, J. S. McCarthy, J. S. Richards, and J. G. Beeson (June 2018). "Human Immunization With a Polymorphic Malaria Vaccine Candidate Induced Antibodies to Conserved Epitopes That Promote Functional Antibodies to Multiple Parasite Strains." In: *The Journal of infectious diseases* 218.1, pp. 35–43.

Feng, G., B. D. Wines, L. Kurtovic, J.-A. Chan, P. Boeuf, V. Mollard, A. Cozijnsen, D. R. Drew, R. J. Center, D. L. Marshall, S. Chishimba, G. I. McFadden, A. E. Dent, K. Chelimo, M. J. Boyle, J. W. Kazura, P. M. Hogarth, and J. G. Beeson (2021). "Mechanisms and targets of Fcgamma-receptor mediated immunity to malaria sporozoites". In: *Nature Communications* 12.1, p. 1742.

Fernandez, V., M. Hommel, Q. Chen, P. Hagblom, and M. Wahlgren (Nov. 1999). "Small, clonally variant antigens expressed on the surface of the Plasmodium falciparum-infected erythrocyte are encoded by the rif gene family and are the target of human immune responses." In: *The Journal of experimental medicine* 190.10, pp. 1393–404.

Fernandez, V., C. J. Treutiger, G. B. Nash, and M. Wahlgren (June 1998). "Multiple adhesive phenotypes linked to rosetting binding of erythrocytes in Plasmodium falciparum malaria." In: *Infection and immunity* 66.6, pp. 2969–75.

Fernandez-Reyes, D., A. G. Craig, S. A. Kyes, N. Peshu, R. W. Snow, A. R. Berendt, K. Marsh, and C. I. Newbold (1997). “A high frequency African coding polymorphism in the N-terminal domain of ICAM-1 predisposing to cerebral malaria in Kenya”. In: *Human Molecular Genetics* 6.8, pp. 1357–1360.

Fischinger, S., D. Cizmeci, S. Shin, L. Davies, P. S. Grace, A. Sivro, N. Yende-Zuma, H. Streeck, S. M. Fortune, D. A. Lauffenburger, et al. (2021). “A Mycobacterium Tuberculosis Specific IgG3 Signature of Recurrent Tuberculosis”. In: *Frontiers in Immunology* 12, p. 729186.

Fowkes, F. J. I., P. Michon, L. Pilling, R. M. Ripley, L. Tavul, H. J. Imrie, C. M. Woods, C. S. Mgone, A. J. F. Luty, and K. P. Day (Jan. 2008). “Host erythrocyte polymorphisms and exposure to Plasmodium falciparum in Papua New Guinea.” In: *Malaria journal* 7, p. 1.

Fowkes, F. J., J. S. Richards, J. A. Simpson, and J. G. Beeson (2010). “The relationship between anti-merozoite antibodies and incidence of Plasmodium falciparum malaria: a systematic review and meta-analysis”. In: *PLoS medicine* 7.1, e1000218.

Francis, S. E., D. J. Sullivan, and D. E. Goldberg (1997). “Hemoglobin metabolism in the malaria parasite Plasmodium falciparium”. In: *Annual Review of Microbiology* 51, pp. 97–123.

Fraser, M., W. Jing, S. Bröer, F. Kurth, L.-E. Sander, K. Matuschewski, and A. G. Maier (Feb. 2021). “Breakdown in membrane asymmetry regulation leads to monocyte recognition of P. falciparum-infected red blood cells”. In: *PLOS Pathogens* 17.2, e1009259.

Fried, M. and P. E. Duffy (June 1996). “Adherence of Plasmodium falciparum to Chondroitin Sulfate A in the Human Placenta”. In: *Science* 272.5267, pp. 1502–1504.

— (2015). “Designing a VAR2CSA-based vaccine to prevent placental malaria”. In: *Vaccine* 33.52, pp. 7483–8.

Fujioka, H. and M. Aikawa (1999). *Malaria - Molecular and Clinical Aspects*. Ed. by W. Mats and P. Perlmann. Amsterdam: Harwood academic publishers, pp. 19–55.

Garcia-Senosiain, A., I. H. Kana, S. Singh, M. K. Das, M. H. Dziegiel, S. Hertegonne, B. Adu, and M. Theisen (Aug. 2021). “Neutrophils dominate in opsonic phagocytosis of P. falciparum blood-stage merozoites and protect against febrile malaria.” In: *Communications biology* 4.1, p. 984.

Garred, P., M. A. Nielsen, J. A. L. Kurtzhals, R. Malhotra, H. O. Madsen, B. Q. Goka, B. D. Akanmori, R. B. Sim, and L. Hviid (Sept. 2003). “Mannose-binding lectin is a disease modifier in clinical malaria and may function as opsonin for Plasmodium falciparum-infected erythrocytes.” In: *Infection and immunity* 71.9, pp. 5245–5253.

Genton, B., F. Al-Yaman, C. S. Mgone, N. Alexander, M. M. Paniu, M. P. Alpers, and D. Mokela (Dec. 1995). “Ovalocytosis and cerebral malaria.” In: 378.6557, pp. 564–565.

Ghazanfari, N., S. N. Mueller, and W. R. Heath (Sept. 2018). “Cerebral Malaria in Mouse and Man”. In: *Frontiers in immunology* 9, p. 2016.

Ghumra, A., P. Khunrae, R. Ataide, A. Raza, S. J. Rogerson, M. K. Higgins, and J. A. Rowe (Jan. 2011). “Immunisation with recombinant PfEMP1 domains elicits functional rosette-inhibiting and phagocytosis-inducing antibodies to Plasmodium falciparum.” In: *PloS one* 6.1, e16414.

Ghumra, A., J. P. Semblat, R. Ataide, C. Kifude, Y. Adams, A. Claessens, D. N. Anong, P. C. Bull, C. Fennell, M. Arman, A. Amambua-Ngwa, M. Walther, D. J. Conway, L. Kassambara, O. K. Doumbo, A. Raza, and J. A. Rowe (2012). “Induction of strain-transcending antibodies against group A PfEMP1 surface antigens from virulent malaria parasites”. In: *PLoS Pathogens* 8.4, e1002665.

Giha, H. A., A. Nasr, N. C. Iriemenam, D. Arnot, M. Troye-Blomberg, T. G. Theander, K. Berzins, G. ElGhazali, and J. P. Pandey (2009). "Antigen-specific influence of GM/KM allotypes on IgG isotypes and association of GM allotypes with susceptibility to Plasmodium falciparum malaria". In: *Malaria Journal* 8, pp. 1–10.

Gil Gonzalez, L., Y. Fernandez-Marrero, P. A. A. Norris, Z. Tawhidi, Y. Shan, Y. Cruz-Leal, K. D. Won, K. Frias-Boligan, D. R. Branch, and A. H. Lazarus (2022). "THP-1 cells transduced with CD16A utilize Fcgamma receptor I and III in the phagocytosis of IgG-sensitized human erythrocytes and platelets." In: *PloS one* 17.12, e0278365.

Gillis, C., A. Gouel-Chéron, F. Jönsson, and P. Bruhns (2014). "Contribution of Human FcgammaRs to Disease with Evidence from Human Polymorphisms and Transgenic Animal Studies ". In: 5.

Gillrie, M. R., B. Renaux, E. Russell-Goldman, M. Avril, A. J. Brazier, K. Mihara, E. Di Cera, D. A. J. Milner, M. D. Hollenberg, J. D. Smith, and M. Ho (Sept. 2016). "Thrombin Cleavage of Plasmodium falciparum Erythrocyte Membrane Protein 1 Inhibits Cytoadherence." In: *mBio* 7.5.

Gillrie, M. R., K. Lee, D. C. Gowda, S. P. Davis, M. Monestier, L. Cui, T. T. Hien, N. P. Day, and M. Ho (Mar. 2012). "Plasmodium falciparum histones induce endothelial proinflammatory response and barrier dysfunction". In: *American Journal of Pathology* 180.3, pp. 1028–1039.

Giribaldi, G., D. Ulliers, F. Mannu, P. Arese, and F. Turrini (2001). "Growth of Plasmodium falciparum induces stage-dependent haemichrome formation, oxidative aggregation of band 3, membrane deposition of complement and antibodies, and phagocytosis of parasitized erythrocytes". In: *British Journal of Haematology* 113.2, pp. 492–499.

Gowda, D. C. and X. Wu (2018). "Parasite Recognition and Signaling Mechanisms in Innate Immune Responses to Malaria ". In: 9, p. 3006.

Gowda, N. M., X. Wu, S. Kumar, M. Febbraio, and D. C. Gowda (Oct. 2013). "CD36 Contributes to Malaria Parasite-Induced Pro-Inflammatory Cytokine Production and NK and T Cell Activation by Dendritic Cells". In: *PLOS ONE* 8.10, e77604.

Greenwood, B. M. (1989). "The microepidemiology of malaria and its importance to malaria control." In: *Transactions of the Royal Society of Tropical Medicine and Hygiene* 83 Suppl, pp. 25–29.

Griffin, J. T., T. Déirdre Hollingsworth, H. Reyburn, C. J. Drakeley, E. M. Riley, and A. C. Ghani (2015). "Gradual acquisition of immunity to severe malaria with increasing exposure". In: *Proceedings of the Royal Society B: Biological Sciences* 282.1801, p. 20142657.

Gunn, B. M., W. H. Yu, M. M. Karim, J. M. Brannan, A. S. Herbert, A. Z. Wec, P. J. Halfmann, M. L. Fusco, S. L. Schendel, K. Gangavarapu, T. Krause, X. Qiu, S. He, J. Das, T. J. Suscovich, J. Lai, K. Chandran, L. Zeitlin, J. E. Crowe, D. Lauffenburger, Y. Kawaoka, G. P. Kobinger, K. G. Andersen, J. M. Dye, E. O. Saphire, and G. Alter (2018). "A Role for Fc Function in Therapeutic Monoclonal Antibody-Mediated Protection against Ebola Virus". In: *Cell Host and Microbe* 24.2, 221–233.e5.

Gupta, S., R. W. Snow, C. A. Donnelly, K. Marsh, and C. Newbold (1999). "Immunity to non-cerebral severe malaria is acquired after one or two infections". In: *Nature Medicine* 5.3, pp. 340–343.

Guyatt, H. L. and R. W. Snow (Oct. 2004). "Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa." In: *Clinical microbiology reviews* 17.4, pp. 760–9.

Hamre, K. E. S., B. N. Ondigo, J. S. Hodges, S. Dutta, M. Theisen, G. Ayodo, and C. C. John (Dec. 2020). "Antibody Correlates of Protection from Clinical Plasmodium falciparum

Malaria in an Area of Low and Unstable Malaria Transmission.” In: *The American journal of tropical medicine and hygiene* 103.6, pp. 2174–2182.

Harawa, V., M. Njie, A. Kessler, A. Choko, B. Kumwenda, S. Kampondeni, M. Potchen, K. Kim, A. Jaworowski, T. Taylor, W. Mandala, K. Seydel, and S. Rogerson (Nov. 2018). “Brain swelling is independent of peripheral plasma cytokine levels in Malawian children with cerebral malaria”. In: *Malaria journal* 17.1, p. 435.

Harmsen, C., L. Turner, S. Thrane, A. F. Sander, T. G. Theander, and T. Lavstsen (2020). “Immunization with virus-like particles conjugated to CIDRalpha-1 domain of Plasmodium falciparum erythrocyte membrane protein 1 induces inhibitory antibodies”. In: *Malaria Journal* 19.1, pp. 1–11.

Hasdemir, D., R. A. van den Berg, A. van Kampen, and A. K. Smilde (2020). “Modeling adaptive response profiles in a vaccine clinical trial”. In: *BMC Medical Research Methodology* 20.1, pp. 1–13.

Hawkes, M., R. E. Elphinstone, A. L. Conroy, and K. C. Kain (Aug. 2013). “Contrasting pediatric and adult cerebral malaria: the role of the endothelial barrier.” In: *Virulence* 4.6, pp. 543–55.

Healer, J., A. Graszynski, and E. Riley (May 1999). “Phagocytosis does not play a major role in naturally acquired transmission-blocking immunity to Plasmodium falciparum malaria.” In: *Infection and immunity* 67.5, pp. 2334–2339.

Hedberg, P., M. Sirel, K. Moll, M. S. Kiwuwa, P. Höglund, U. Ribacke, and M. Wahlgren (Nov. 2021). “Red blood cell blood group A antigen level affects the ability of heparin and PfEMP1 antibodies to disrupt Plasmodium falciparum rosettes.” In: *Malaria journal* 20.1, p. 441.

Heddini, A., Q. Chen, J. Obiero, O. Kai, V. Fernandez, K. Marsh, M. Wahlgren, and W. A. Muller (July 2001). “Binding of Plasmodium falciparum-infected erythrocytes to soluble platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31): frequent recognition by clinical isolates.” In: *The American Journal of Tropical Medicine and Hygiene* 65.1, pp. 47–51.

Heinberg, A., I. Amit-Avraham, V. Mitesser, K. Simantov, M. Goyal, Y. Nevo, S. Kandelis-Shalev, E. Thompson, and R. Dzikowski (2022). “A nuclear redox sensor modulates gene activation and var switching in Plasmodium falciparum”. In: *Proceedings of the National Academy of Sciences* 119.33, e2201247119.

Hill, D. L., E. J. Carr, T. Rutishauser, G. Moncunill, J. J. Campo, S. Innocentin, M. Mpina, A. Nhabomba, A. Tumbo, C. Jairoce, H. A. Moll, M. C. van Zelm, C. Dobaño, C. Daubenberger, and M. A. Linterman (Feb. 2020). “Immune system development varies according to age, location, and anemia in African children.” In: *Science translational medicine* 12.529.

Hill, D. L., E. M. Eriksson, C. S. N. Li Wai Suen, C. Y. Chiu, V. Ryg-Cornejo, L. J. Robinson, P. M. Siba, I. Mueller, D. S. Hansen, and L. Schofield (Sept. 2013). “Opsonising Antibodies to P. falciparum Merozoites Associated with Immunity to Clinical Malaria”. In: *PLOS ONE* 8.9, e74627.

Hirayama, K., R. Ubalee, M. Kikuchi, A. Kimura, S. Looareesuwan, K. Na-Bangchang, M. Aikawa, O. Tasanor, Y. Wattanagoon, and F. Suzuki (2002). “Association of adhesion molecule PECAM-1/CD31 polymorphism with susceptibility to cerebral malaria in Thais”. In: *Parasitology International* 50.4, pp. 235–239.

Hochman, S. E., T. F. Madaline, S. C. Wassmer, E. Mbale, N. Choi, K. B. Seydel, R. O. Whitten, J. Varughese, G. E. Grau, S. Kamiza, et al. (2015). “Fatal pediatric cerebral malaria is associated with intravascular monocytes and platelets that are increased with HIV coinfection". In: *MBio* 6.5, e01390–15.

Hoehn, K. B., A. Fowler, G. Lunter, and O. G. Pybus (May 2016). "The Diversity and Molecular Evolution of B-Cell Receptors during Infection". In: *Molecular biology and evolution* 33.5, pp. 1147–1157.

Hogarth, P. M. and G. A. Pietersz (2012). "Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond". In: *Nature Reviews Drug Discovery* 11.4, pp. 311–331.

Holmberg, V., F. Schuster, E. Dietz, J. C. Sagarriga Visconti, S. D. Anemana, U. Bienzle, and F. P. Mockenhaupt (Apr. 2008). "Mannose-binding lectin variant associated with severe malaria in young African children." In: *Microbes and infection* 10.4, pp. 342–348.

Hopp, C. S., P. Sekar, A. Diouf, K. Miura, K. Boswell, J. Skinner, C. M. Tipton, M. E. Peterson, M. J. Chambers, S. Andrews, J. Lu, J. Tan, S. Li, S. Doumbo, K. Kayentao, A. Ongoiba, B. Traore, S. Portugal, P. D. Sun, C. Long, R. A. Koup, E. O. Long, A. B. McDermott, and P. D. Crompton (Apr. 2021). "Plasmodium falciparum-specific IgM B cells dominate in children, expand with malaria, and produce functional IgM". In: *The Journal of experimental medicine* 218.4, e20200901.

Howell, D. P.-G., E. A. Levin, A. L. Springer, S. M. Kraemer, D. J. Phippard, W. R. Schief, and J. D. Smith (Nov. 2007). "Mapping a common interaction site used by Plasmodium falciparum Duffy binding-like domains to bind diverse host receptors". In: *Molecular Microbiology* 67.1, pp. 78–87.

Howland, S. W., C. M. Poh, and L. Rénia (June 2015). "Activated Brain Endothelial Cells Cross-Present Malaria Antigen". In: *PLOS Pathogens* 11.6, e1004963.

Ikeda, M., M. Kaneko, S.-I. Tachibana, B. Balikagala, M. Sakurai-Yatsushiro, S. Yatsushiro, N. Takahashi, M. Yamauchi, M. Sekihara, M. Hashimoto, O. T. Katuro, A. Olia, P. S. Obwoya, M. A. Auma, D. A. Anywar, E. I. Odongo-Aginya, J. Okello-Onen, M. Hirai, J. Ohashi, N. M. Q. Palacpac, M. Kataoka, T. Tsuboi, E. Kimura, T. Horii, and T. Mita (Apr. 2018). "Artemisinin-Resistant Plasmodium falciparum with High Survival Rates, Uganda, 2014-2016." In: *Emerging infectious diseases* 24.4, pp. 718–726.

Illingworth, J. J., D. G. Alanine, R. Brown, J. M. Marshall, H. E. Bartlett, S. E. Silk, G. M. Labbé, D. Quinkert, J. S. Cho, J. P. Wendler, et al. (2019). "Functional comparison of blood-stage Plasmodium falciparum malaria vaccine candidate antigens". In: *Frontiers in immunology* 10, p. 1254.

Ippolito, M. M., M. E. Gebhardt, E. Ferriss, J. L. Schue, T. Kobayashi, M. Chaponda, J.-B. Kabuya, M. Muleba, M. Mburu, J. Matoba, et al. (2022). "Scientific Findings of the Southern and Central Africa International Center of Excellence for Malaria Research: Ten Years of Malaria Control Impact Assessments in Hypo-, Meso-, and Holoendemic Transmission Zones in Zambia and Zimbabwe". In: *The American Journal of Tropical Medicine and Hygiene* 107.4 Suppl, p. 55.

Irani, V., A. J. Guy, D. Andrew, J. G. Beeson, P. A. Ramsland, and J. S. Richards (Oct. 2015). "Molecular properties of human IgG subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases". In: *Molecular Immunology* 67.2, pp. 171–182.

Ishizuka, A. S., K. E. Lyke, A. DeZure, A. A. Berry, T. L. Richie, F. H. Mendoza, M. E. Enama, I. J. Gordon, L.-J. Chang, U. N. Sarwar, K. L. Zephir, L. A. Holman, E. R. James, P. F. Billingsley, A. Gunasekera, S. Chakravarty, A. Manoj, M. Li, A. J. Ruben, T. Li, A. G. Eappen, R. E. Stafford, N. K C, T. Murshedkar, H. DeCederfelt, S. H. Plummer, C. S. Hendel, L. Novik, P. J. M. Costner, J. G. Saunders, M. B. Laurens, C. V. Plowe, B. Flynn,

W. R. Whalen, J. P. Todd, J. Noor, S. Rao, K. Sierra-Davidson, G. M. Lynn, J. E. Epstein, M. A. Kemp, G. A. Fahle, S. A. Mikolajczak, M. Fishbaugher, B. K. Sack, S. H. I. Kappe, S. A. Davidson, L. S. Garver, N. K. Björkström, M. C. Nason, B. S. Graham, M. Roederer, B. K. L. Sim, S. L. Hoffman, J. E. Ledgerwood, and R. A. Seder (June 2016). "Protection against malaria at 1 year and immune correlates following PfSPZ vaccination." In: *Nature medicine* 22.6, pp. 614–623.

Ismail, H. A., M. K. Tijani, C. Langer, L. Reiling, M. T. White, J. G. Beeson, M. Wahlgren, R. Nwuba, and K. E. Persson (2014). "Subclass responses and their half-lives for antibodies against EBA175 and PfRh2 in naturally acquired immunity against Plasmodium falciparum malaria". In: *Malaria Journal* 13.1, pp. 1–10.

Jafarshad, A., M. H. Dziegiel, R. Lundquist, L. K. Nielsen, S. Singh, and P. L. Druilhe (Mar. 2007). "A Novel Antibody-Dependent Cellular Cytotoxicity Mechanism Involved in Defense against Malaria Requires Costimulation of Monocytes FcgammaRII and FcgammaRIII". In: *The Journal of Immunology* 178.5, pp. 3099–3106.

Jakubzick, C. V., G. J. Randolph, and P. M. Henson (June 2017). "Monocyte differentiation and antigen-presenting functions." In: *Nature reviews. Immunology* 17.6, pp. 349–362.

Janes, J. H., C. P. Wang, E. Levin-Edens, I. Vigan-Womas, M. Guillotte, M. Melcher, O. Mercereau-Puijalon, and J. D. Smith (2011). "Investigating the host binding signature on the Plasmodium falciparum PfEMP1 protein family". In: *PLoS Pathogens* 7.5, e1002032.

Janeway, C. A., P. Travers, M. Walport, and M. J. Shlomchik (2015). "Immunobiology - the immune system in health and disease". In: ed. by E. Lawrence, G. Bushell, M. Morales, and B. Goatly, pp. 103–165, 369–408.

Jassim, A., K. Hassan, and D. Catty (Nov. 1987). "Antibody isotypes in human schistosomiasis mansoni". In: *Parasite Immunology* 9.6, pp. 627–650.

Jensen, A. R., Y. Adams, and L. Hviid (Sept. 2019). "Cerebral Plasmodium falciparum malaria: The role of PfEMP1 in its pathogenesis and immunity, and PfEMP1-based vaccines to prevent it". In: *Immunological Reviews* 293.1, pp. 230–252.

— (Jan. 2020). "Cerebral Plasmodium falciparum malaria: The role of PfEMP1 in its pathogenesis and immunity, and PfEMP1-based vaccines to prevent it". In: 293.1, pp. 230–252.

Jensen, A. T., T. G. Theander, M. A. Nielsen, A. Salanti, J. Christensen, L. Joergensen, L. Hviid, S. Sharp, C. Sutherland, A. Chiucchiuini, L. S. Vestergaard, R. Hermsen, T. Lavstsen, R. Sauerwein, J. P. Lusingu, T. Staalsoe, and P. Magistrado (2004). "Plasmodium falciparum Associated with Severe Childhood Malaria Preferentially Expresses PfEMP1 Encoded by Group A var Genes". In: *The Journal of Experimental Medicine* 199.9, pp. 1179–1190.

Jensen, J. B. (2002). "In Vitro Culture of Plasmodium Parasites BT - Malaria Methods and Protocols". In: 72, pp. 477–488.

Jespersen, J. S., C. W. Wang, S. I. Mkumbaye, D. T. Minja, B. Petersen, L. Turner, J. E. Petersen, J. P. Lusingu, T. G. Theander, and T. Lavstsen (Aug. 2016). "Plasmodium falciparum var genes expressed in children with severe malaria encode CIDRalpha-1 domains". In: *EMBO Molecular Medicine* 8.8, pp. 839–850.

Joergensen, L., D. C. Bengtsson, A. Bengtsson, E. Ronander, S. S. Berger, L. Turner, M. B. Dalgaard, G. K. Cham, M. E. Victor, T. Lavstsen, T. G. Theander, D. E. Arnot, and A. T. Jensen (2010). "Surface co-expression of two different PfEMP1 antigens on single plasmodium falciparum-infected erythrocytes facilitates binding to ICAM1 and PECAM1". In: *PLoS Pathogens* 6.9.

Joice, R., S. K. Nilsson, J. Montgomery, S. Dankwa, E. Egan, B. Morahan, K. B. Seydel, L. Bertuccini, P. Alano, K. C. Williamson, M. T. Duraisingh, T. E. Taylor, D. A. Milner, and M. Marti (July 2014). "Plasmodium falciparum transmission stages accumulate in the human bone marrow." In: *Science translational medicine* 6.244, 244re5.

Jong, R. M. de, L. Meerstein-Kessel, D. F. Da, S. Nsango, J. D. Challenger, M. van de Vegte-Bolmer, G.-J. van Gemert, E. Duarte, N. Teyssier, R. W. Sauerwein, T. S. Churcher, R. K. Dabire, I. Morlais, E. Locke, M. A. Huynen, T. Bousema, and M. M. Jore (2021). "Monoclonal antibodies block transmission of genetically diverse Plasmodium falciparum strains to mosquitoes". In: *NPJ Vaccines* 6.1, p. 101.

Jong, R. M. de, S. K. Tebeje, L. Meerstein-Kessel, F. G. Tadesse, M. M. Jore, W. Stone, and T. Bousema (2020). "Immunity against sexual stage Plasmodium falciparum and Plasmodium vivax parasites". In: *Immunological reviews* 293.1, pp. 190–215.

Joste, V., E. Guillochon, J. Fraering, B. Vianou, L. Watier, S. Jafari-guemouri, M. Cot, and S. Houzé (2020). "PfEMP1 A-Type ICAM-1-Binding Domains Are Not Associated with Cerebral Malaria in Beninese Children". In: *mBio* 11.6, e02103–20.

Kaestli, M., I. A. Cockburn, A. Cortés, K. Baea, J. A. Rowe, and H.-P. Beck (June 2006). "Virulence of Malaria Is Associated with Differential Expression of Plasmodium falciparum var Gene Subgroups in a Case-Control Study". In: *The Journal of Infectious Diseases* 193.11, pp. 1567–1574.

Kana, I. H., A. Garcia-Senosiain, S. K. Singh, R. W. Tiendrebeogo, B. K. Chourasia, P. Malhotra, S. K. Sharma, M. K. Das, S. Singh, B. Adu, and M. Theisen (Aug. 2018). "Cytophilic Antibodies Against Key Plasmodium falciparum Blood Stage Antigens Contribute to Protection Against Clinical Malaria in a High Transmission Region of Eastern India." In: *The Journal of infectious diseases* 218.6, pp. 956–965.

Kanoi, B. N., H. Nagaoka, M. T. White, M. Morita, N. M. Q. Palacpac, E. H. Ntege, B. Balikagala, A. Yeka, T. G. Egwang, T. Horii, T. Tsuboi, and E. Takashima (2020). "Global Repertoire of Human Antibodies Against Plasmodium falciparum RIFINs, SURFINs, and STEVORs in a Malaria Exposed Population." In: *Frontiers in immunology* 11, p. 893.

Kanoi, B. N., H. Nagaoka, M. Morita, M. T. White, N. M. Palacpac, E. H. Ntege, B. Balikagala, A. Yeka, T. G. Egwang, T. Horii, T. Tsuboi, and E. Takashima (2018). "Comprehensive analysis of antibody responses to Plasmodium falciparum erythrocyte membrane protein 1 domains". In: *Vaccine* 36.45, pp. 6826–6833.

Kapelski, S., T. Klockenbring, R. Fischer, S. Barth, and R. Fendel (Dec. 2014). "Assessment of the neutrophilic antibody-dependent respiratory burst (ADRB) response to Plasmodium falciparum." In: *Journal of leukocyte biology* 96.6, pp. 1131–42.

Kapesa, A., E. J. Kweka, H. Atieli, Y. A. Afrane, E. Kamugisha, M.-C. Lee, G. Zhou, A. K. Githeko, and G. Yan (Aug. 2018). "The current malaria morbidity and mortality in different transmission settings in Western Kenya". In: *PLOS ONE* 13.8. Ed. by A. P. Arez, e0202031.

Kapisi, J., A. Kakuru, P. Jagannathan, M. K. Muhindo, P. Natureeba, P. Awori, M. Nakalembe, R. Ssekitoleko, P. Olwoch, J. Ategeka, P. Nayebare, T. D. Clark, G. Rizzuto, A. Muehlenbachs, D. V. Havlir, M. R. Kamya, G. Dorsey, and S. L. Gaw (Dec. 2017). "Relationships between infection with Plasmodium falciparum during pregnancy, measures of placental malaria, and adverse birth outcomes". In: *Malaria Journal* 16.1, p. 400.

Kassa, M. W., W. Hasang, and S. J. Rogerson (2022). "Antibody-Dependent THP-1 Cell-Mediated Phagocytosis of Plasmodium falciparum-Infected Erythrocytes." In: *Methods in molecular biology (Clifton, N.J.)* 2470, pp. 617–628.

Kessler, A., J. J. Campo, V. Harawa, W. L. Mandala, S. J. Rogerson, W. B. Mowrey, K. B. Seydel, and K. Kim (Apr. 2018). “Convalescent Plasmodium falciparum-specific seroreactivity does not correlate with paediatric malaria severity or Plasmodium antigen exposure.” In: *Malaria journal* 17.1, p. 178.

Kessler, A., S. Dankwa, M. Bernabeu, V. Harawa, S. A. Danziger, F. Duffy, S. D. Kampondeni, M. J. Potchen, N. Dambrauskas, V. Vigdorovich, B. G. Oliver, S. E. Hochman, W. B. Mowrey, I. J. C. MacCormick, W. L. Mandala, S. J. Rogerson, D. N. Sather, J. D. Aitchison, T. E. Taylor, K. B. Seydel, J. D. Smith, and K. Kim (Nov. 2017). “Linking EPCR-Binding PfEMP1 to Brain Swelling in Pediatric Cerebral Malaria.” In: *Cell host microbe* 22.5, 601–614.e5.

Kho, S., L. Qotrunnada, L. Leonardo, B. Andries, P. A. I. Wardani, A. Fricot, B. Henry, D. Hardy, N. I. Margyaningsih, D. Apriyanti, A. M. Puspitasari, P. Prayoga, L. Trianty, E. Kenangalem, F. Chretien, I. Safeukui, H. A. Del Portillo, C. Fernandez-Becerra, E. Meibalan, M. Marti, R. N. Price, T. Woodberry, P. A. Ndour, B. M. Russell, T. W. Yeo, G. Minigo, R. Noviyanti, J. R. Poespoprodjo, N. C. Siregar, P. A. Buffet, and N. M. Anstey (May 2021). “Hidden Biomass of Intact Malaria Parasites in the Human Spleen.” In: *The New England journal of medicine* 384.21, pp. 2067–2069.

Kilejian, A. (Sept. 1979). “Characterization of a protein correlated with the production of knob-like protrusions on membranes of erythrocytes infected with Plasmodium falciparum.” In: *Proceedings of the National Academy of Sciences of the United States of America* 76.9, pp. 4650–4653.

Kinyanjui, S. M., P. Bull, C. I. Newbold, and K. Marsh (Feb. 2003). “Kinetics of antibody responses to Plasmodium falciparum-infected erythrocyte variant surface antigens.” In: *The Journal of infectious diseases* 187.4, pp. 667–674.

Klejnstrup, N. R., J. Buhl-Wiggers, S. Jones, and J. Rand (2018). “Early life malaria exposure and academic performance”. In: *PloS one* 13.6, e0199542.

Kolk, M. van der, S. J. de Vlas, and R. W. Sauerwein (2006). “Reduction and enhancement of Plasmodium falciparum transmission by endemic human sera”. In: *International journal for parasitology* 36.10-11, pp. 1091–1095.

Korenromp, E. L., J. R. M. Armstrong-Schellenberg, B. G. Williams, B. L. Nahlen, and R. W. Snow (Oct. 2004). “Impact of malaria control on childhood anaemia in Africa – a quantitative review”. In: *Tropical Medicine International Health* 9.10, pp. 1050–1065.

Korir, J. C., N. K. Nyakoe, G. Awinda, and J. N. Waitumbi (Aug. 2014). “Complement Activation by Merozoite Antigens of Plasmodium falciparum”. In: *PLOS ONE* 9.8, e105093.

Krishnegowda, G., A. M. Hajjar, J. Zhu, E. J. Douglass, S. Uematsu, S. Akira, A. S. Woods, and D. C. Gowda (Mar. 2005). “Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of Plasmodium falciparum: cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity.” In: *The Journal of biological chemistry* 280.9, pp. 8606–8616.

Kristensen, A. B., W. N. Lay, F. Ana-Sosa-Batiz, H. A. Vanderven, V. Madhavi, K. L. Laurie, L. Carolan, B. D. Wines, M. Hogarth, A. K. Wheatley, et al. (2016). “Antibody responses with Fc-mediated functions after vaccination of HIV-infected subjects with trivalent influenza vaccine”. In: *Journal of virology* 90.12, pp. 5724–5734.

Kumar, R., J. R. Loughland, S. S. Ng, M. J. Boyle, and C. R. Engwerda (2020). “The regulation of CD4+ T cells during malaria”. In: *Immunological Reviews* 293.1, pp. 70–87.

Kumaratilake, L. M. and A. Ferrante (Jan. 2000). "Opsonization and phagocytosis of Plasmodium falciparum merozoites measured by flow cytometry." In: *Clinical and diagnostic laboratory immunology* 7.1, pp. 9–13.

Kumsiri, R., M. Troye-Blomberg, K. Pattanapanyasat, S. Krudsood, and Y. Maneerat (Feb. 2016). "IgE low affinity receptor (CD23) expression, Plasmodium falciparum specific IgE and tumor necrosis factor-alpha production in Thai uncomplicated and severe falciparum malaria patients." In: *Acta tropica* 154, pp. 25–33.

Kun, J. F. J., J. Klabunde, B. Lell, D. Luckner, M. Alpers, J. May, C. Meyer, and P. G. Kremsner (1999). "Association of the ICAM:1(KILIFI) mutation with protection against severe malaria in lambarene, gabon". In: *American Journal of Tropical Medicine and Hygiene* 61.5, pp. 776–779.

Kurtis, J. D., R. Mtalib, F. K. Onyango, and P. E. Duffy (Jan. 2001). "Human resistance to Plasmodium falciparum increases during puberty and is predicted by dehydroepiandrosterone sulfate levels". In: *Infection and immunity* 69.1, pp. 123–128.

Kurtovic, L., T. Atre, G. Feng, B. D. Wines, J.-A. Chan, M. J. Boyle, D. R. Drew, P. M. Hogarth, F. J. I. Fowkes, E. S. Bergmann-Leitner, and J. G. Beeson (Oct. 2021). "Multifunctional Antibodies Are Induced by the RTS,S Malaria Vaccine and Associated With Protection in a Phase 1/2a Trial." In: *The Journal of infectious diseases* 224.7, pp. 1128–1138.

Kurtovic, L., M. J. Boyle, D. H. Opi, A. T. Kennedy, W.-H. Tham, L. Reiling, J.-A. Chan, and J. G. Beeson (Jan. 2020). "Complement in malaria immunity and vaccines". In: *Immunological reviews* 293.1, pp. 38–56.

Kwenti, T. E., T. D. B. Kwenti, A. Latz, L. A. Njunda, and T. Nkuo-Akenji (July 2017). "Epidemiological and clinical profile of paediatric malaria: a cross sectional study performed on febrile children in five epidemiological strata of malaria in Cameroon." In: *BMC infectious diseases* 17.1, p. 499.

Kyei-Baafour, E., M. Oppong, K. A. Kusi, A. F. Frempong, B. Aculley, F. K. N. Arthur, R. W. Tiendrebeogo, S. K. Singh, M. Theisen, M. Kweku, B. Adu, L. Hviid, and M. F. Ofori (2021). "Suitability of IgG responses to multiple Plasmodium falciparum antigens as markers of transmission intensity and pattern". In: *PLoS One* 16.4, e0249936.

Kyriacou, H. M., O. K. Doumbo, R. J. Challis, M. A. Thera, A. K. Koné, A. Raza, G. N. Stone, C. V. Plowe, J. A. Rowe, and K. E. Lyke (2006). "Differential var gene transcription in Plasmodium falciparum isolates from patients with cerebral malaria compared to hyperparasitaemia". In: *Molecular and Biochemical Parasitology* 150.2, pp. 211–218.

Lambert, L. H., J. L. Bullock, S. T. Cook, K. Miura, D. N. Garboczi, M. Diakite, R. M. Fairhurst, K. Singh, and C. A. Long (Nov. 2014). "Antigen reversal identifies targets of opsonizing IgGs against pregnancy-associated malaria". In: *Infection and immunity* 82.11, pp. 4842–4853.

Lamikanra, A. A., A. T. Merryweather-Clarke, A. J. Tipping, and D. J. Roberts (Mar. 2015). "Distinct mechanisms of inadequate erythropoiesis induced by tumor necrosis factor alpha or malarial pigment". In: *PloS one* 10.3, e0119836–e0119836.

Larsen, M. D., M. del Pilar Quintana, S. B. Ditlev, R. Bayarri-Olmos, M. F. Ofori, L. Hviid, and P. Garred (2019). "Evasion of classical complement pathway activation on Plasmodium falciparum-infected erythrocytes opsonized by PfEMP1-Specific IgG". In: *Frontiers in Immunology* 10.JAN, pp. 1–10.

Larsen, M. D., M. Lopez-Perez, E. K. Dickson, P. Ampomah, N. Tuikue Ndam, J. Nouta, C. A. M. Koeleman, A. L. H. Ederveen, B. Mordmüller, A. Salanti, M. A. Nielsen, A.

Massougbodji, C. E. van der Schoot, M. F. Ofori, M. Wuhrer, L. Hviid, and G. Vidarsson (Oct. 2021). “Afucosylated Plasmodium falciparum-specific IgG is induced by infection but not by subunit vaccination.” In: *Nature communications* 12.1, p. 5838.

Lau, C. K., L. Turner, J. S. Jespersen, E. D. Lowe, B. Petersen, C. W. Wang, J. E. Petersen, J. Lusingu, T. G. Theander, T. Lavstsen, and M. K. Higgins (2015). “Structural conservation despite huge sequence diversity allows EPCR binding by the PfEMP1 family implicated in severe childhood malaria”. In: *Cell Host and Microbe* 17.11, pp. 118–29.

Lavstsen, T., A. Salanti, A. T. R. Jensen, D. E. Arnot, and T. G. Theander (Sept. 2003). “Sub-grouping of Plasmodium falciparum 3D7 var genes based on sequence analysis of coding and non-coding regions.” In: *Malaria journal* 2, p. 27.

Lavstsen, T., L. Turner, F. Saguti, P. Magistrado, T. S. Rask, J. S. Jespersen, C. W. Wang, S. S. Berger, V. Baraka, A. M. Marquard, A. Seguin-Orlando, E. Willerslev, M. T. P. Gilbert, J. Lusingu, and T. G. Theander (June 2012). “Plasmodium falciparum erythrocyte membrane protein 1 domain cassettes 8 and 13 are associated with severe malaria in children.” In: *Proceedings of the National Academy of Sciences of the United States of America* 109.26, E1791–800.

Lee, C. H., G. Romain, W. Yan, M. Watanabe, W. Charab, B. Todorova, J. Lee, K. Triplett, M. Donkor, O. I. Lungu, A. Lux, N. Marshall, M. A. Lindorfer, O. R. L. Goff, B. Balbino, T. H. Kang, H. Tanno, G. Delidakis, C. Alford, R. P. Taylor, F. Nimmerjahn, N. Varadarajan, P. Bruhns, Y. J. Zhang, and G. Georgiou (July 2017). “IgG Fc domains that bind C1q but not effector Fc3 receptors delineate the importance of complement-mediated effector functions”. In: *Nature Immunology* 18.8, pp. 889–898.

Lencer, W. I. and R. S. Blumberg (2005). “A passionate kiss, then run: exocytosis and recycling ofIgG by FcRn”. In: *Trends in Cell Biology* 15.1, pp. 5–9.

Lennartz, F., Y. Adams, A. Bengtsson, R. W. Olsen, L. Turner, N. T. Ndam, G. Ecklu-Mensah, A. Moussiliou, M. F. Ofori, B. Gamain, J. P. Lusingu, J. E. Petersen, C. W. Wang, S. Nunes-Silva, J. S. Jespersen, C. K. Lau, T. G. Theander, T. Lavstsen, L. Hviid, M. K. Higgins, and A. T. Jensen (2017). “Structure-Guided Identification of a Family of Dual Receptor-Binding PfEMP1 that Is Associated with Cerebral Malaria”. In: *Cell Host and Microbe* 21.3, pp. 403–414.

Lennartz, F., A. Bengtsson, R. W. Olsen, L. Joergensen, A. Brown, L. Remy, P. Man, E. Forest, L. K. Barfod, Y. Adams, M. K. Higgins, and A. T. R. Jensen (2015). “Mapping the Binding Site of a Cross-Reactive Plasmodium falciparum PfEMP1 Monoclonal Antibody Inhibitory of ICAM-1 Binding”. In: *The Journal of Immunology.*

Leoni, S., D. Buonfrate, A. Angheben, F. Gobbi, and Z. Bisoffi (2015). “The hyper-reactive malarial splenomegaly: a systematic review of the literature”. In: *Malaria journal* 14.1, pp. 1–11.

Liew, P. X. and P. Kubes (Apr. 2019). “The Neutrophil’s Role During Health and Disease.” In: *Physiological reviews* 99.2, pp. 1223–1248.

Lopaticki, S., A. G. Maier, J. Thompson, D. W. Wilson, W.-H. Tham, T. Triglia, A. Gout, T. P. Speed, J. G. Beeson, J. Healer, and A. F. Cowman (Mar. 2011). “Reticulocyte and erythrocyte binding-like proteins function cooperatively in invasion of human erythrocytes by malaria parasites.” In: *Infection and immunity* 79.3, pp. 1107–1117.

Ludlow, L. E., J. Zhou, E. Tippett, W.-J. Cheng, W. Hasang, S. J. Rogerson, and A. Jaworowski (2012). “HIV-1 inhibits phagocytosis and inflammatory cytokine responses of human monocyte-derived macrophages to P. falciparum infected erythrocytes”. In: *PloS one* 7.2, e32102.

Lusingu, J. P. A., A. T. R. Jensen, L. S. Vestergaard, D. T. Minja, M. B. Dalgaard, S. Gesase, B. P. Mmbando, A. Y. Kitua, M. M. Lemnge, D. Cavanagh, L. Hviid, and T. G. Theander (May 2006). "Levels of plasma immunoglobulin G with specificity against the cysteine-rich interdomain regions of a semiconserved Plasmodium falciparum erythrocyte membrane protein 1, VAR4, predict protection against malarial anemia and febrile episodes." In: *Infection and immunity* 74.5, pp. 2867–75.

MacCormick, I. J. C., V. Barrera, N. A. V. Beare, G. Czanner, M. Potchen, S. Kampondeni, R. S. Heyderman, A. G. Craig, M. E. Molyneux, M. Mallewa, V. A. White, D. Milner, P. Hiscott, T. E. Taylor, K. B. Seydel, and S. P. Harding (Mar. 2022). "How Does Blood-Retinal Barrier Breakdown Relate to Death and Disability in Pediatric Cerebral Malaria?" In: *The Journal of infectious diseases* 225.6, pp. 1070–1080.

Madanitsa, M., L. Kalilani, V. Mwapasa, A. M. van Eijk, C. Khairallah, D. Ali, C. Pace, J. Smedley, K.-L. Thwai, B. Levitt, et al. (2016). "Scheduled intermittent screening with rapid diagnostic tests and treatment with dihydroartemisinin-piperaquine versus intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy in Malawi: an open-label randomized controlled trial". In: *PLoS medicine* 13.9, e1002124.

Madkhali, A. M., M. O. Alkurbi, T. Szestak, A. Bengtsson, P. R. Patil, Y. Wu, S. Alharthi, A. T. Jensen, R. Pleass, and A. G. Craig (2014). "An analysis of the binding characteristics of a panel of recently selected ICAM-1 binding Plasmodium falciparum patient isolates". In: *PLoS ONE* 9.10, pp. 4–11.

Magistrado, P. A., J. Lusingu, L. S. Vestergaard, M. Lemnge, T. Lavstsen, L. Turner, L. Hviid, A. T. Jensen, and T. G. Theander (2007). "Immunoglobulin G antibody reactivity to a group A Plasmodium falciparum erythrocyte membrane protein 1 and protection from P. falciparum malaria". In: *Infection and Immunity* 75.5, pp. 2415–2420.

Mahamar, A., O. Attaher, B. Swihart, A. Barry, B. S. Diarra, M. B. Kanoute, K. B. Cisse, A. B. Dembele, S. Keita, B. Gamain, S. Gaoussou, D. Issiaka, A. Dicko, P. E. Duffy, and M. Fried (2017). "Host factors that modify Plasmodium falciparum adhesion to endothelial receptors". In: *Scientific Reports* 7.1, p. 13872.

Malkin, E. M., A. P. Durbin, D. J. Diemert, J. Sattabongkot, Y. Wu, K. Miura, C. A. Long, L. Lambert, A. P. Miles, J. Wang, A. Stowers, L. H. Miller, and A. Saul (2005). "Phase 1 vaccine trial of Pvs25H: a transmission blocking vaccine for Plasmodium vivax malaria". In: *Vaccine* 23.24, pp. 3131–3138.

Manning, L., M. Laman, I. Law, C. Bona, S. Aipit, D. Teine, J. Warrell, A. Rosanas-Urgell, E. Lin, B. Kiniboro, J. Vince, I. Hwaiwhanje, H. Karunajeewa, P. Michon, P. Siba, I. Mueller, and T. M. E. Davis (2011). "Features and prognosis of severe malaria caused by Plasmodium falciparum, Plasmodium vivax and mixed Plasmodium species in Papua New Guinean children." In: *PloS one* 6.12, e29203.

Marsh, K., L. Otoo, R. J. Hayes, D. C. Carson, and B. M. Greenwood (1989). "Antibodies to blood stage antigens of Plasmodium falciparum in rural Gambians and their relation to protection against infection." In: *Transactions of the Royal Society of Tropical Medicine and Hygiene* 83.3, pp. 293–303.

Mavoungou, E., A. J. F. Luty, and P. G. Kremsner (2003). "Natural killer (NK) cell-mediated cytolysis of Plasmodium falciparum-infected human red blood cells in vitro." In: *European cytokine network* 14.3, pp. 134–142.

Mayor, A., U. Kumar, A. Bardají, P. Gupta, A. Jiménez, A. Hamad, B. Sigaúque, B. Singh, L. Quintó, S. Kumar, P. K. Gupta, V. S. Chauhan, C. Dobaño, P. L. Alonso, C. Menéndez, and C. E. Chitnis (2013). "Improved pregnancy outcomes in women exposed to malaria with high antibody levels against plasmodium falciparum". In: *Journal of Infectious Diseases* 207.11, pp. 1664–1674.

Mbengue, B., M. Fall, M.-L. Varela, C. Loucoubar, C. Joos, B. Fall, M. Niang, B. Niang, M. Mbow, A. Dieye, et al. (2019a). "Analysis of antibody responses to selected Plasmodium falciparum merozoite surface antigens in mild and cerebral malaria and associations with clinical outcomes". In: *Clinical & Experimental Immunology* 196.1, pp. 86–96.

Mbengue, B., M. M. Fall, M. L. Varela, C. Loucoubar, C. Joos, B. Fall, M. S. Niang, B. Niang, M. Mbow, A. Dieye, and R. Perraut (Apr. 2019b). "Analysis of antibody responses to selected Plasmodium falciparum merozoite surface antigens in mild and cerebral malaria and associations with clinical outcomes". In: *Clinical and Experimental Immunology* 196.1, pp. 86–96.

McCallum, F. J., K. E. M. Persson, F. J. I. Fowkes, L. Reiling, C. K. Mugyenyi, J. S. Richards, J. A. Simpson, T. N. Williams, P. R. Gilson, A. N. Hodder, P. R. Sanders, R. F. Anders, D. L. Narum, C. Chitnis, B. S. Crabb, K. Marsh, and J. G. Beeson (Apr. 2017). "Differing rates of antibody acquisition to merozoite antigens in malaria: implications for immunity and surveillance." In: *Journal of leukocyte biology* 101.4, pp. 913–925.

McGilvray, I. D., L. Serghides, A. Kapus, O. D. Rotstein, and K. C. Kain (Nov. 2000). "Nonopsonic monocyte/macrophage phagocytosis of Plasmodium falciparum-parasitized erythrocytes: a role for CD36 in malarial clearance." In: *Blood* 96.9, pp. 3231–3240.

McIntosh, R. S., J. Shi, R. M. Jennings, J. C. Chappel, T. F. De Koning-Ward, T. Smith, J. Green, M. Van Egmond, J. H. Leusen, M. Lazarou, J. Van De Winkel, T. S. Jones, B. S. Crabb, A. A. Holder, and R. J. Pleass (2007). "The importance of human FcgammaRI in mediating protection to malaria". In: *PLoS Pathogens* 3.5, pp. 0647–0658.

McLean, M. R., V. Madhavi, B. D. Wines, P. M. Hogarth, A. W. Chung, and S. J. Kent (July 2017). "Dimeric Fcgamma Receptor Enzyme-Linked Immunosorbent Assay To Study HIV-Specific Antibodies: A New Look into Breadth of Fcgamma Receptor Antibodies Induced by the RV144 Vaccine Trial". In: *The Journal of Immunology* 199.2, pp. 816–826.

McQuaid, F. and J. A. Rowe (2020). "Rosetting revisited: a critical look at the evidence for host erythrocyte receptors in Plasmodium falciparum rosetting". In: *Parasitology* 147.1, pp. 1–11.

Megnekou, R., T. Staalsoe, D. W. Taylor, R. Leke, and L. Hviid (July 2005). "Effects of pregnancy and intensity of Plasmodium falciparum transmission on immunoglobulin G subclass responses to variant surface antigens". In: *Infection and immunity* 73.7, pp. 4112–4118.

Melancon-Kaplan, J., J. M. Burns Jr, A. B. Vaidya, H. K. Webster, and W. P. Weidanz (1993). "Malaria". In: ed. by K. Warren, pp. 302–351.

Messina, V., M. Valtieri, M. Rubio, M. Falchi, F. Mancini, A. Mayor, P. Alano, and F. Silvestrini (2018a). "Gametocytes of the malaria parasite Plasmodium falciparum interact with and stimulate bone marrow mesenchymal cells to secrete angiogenetic factors". In: *Frontiers in cellular and infection microbiology* 8, p. 50.

— (2018b). "Gametocytes of the Malaria Parasite Plasmodium falciparum Interact With and Stimulate Bone Marrow Mesenchymal Cells to Secrete Angiogenetic Factors." In: *Frontiers in cellular and infection microbiology* 8, p. 50.

Michelow, I. C., S. Park, S.-W. Tsai, B. Rayta, C. F. A. Pasaje, S. Nelson, A. M. Early, A. P. Frosch, G. Ayodo, D. K. Raj, C. E. Nixon, C. P. Nixon, S. Pond-Tor, J. F. Friedman, M. Fried, P. E. Duffy, K. G. Le Roch, J. C. Niles, and J. D. Kurtis (Sept. 2021). "A newly characterized malaria antigen on erythrocyte and merozoite surfaces induces parasite inhibitory antibodies." In: *The Journal of experimental medicine* 218.9, e20200170.

Michon, P., J. L. Cole-Tobian, E. Dabod, S. Schoepflin, J. Igu, M. Susapu, N. Tarongka, P. A. Zimmerman, J. C. Reeder, J. G. Beeson, L. Schofield, C. L. King, and I. Mueller (June 2007). "The risk of malarial infections and disease in Papua New Guinean children." In: *The American journal of tropical medicine and hygiene* 76.6, pp. 997–1008.

Migot-Nabias, F., A. G. Lokossou, I. Vigan-Womas, E. Guitard, M. Guillotte, J. M. Noukpo, O. Mercereau-Puijalon, J.-M. Dugoujon, and A. Garcia (2011). "Combined effects of Gm or Km immunoglobulin allotypes and age on antibody responses to Plasmodium falciparum VarO rosetting variant in Benin". In: *Microbes and infection* 13.8-9, pp. 771–775.

Migot-Nabias, F., J. M. Noukpo, E. Guitard, J. Doritchamou, A. Garcia, and J.-M. Dugoujon (Dec. 2008). " Imbalanced Distribution of GM Immunoglobulin Allotypes According to the Clinical Presentation of Plasmodium falciparum Malaria in Beninese Children ". In: *The Journal of Infectious Diseases* 198.12, pp. 1892–1895.

Milner, D. A., R. O. Whitten, S. Kamiza, R. Carr, G. Liomba, C. Dzamalala, K. B. Seydel, M. E. Molyneux, and T. E. Taylor (2014). "The systemic pathology of cerebral malaria in African children". In: *Frontiers in Cellular and Infection Microbiology* 4.August, pp. 1–13.

Minassian, A. M., S. E. Silk, J. R. Barrett, C. M. Nielsen, K. Miura, A. Diouf, C. Loos, J. K. Fallon, A. R. Michell, M. T. White, N. J. Edwards, I. D. Poulton, C. H. Mitton, R. O. Payne, M. Marks, H. Maxwell-Scott, A. Querol-Rubiera, K. Bisnauthsing, R. Batra, T. Ogrina, N. J. Brendish, Y. Themistocleous, T. A. Rawlinson, K. J. Ellis, D. Quinkert, M. Baker, R. Lopez Ramon, F. Ramos Lopez, L. Barfod, P. M. Folegatti, D. Silman, M. Datoo, I. J. Taylor, J. Jin, D. Pulido, A. D. Douglas, W. A. de Jongh, R. Smith, E. Berrie, A. R. Noe, C. L. Diggs, L. A. Soisson, R. Ashfield, S. N. Faust, A. L. Goodman, A. M. Lawrie, F. L. Nugent, G. Alter, C. A. Long, and S. J. Draper (June 2021). "Reduced blood-stage malaria growth and immune correlates in humans following RH5 vaccination." In: *Med* 2.6, 701–719.e19.

Mishra, R., S. K. Panda, P. K. Sahoo, S. Mishra, and A. K. Satapathy (July 2019). "Self-reactive IgG4 antibodies are associated with blocking of pathology in human lymphatic filariasis." In: *Cellular immunology* 341, p. 103927.

Mishra, S., R. S. Nussenzweig, and V. Nussenzweig (Mar. 2012). "Antibodies to Plasmodium circumsporozoite protein (CSP) inhibit sporozoite's cell traversal activity." In: *Journal of immunological methods* 377.1-2, pp. 47–52.

Mkumbaye, S. I., C. W. Wang, E. Lyimo, J. S. Jespersen, A. Manjurano, J. Mosha, R. A. Kavishe, S. B. Mwakalinga, D. T. R. Minja, J. P. Lusingu, T. G. Theander, and T. Lavstsen (2017a). "The Severity of Plasmodium falciparum Infection Is Associated with Transcript Levels of var Genes Encoding Endothelial Protein C Receptor-Binding P. falciparum Erythrocyte Membrane Protein 1." In: *Infection and immunity* 85.4.

— (2017b). "The severity of Plasmodium falciparum infection is associated with transcript levels of var genes encoding endothelial protein C receptor-binding P. falciparum erythrocyte membrane protein 1". In: *Infection and Immunity* 85.4, pp. 1–14.

Mohan Rao, L. V., C. T. Esmon, and U. R. Pendurthi (Sept. 2014). "Endothelial cell protein C receptor: a multiliganded and multifunctional receptor." In: *Blood* 124.10, pp. 1553–62.

Moll, K., M. Palmkvist, J. Ch'ng, M. S. Kiwuwa, and M. Wahlgren (2015). "Evasion of Immunity to Plasmodium falciparum: Rosettes of Blood Group A Impair Recognition of PfEMP1." In: *PloS one* 10.12, e0145120.

Molyneux, M. E., T. E. Taylor, J. J. Wirimaf, and A. Borgsteinj (1989). "Clinical Features and Prognostic Indicators in Paediatric Cerebral Malaria A Study of 131 Comatose Malawian Children". In: *Quarterly Journal of Medicine* 71.265, pp. 441–459.

Moon, J. E., M. E. Greenleaf, J. A. Regules, M. Debois, E. H. Duncan, M. Sedegah, I. Chuang, C. K. Lee, A. K. Sikaffy, L. S. Garver, K. Ivinson, E. Angov, D. Morelle, M. Lievens, C. F. Ockenhouse, V. Ngauy, and O. Ofori-Anyinam (Oct. 2021). "A phase IIA extension study evaluating the effect of booster vaccination with a fractional dose of RTS,S/AS01(E) in a controlled human malaria infection challenge." In: *Vaccine* 39.43, pp. 6398–6406.

Mordmuller, B., M. Sulyok, D. Egger-Adam, M. Resende, W. A. de Jongh, M. H. Jensen, H. H. Smedegaard, S. B. Ditlev, M. Soegaard, L. Poulsen, C. Dyring, C. L. Calle, A. Knoblich, J. Ibanez, M. Esen, P. Deloron, N. Ndam, S. Issifou, S. Houard, R. F. Howard, S. G. Reed, O. Leroy, A. J. F. Luty, T. G. Theander, P. G. Kremsner, A. Salanti, and M. A. Nielsen (2019). "First-in-human, Randomized, Double-blind Clinical Trial of Differentially Adjuvanted PAMVAC, A Vaccine Candidate to Prevent Pregnancy-associated Malaria". In: *Clin Infect Dis* 69.9, pp. 1509–1516.

Mudenda, S. S., S. Kamocha, R. Mswia, M. Conkling, P. Sikanyiti, D. Potter, W. C. Mayaka, and M. A. Marx (Aug. 2011). "Feasibility of using a World Health Organization-standard methodology for Sample Vital Registration with Verbal Autopsy (SAVVY) to report leading causes of death in Zambia: Results of a pilot in four provinces, 2010". In: *Population Health Metrics* 9.

Mueller, I., S. Schoepflin, T. A. Smith, K. L. Benton, M. T. Bretscher, E. Lin, B. Kiniboro, P. A. Zimmerman, T. P. Speed, P. Siba, and I. Felger (June 2012). "Force of infection is key to understanding the epidemiology of Plasmodium falciparum malaria in Papua New Guinean children". In: *Proceedings of the National Academy of Sciences* 109.25, pp. 10030–10035.

Muniz-Junqueira, M. I., L. L. dos Santos-Neto, and C. E. Tosta (Mar. 2001). "Influence of tumor necrosis factor-alpha on the ability of monocytes and lymphocytes to destroy intraerythrocytic Plasmodium falciparum in vitro." In: *Cellular immunology* 208.2, pp. 73–79.

Murungi, L. M., K. Sondén, D. Llewellyn, J. Rono, F. Guleid, A. R. Williams, E. Ogada, A. Thairu, A. Färnert, K. Marsh, et al. (2016). "Targets and mechanisms associated with protection from severe Plasmodium falciparum malaria in Kenyan children". In: *Infection and immunity* 84.4, pp. 950–963.

Nacher, M., F. Gay, P. Singhasivanon, S. Krudsood, S. Treeprasertsuk, D. Mazier, I. Vouldoukis, and S. Looareesuwan (Mar. 2000). "Ascaris lumbricoides infection is associated with protection from cerebral malaria." In: *Parasite immunology* 22.3, pp. 107–13.

Nagaoka, H., B. N. Kanoi, K. Jinoka, M. Morita, T. U. Arumugam, N. M. Q. Palacpac, T. G. Egwang, T. Horii, T. Tsuboi, and E. Takashima (2019). "The N-Terminal Region of Plasmodium falciparum MSP10 Is a Target of Protective Antibodies in Malaria and Is Important for PfGAMA/PfMSP10 Interaction." In: *Frontiers in immunology* 10, p. 2669.

Nash, S. D., D. R. Prevots, E. Kabyemela, Y. P. Khasa, K.-L. Lee, M. Fried, and P. E. Duffy (May 2017). "A Malaria-Resistant Phenotype with Immunological Correlates in a Tanzanian Birth Cohort Exposed to Intense Malaria Transmission." In: *The American journal of tropical medicine and hygiene* 96.5, pp. 1190–1196.

Nasr, A., O. Hamid, A. Al-Ghamdi, and G. Allam (Mar. 2013). "Anti-malarial IgG subclasses pattern and FcgammaRIIa (CD32) polymorphism among pregnancy-associated malaria in semi-immune Saudi women." In: *Malaria journal* 12, p. 110.

Nduati, E., A. Gwela, H. Karanja, C. Mugyenyi, J. Langhorne, K. Marsh, and B. C. Urban (2011). “The plasma concentration of the B cell activating factor is increased in children with acute malaria”. In: *Journal of Infectious Diseases* 204.6, pp. 962–970.

Ndungu, F. M., P. C. Bull, A. Ross, B. S. Lowe, E. Kabiru, and K. Marsh (Feb. 2002). “Naturally acquired immunoglobulin (Ig)G subclass antibodies to crude asexual Plasmodium falciparum lysates: evidence for association with protection for IgG1 and disease for IgG2.” In: *Parasite immunology* 24.2, pp. 77–82.

Ndungu, F. M., A. Olotu, J. Mwacharo, M. Nyonda, J. Apfeld, L. K. Mramba, G. W. Fegan, P. Bejon, and K. Marsh (May 2012). “Memory B cells are a more reliable archive for historical antimalarial responses than plasma antibodies in no-longer exposed children”. In: *Proceedings of the National Academy of Sciences* 109.21, pp. 8247–8252.

Nebie, I., A. Diarra, A. Ouedraogo, I. Soulama, E. C. Bougouma, A. B. Tiono, A. T. Konate, R. Chilengi, M. Theisen, D. Dodoo, E. Remarque, S. Bosomprah, P. Milligan, and S. B. Sirima (Feb. 2008). “Humoral responses to Plasmodium falciparum blood-stage antigens and association with incidence of clinical malaria in children living in an area of seasonal malaria transmission in Burkina Faso, West Africa.” In: *Infection and immunity* 76.2, pp. 759–766.

Newbold, C., P. Warn, G. Black, A. Berendt, A. Craig, B. Snow, M. Msobo, N. Peshu, and K. Marsh (1997). “Receptor-specific adhesion and clinical disease in Plasmodium falciparum”. In: *The American journal of tropical medicine and hygiene* 57.4, pp. 389–398.

Nhabomba, A. J., C. Guinovart, A. Jiménez, M. N. Manaca, L. Quintó, P. Cisteró, R. Aguilar, A. Barbosa, M. H. Rodríguez, Q. Bassat, J. J. Aponte, A. Mayor, C. E. Chitnis, P. L. Alonso, and C. Dobaño (2014). “Impact of age of first exposure to Plasmodium falciparum on antibody responses to malaria in children: a randomized, controlled trial in Mozambique”. In: *Malaria Journal* 13.1, p. 121.

Niang, M., A. K. Bei, K. G. Madnani, S. Pelly, S. Dankwa, U. Kanjee, K. Gunalan, A. Amaladoss, K. P. Yeo, N. S. Bob, B. Malleret, M. T. Duraisingh, and P. R. Preiser (July 2014). “STEVOR is a Plasmodium falciparum erythrocyte binding protein that mediates merozoite invasion and rosetting.” In: *Cell host microbe* 16.1, pp. 81–93.

Nielsen, M. A., M. Resende, W. A. de Jongh, S. B. Ditlev, B. Mordmüller, S. Houard, N. T. Ndam, M. Ø. Agerbæk, M. Hamborg, A. Massougbodji, et al. (2015a). “The influence of sub-unit composition and expression system on the functional antibody response in the development of a VAR2CSA based Plasmodium falciparum placental malaria vaccine”. In: *PLoS One* 10.9, e0135406.

Nielsen, M. A., M. Resende, W. A. de Jongh, S. B. Ditlev, B. Mordmüller, S. Houard, N. T. Ndam, M. Ø. Agerbæk, M. Hamborg, A. Massougbodji, S. Issifou, A. Strøbæk, L. Poulsen, O. Leroy, P. G. Kremsner, J.-P. Chippaux, A. J. F. Luty, P. Deloron, T. G. Theander, C. Dyring, and A. Salanti (Sept. 2015b). “The Influence of Sub-Unit Composition and Expression System on the Functional Antibody Response in the Development of a VAR2CSA Based Plasmodium falciparum Placental Malaria Vaccine”. In: *PLOS ONE* 10.9. Ed. by K. K. Tetteh, e0135406.

Nielsen, M. A., T. Staalsoe, J. A. L. Kurtzhals, B. Q. Goka, D. Dodoo, M. Alifrangis, T. G. Theander, B. D. Akanmori, and L. Hviid (Apr. 2002). “ Plasmodium falciparum Variant Surface Antigen Expression Varies Between Isolates Causing Severe and Nonsevere Malaria and Is Modified by Acquired Immunity ”. In: *The Journal of Immunology* 168.7, pp. 3444–3450.

Normark, J., D. Nilsson, U. Ribacke, G. Winter, K. Moll, C. E. Wheelock, J. Bayarugaba, F. Kironde, T. G. Egwang, Q. Chen, B. Andersson, and M. Wahlgren (Oct. 2007). "PfEMP1-DBL1alpha amino acid motifs in severe disease states of Plasmodium falciparum malaria." In: *Proceedings of the National Academy of Sciences of the United States of America* 104.40, pp. 15835–15840.

Nunes-Silva, S., S. Dechavanne, A. Moussiliou, N. Pstrag, J. P. Semblat, S. Gangnard, N. Tuikue-Ndam, P. Deloron, A. Chêne, and B. Gamain (2015). "Beninese children with cerebral malaria do not develop humoral immunity against the IT4-VAR19-DC8 PfEMP1 variant linked to EPCR and brain endothelial binding". In: *Malaria Journal*.

Nurieva, R. I. and Y. Chung (2010). "Understanding the development and function of T follicular helper cells". In: *Cellular and Molecular Immunology* 7.3, pp. 190–197.

O'Brien, N. F., Y. Fonseca, H. C. Johnson, D. Postels, G. L. Birbeck, Y. Chimalizeni, K. B. Seydel, M. Bernard Gushu, T. Phiri, S. June, et al. (2022). "Mechanisms of Transcranial Doppler Ultrasound phenotypes in paediatric cerebral malaria remain elusive". In: *Malaria journal* 21.1, p. 196.

Obeng-Adjei, N., D. B. Larremore, L. Turner, A. Ongoiba, S. Li, S. Doumbo, T. B. Yazew, K. Kayentao, L. H. Miller, B. Traore, S. K. Pierce, C. O. Buckee, T. Lavstsen, P. D. Crompton, and T. M. Tran (2020). "Longitudinal analysis of naturally acquired PfEMP1 CIDR domain variant antibodies identifies associations with malaria protection". In: *JCI Insight* 5.12, e137262.

Obeng-Adjei, N., S. Portugal, T. M. Tran, T. B. Yazew, J. Skinner, S. Li, A. Jain, P. L. Felgner, O. K. Doumbo, K. Kayentao, A. Ongoiba, B. Traore, and P. D. Crompton (Oct. 2015). "Circulating Th1-Cell-type Tfh Cells that Exhibit Impaired B Cell Help Are Preferentially Activated during Acute Malaria in Children." In: *Cell reports* 13.2, pp. 425–439.

Offeddu, V., A. Olotu, F. Osier, K. Marsh, K. Matuschewski, and V. Thathy (2017). "High Sporozoite Antibody Titers in Conjunction with Microscopically Detectable Blood Infection Display Signatures of Protection from Clinical Malaria ". In: 8, p. 488.

Ofori, M. F., D. Dodoo, T. Staalsoe, J. A. L. Kurtzhals, K. Koram, T. G. Theander, B. D. Akanmori, and L. Hviid (June 2002). "Malaria-induced acquisition of antibodies to Plasmodium falciparum variant surface antigens." In: *Infection and immunity* 70.6, pp. 2982–8.

Ogonda, L. A., A. S. S. Orago, M. F. Otieno, C. Adhiambo, W. Otieno, and J. A. Stoute (May 2010). "The levels of CD16/Fc gamma receptor IIIA on CD14+ CD16+ monocytes are higher in children with severe Plasmodium falciparum anemia than in children with cerebral or uncomplicated malaria". In: *Infection and immunity* 78.5, pp. 2173–2181.

Ohashi, J., I. Naka, H. Hananantachai, and J. Patarapotikul (2016). "Association of PECAM1/CD31 polymorphisms with cerebral malaria." In: *International journal of molecular epidemiology and genetics* 7.2, pp. 87–94.

Oleinikov, A. V., E. Amos, I. T. Frye, E. Rossnagle, T. K. Mutabingwa, M. Fried, and P. E. Duffy (Apr. 2009). "High Throughput Functional Assays of the Variant Antigen PfEMP1 Reveal a Single Domain in the 3D7 Plasmodium falciparum Genome that Binds ICAM1 with High Affinity and Is Targeted by Naturally Acquired Neutralizing Antibodies". In: *PLoS Pathogens* 5.4. Ed. by J. W. Kazura, e1000386.

Oleinikov, A. V., R. Morrison, E. Amos, P. E. Duffy, M. Fried, V. V. Voronkova, and I. T. Frye (2012). "A Plasma Survey Using 38 PfEMP1 Domains Reveals Frequent Recognition of the Plasmodium falciparum Antigen VAR2CSA among Young Tanzanian Children". In: *PLoS ONE* 7.1, e31011.

Olsen, R. W., G. Ecklu-Mensah, A. Bengtsson, M. F. Ofori, K. A. Kusi, K. A. Koram, L. Hviid, Y. Adams, and A. T. Jensen (Oct. 2019). "Acquisition of IGG to ICAM-1-Binding DBLB Domains in the plasmodium falciparum erythrocyte membrane protein 1 antigen family varies between groups A, B, and C". In: *Infection and Immunity* 87.10.

Olsen, R. W., G. Ecklu-Mensah, A. Bengtsson, M. F. Ofori, J. P. Lusingu, F. C. Castberg, L. Hviid, Y. Adams, and A. T. R. Jensen (2018). "Natural and Vaccine-Induced Acquisition of Cross-Reactive IgG-Inhibiting ICAM-1-Specific Binding of a Plasmodium falciparum PfEMP1 Subtype Associated Specifically with Cerebral Malaria". In: *Infection and immunity* 86.4, pp. 1–17.

Omi, K., J. Ohashi, J. Patarapotikul, H. Hananantachai, I. Naka, S. Looareesuwan, and K. Tokunaga (Dec. 2002). "Fcgamma receptor IIA and IIIB polymorphisms are associated with susceptibility to cerebral malaria." In: *Parasitology international* 51.4, pp. 361–6.

Omosun, Y. O., C. I. Anumudu, S. Adoro, A. B. Odaibo, O. Sodeinde, A. A. Holder, M. Nwagwu, and R. I. Nwuba (Sept. 2005). "Variation in the relationship between anti-MSP-1(19) antibody response and age in children infected with Plasmodium falciparum during the dry and rainy seasons." In: *Acta tropica* 95.3, pp. 233–247.

Oosterhoff, J. J., M. D. Larsen, C. E. van der Schoot, and G. Vidarsson (Oct. 2022). "Afucosylated IgG responses in humans - structural clues to the regulation of humoral immunity." In: *Trends in immunology* 43.10, pp. 800–814.

Opi, D. H., M. J. Boyle, A. R. D. McLean, L. Reiling, J. A. Chan, D. I. Stanisic, A. Ura, I. Mueller, F. J. I. Fowkes, S. J. Rogerson, and J. G. Beeson (2021). "Reduced risk of placental parasitemia associated with complement fixation on Plasmodium falciparum by antibodies among pregnant women". In: *BMC Med* 19.1, p. 201.

Oppenheimer, S. J., D. R. Higgs, D. J. Weatherall, J. Barker, and R. A. Spark (Feb. 1984). "Alpha thalassaemia in Papua New Guinea." In: *Lancet (London, England)* 1.8374, pp. 424–426.

Orago, A. S. and C. A. Facer (Oct. 1991). "Cytotoxicity of human natural killer (NK) cell subsets for Plasmodium falciparum erythrocytic schizonts: stimulation by cytokines and inhibition by neomycin". In: *Clinical and experimental immunology* 86.1, pp. 22–29.

Ortega-Pajares, A. and S. J. Rogerson (2018). "The Rough Guide to Monocytes in Malaria Infection ". In: 9, p. 2888.

Osier, F. H. A., G. Feng, M. J. Boyle, C. Langer, J. Zhou, J. S. Richards, F. J. McCallum, L. Reiling, A. Jaworowski, R. F. Anders, K. Marsh, and J. G. Beeson (2014). "Opsonic phagocytosis of Plasmodium falciparum merozoites: Mechanism in human immunity and a correlate of protection against malaria". In: *BMC Medicine* 12.1, pp. 1–15.

Otto, T. D., S. A. Assefa, U. Böhme, M. J. Sanders, D. Kwiatkowski, M. Berriman, and C. Newbold (2019). "Evolutionary analysis of the most polymorphic gene family in falciparum malaria." In: *Wellcome open research* 4, p. 193.

Otto, T. D., U. Böhme, M. Sanders, A. Reid, E. I. Bruske, C. W. Duffy, P. C. Bull, R. D. Pearson, A. Abdi, S. Dimonte, et al. (2018). "Long read assemblies of geographically dispersed Plasmodium falciparum isolates reveal highly structured subtelomeres". In: *Wellcome open research* 3.

Owusu-Agyei, S., K. A. Koram, J. K. Baird, G. C. Utz, F. N. Binka, F. K. Nkrumah, D. J. Fryauff, and S. L. Hoffman (Sept. 2001). "Incidence of symptomatic and asymptomatic Plasmodium falciparum infection following curative therapy in adult residents of northern Ghana." In: *The American journal of tropical medicine and hygiene* 65.3, pp. 197–203.

Oyong, D. A., E. Kenangalem, J. R. Poespoprodjo, J. G. Beeson, N. M. Anstey, R. N. Price, and M. J. Boyle (Nov. 2018). "Loss of complement regulatory proteins on uninfected erythrocytes in vivax and falciparum malaria anemia". In: *JCI insight* 3.22, e124854.

Paget-McNicol, S., M. Gatton, I. Hastings, and A. Saul (Mar. 2002). "The Plasmodium falciparum var gene switching rate, switching mechanism and patterns of parasite recrudescence described by mathematical modelling." In: *Parasitology* 124.Pt 3, pp. 225–35.

Pain, A., D. J. Ferguson, O. Kai, B. C. Urban, B. Lowe, K. Marsh, and D. J. Roberts (Feb. 2001). "Platelet-mediated clumping of Plasmodium falciparum-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria." In: *Proceedings of the National Academy of Sciences of the United States of America* 98.4, pp. 1805–10.

Papadea, C. and I. J. Check (1989). "Human immunoglobulin g and immunoglobulin g subclasses: Biochemical, genetic, and clinical aspects". In: *Critical Reviews in Clinical Laboratory Sciences* 27.1, pp. 27–58.

Partey, F. D., F. C. Castberg, E. W. Sarbah, S. E. Silk, G. A. Awandare, S. J. Draper, N. Opoku, M. Kweku, M. F. Ofori, L. Hviid, and L. Barfod (2018). "Kinetics of antibody responses to PfRH5-complex antigens in Ghanaian children with Plasmodium falciparum malaria." In: *PloS one* 13.6, e0198371.

Paul, G., A. Deshmukh, B. Kumar Chourasia, M. Kalamuddin, A. Panda, S. Kumar Singh, P. K. Gupta, A. Mohmmed, V. S. Chauhan, M. Theisen, and P. Malhotra (Mar. 2018). "Protein-protein interaction studies reveal the Plasmodium falciparum merozoite surface protein-1 region involved in a complex formation that binds to human erythrocytes." In: *The Biochemical journal* 475.6, pp. 1197–1209.

Pawluczkowycz, A. W., M. A. Lindorfer, J. N. Waitumbi, and R. P. Taylor (2007). "Hematin promotes complement alternative pathway-mediated deposition of C3 activation fragments on human erythrocytes: potential implications for the pathogenesis of anemia in malaria". In: *The Journal of Immunology* 179.8, pp. 5543–5552.

Pérez-Mazliah, D., F. M. Ndungu, R. Aye, and J. Langhorne (Jan. 2020). "B-cell memory in malaria: Myths and realities". In: *Immunological reviews* 293.1, pp. 57–69.

Pérez-Mazliah, D., F. M. Ndungu, R. Aye, and J. Langhorne (Nov. 2019). "B-cell memory in malaria: Myths and realities". In: *Immunological Reviews*, imr.12822.

Perlmann, P., H. Perlmann, B. W. Flyg, M. Hagstedt, G. Elghazali, S. Worku, V. Fernandez, A. S. Rutta, and M. Troye-Blomberg (Jan. 1997). "Immunoglobulin E, a pathogenic factor in Plasmodium falciparum malaria." In: *Infection and immunity* 65.1, pp. 116–21.

Pillai, S. (2023). "Is it bad, is it good, or is IgG4 just misunderstood?" In: *Science Immunology*, eadg7327.

Piper, K. P., D. J. Roberts, and K. P. Day (Feb. 1999). "Plasmodium falciparum: analysis of the antibody specificity to the surface of the trophozoite-infected erythrocyte." In: *Experimental parasitology* 91.2, pp. 161–169.

Pologe, L. G., A. Pavlovec, H. Shio, and J. V. Ravetch (Oct. 1987). "Primary structure and subcellular localization of the knob-associated histidine-rich protein of Plasmodium falciparum." In: *Proceedings of the National Academy of Sciences of the United States of America* 84.20, pp. 7139–7143.

Ponsford, M. J., I. M. Medana, P. Prapansilp, T. T. Hien, S. J. Lee, A. M. Dondorp, M. M. Esiri, N. P. J. Day, N. J. White, and G. D. H. Turner (2012). "Sequestration and microvascular congestion are associated with coma in human cerebral malaria". In: *Journal of Infectious Diseases* 205.4, pp. 663–671.

Porter, D. W., F. M. Thompson, T. K. Berthoud, C. L. Hutchings, L. Andrews, S. Biswas, I. Poulton, E. Prieur, S. Correa, R. Rowland, et al. (2011). "A human Phase I/IIa malaria challenge trial of a polyprotein malaria vaccine". In: *Vaccine* 29.43, pp. 7514–7522.

Quintana, M. d. P., D. Angeletti, K. Moll, Q. Chen, and M. Wahlgren (2016). "Phagocytosis-inducing antibodies to Plasmodium falciparum upon immunization with a recombinant PfEMP1 NTS-DBL1alpha- domain". In: *Malaria Journal* 15.1, p. 416.

Quintana, M. d. P., J.-H. Ch'ng, K. Moll, A. Zandian, P. Nilsson, Z. M. Idris, S. Saiwaew, U. Qundos, and M. Wahlgren (2018). "Antibodies in children with malaria to PfEMP1, RIFIN and SURFIN expressed at the Plasmodium falciparum parasitized red blood cell surface". In: *Scientific Reports* 8.1, p. 3262.

Quintana, M. d. P., G. Ecklu-Mensah, S. O. Tcherniuk, S. B. Ditlev, A. V. Oleinikov, L. Hviid, and M. Lopez-Perez (2019). "Comprehensive analysis of Fc-mediated IgM binding to the Plasmodium falciparum erythrocyte membrane protein 1 family in three parasite clones". In: *Scientific Reports* 9.1, pp. 1–11.

Raghavan, M., K. L. Kalantar, E. Duarte, N. Teyssier, S. Takahashi, A. F. Kung, J. V. Rajan, J. Rek, K. K. Tetteh, C. Drakeley, et al. (2023). "Antibodies to repeat-containing antigens in Plasmodium falciparum are exposure-dependent and short-lived in children in natural malaria infections". In: *Elife* 12, e81401.

Ramachandran, A. and A. Sharma (Nov. 2022). "Dissecting the mechanisms of pathogenesis in cerebral malaria." In: *PLoS pathogens* 18.11, e1010919.

Rambhatla, J. S., G. Q. Tonkin-hill, E. Takashima, T. Tsuboi, R. Noviyanti, L. Trianty, B. F. Sebayang, D. A. Lampah, J. Marfurt, R. N. Price, N. M. Anstey, A. T. Papenfuss, T. Damelang, A. W. Chung, M. F. Duffy, and S. J. Rogerson (2022). "Identifying Targets of Protective Antibodies against Severe Malaria in Papua, Indonesia, Using Locally Expressed Domains". In: *Infection and immunity* 90.2, e0043521.

Rambhatla, J. S. (2018). "Antibody immunity to Plasmodium falciparum erythrocyte membrane protein 1 and severe malaria [Doctoral dissertation]". In: *Univesrity of Melbourne Research Respository*.

Rambhatla, J. S., L. Turner, L. Manning, M. Laman, T. M. Davis, J. G. Beeson, I. Mueller, J. Warrel, T. G. Theander, T. Lavstsen, and S. J. Rogerson (2019). "Acquisition of antibodies against endothelial protein c receptor-binding domains of plasmodium falciparum erythrocyte membrane protein 1 in children with severe malaria". In: *Journal of Infectious Diseases* 219.5, pp. 808–818.

Rask, T. S., D. A. Hansen, T. G. Theander, A. G. Pedersen, and T. Lavstsen (2010). "Plasmodium falciparum erythrocyte membrane protein 1 diversity in seven genomes - divide and conquer". In: *PLoS Computational Biology* 6.9, e1000933.

Rathnayake, D., E. H. Aitken, and S. J. Rogerson (2021). "Beyond Binding: The Outcomes of Antibody-Dependent Complement Activation in Human Malaria." In: *Frontiers in immunology* 12, p. 683404.

Rathnayake, R. M. D. M. (2022). "The role of antibody, complement, and innate immune cells in protective immunity to malaria [Doctoral dissertation]". In: *Univesrity of Melbourne Research Respository*.

Recker, M., C. O. Buckee, A. Serazin, S. Kyes, R. Pinches, Z. Christodoulou, A. L. Springer, S. Gupta, and C. I. Newbold (Mar. 2011). "Antigenic variation in Plasmodium falciparum malaria involves a highly structured switching pattern." In: *PLoS pathogens* 7.3, e1001306.

Reece, W. H. H., M. Pinder, P. K. Gothard, P. Milligan, K. Bojang, T. Doherty, M. Plebanski, P. Akinwunmi, S. Everaere, K. R. Watkins, G. Voss, N. Tornieporth, A. Alloueche, B. M.

Greenwood, K. E. Kester, K. P. W. J. McAdam, J. Cohen, and A. V. S. Hill (Apr. 2004). "A CD4(+) T-cell immune response to a conserved epitope in the circumsporozoite protein correlates with protection from natural Plasmodium falciparum infection and disease." In: *Nature medicine* 10.4, pp. 406–410.

Remarque, E. J., B. W. Faber, R. Rodriguez Garcia, H. Oostermeijer, S. B. Sirima, I. Nebie Ouedraogo, L. Kara, O. Launay, S. Houard, O. Leroy, and C. H. M. Kocken (Apr. 2021). "Accelerated phase Ia/b evaluation of the malaria vaccine candidate PfAMA1 DiCo demonstrates broadening of humoral immune responses." In: *NPJ vaccines* 6.1, p. 55.

Richards, J. S., D. I. Stanisic, F. J. I. Fowkes, L. Tavul, E. Dabod, J. K. Thompson, S. Kumar, C. E. Chitnis, D. L. Narum, P. Michon, P. M. Siba, A. F. Cowman, I. Mueller, and J. G. Beeson (Oct. 2010). "Association between naturally acquired antibodies to erythrocyte-binding antigens of Plasmodium falciparum and protection from malaria and high-density parasitemia." In: *Clinical Infectious Diseases* 51.8, e50–60.

Ricke, C. H., T. Staalsoe, K. Koram, B. D. Akanmori, E. M. Riley, T. G. Theander, and L. Hviid (Sept. 2000). "Plasma antibodies from malaria-exposed pregnant women recognize variant surface antigens on Plasmodium falciparum-infected erythrocytes in a parity-dependent manner and block parasite adhesion to chondroitin sulfate A". In: *The Journal of Immunology* 165.6, pp. 3309–3316.

Rispens, T., A. M. Davies, P. Ooijevaar-de Heer, S. Absalah, O. Bende, B. J. Sutton, G. Vidarsson, and R. C. Aalberse (2014). "Dynamics of inter-heavy chain interactions in human immunoglobulin G (IgG) subclasses studied by kinetic Fab arm exchange". In: *Journal of Biological Chemistry* 289.9, pp. 6098–6109.

Roberts, D. J., A. G. Craig, A. R. Berendt, R. Pinches, G. Nash, K. Marsh, and C. I. Newbold (June 1992). "Rapid switching to multiple antigenic and adhesive phenotypes in malaria." In: *Nature* 357.6380, pp. 689–92.

Roberts, J. T. and A. W. Barb (Dec. 2018). "A single amino acid distorts the Fc gamma receptor IIIb/CD16b structure upon binding immunoglobulin G1 and reduces affinity relative to CD16a." In: *The Journal of biological chemistry* 293.51, pp. 19899–19908.

Robinson, B. A., T. L. Welch, and J. D. Smith (Feb. 2003). "Widespread functional specialization of Plasmodium falciparum erythrocyte membrane protein 1 family members to bind CD36 analysed across a parasite genome". In: *Molecular Microbiology* 47.5, pp. 1265–1278.

Roeffen, W., K. Teelen, J. Van As, M. vd Vegte-Bolmer, W. Eling, and R. Sauerwein (2001). "Plasmodium falciparum: production and characterization of rat monoclonal antibodies specific for the sexual-stage Pfs48/45 antigen". In: *Experimental parasitology* 97.1, pp. 45–49.

Roestenberg, M., M. McCall, T. E. Mollnes, M. van Deuren, T. Sprong, I. Klasen, C. C. Hermsen, R. W. Sauerwein, and A. van der Ven (July 2007). "Complement activation in experimental human malaria infection." In: *Transactions of the Royal Society of Tropical Medicine and Hygiene* 101.7, pp. 643–649.

Rogerson, S. J. and G. V. Brown (Feb. 1997). "Chondroitin sulphate A as an adherence receptor for Plasmodium falciparum-infected erythrocytes." In: *Parasitology today (Personal ed.)* 13.2, pp. 70–5.

Rogerson, S. J., S. Plitt, T. E. Taylor, C. Dobaño, R. Tembenu, and M. E. Molyneux (Sept. 1999). "Cytoadherence characteristics of Plasmodium falciparum-infected erythrocytes from Malawian children with severe and uncomplicated malaria." In: *The American Journal of Tropical Medicine and Hygiene* 61.3, pp. 467–472.

Rogerson, S. J., J. G. Beeson, M. Laman, J. R. Poespoprodjo, T. William, J. A. Simpson, R. N. Price, and A. Investigators (July 2020). “Identifying and combating the impacts of COVID-19 on malaria”. In: *BMC medicine* 18.1, p. 239.

Rogerson, S. J., E. Pollina, A. Getachew, E. Tadesse, V. M. Lema, and M. E. Molyneux (Jan. 2003). “Placental monocyte infiltrates in response to Plasmodium falciparum malaria infection and their association with adverse pregnancy outcomes.” In: *The American journal of tropical medicine and hygiene* 68.1, pp. 115–119.

Rogerson, S. J., M. Desai, A. Mayor, E. Sicuri, S. M. Taylor, and A. M. van Eijk (Apr. 2018). “Burden, pathology, and costs of malaria in pregnancy: new developments for an old problem”. In: *The Lancet Infectious Diseases* 18.4, e107–e118.

Rogier, E., D. Nace, P. R. Dimbu, B. Wakeman, J. G. Beeson, C. Drakeley, K. Tetteh, and M. Plucinski (2022). “Antibody dynamics in children with first or repeat Plasmodium falciparum infections.” In: *Frontiers in medicine* 9, p. 869028.

Rosanas-Urgell, A., N. Senn, P. Rarau, J. J. Aponte, J. C. Reeder, P. M. Siba, P. Michon, and I. Mueller (Nov. 2012). “Lack of associations of alpha-(+)-thalassemia with the risk of Plasmodium falciparum and Plasmodium vivax infection and disease in a cohort of children aged 3-21 months from Papua New Guinea.” In: *International journal for parasitology* 42.12, pp. 1107–1113.

Rottmann, M., D. Muller, A. T. R. Jensen, M. Kaestli, H.-P. Beck, T. Lavstsen, J. P. Mugasa, and T. Theander (2006). “Differential Expression of var Gene Groups Is Associated with Morbidity Caused by Plasmodium falciparum Infection in Tanzanian Children”. In: *Infection and Immunity* 74.7, pp. 3904–3911.

Rowe, A., J. Obeiro, C. I. Newbold, and K. Marsh (June 1995). “Plasmodium falciparum rosetting is associated with malaria severity in Kenya.” In: *Infection and immunity* 63.6, pp. 2323–6.

Rowe, J. A., J. Shafi, O. K. Kai, K. Marsh, and A. Raza (June 2002). “Nonimmune IgM, but not IgG binds to the surface of Plasmodium falciparum-infected erythrocytes and correlates with rosetting and severe malaria.” In: *The American journal of tropical medicine and hygiene* 66.6, pp. 692–9.

Rowe, J. A., J. M. Moulds, C. I. Newbold, and L. H. Miller (1997). “P. falciparum rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1.” In: *Nature* 388.6639, pp. 292–295.

Royo, J., M. Rahabi, C. Kamaliddin, S. Ezinmegnon, D. Olagnier, H. Authier, A. Massougbodji, J. Alao, Y. Ladipo, P. Deloron, G. Bertin, B. Pipy, A. Coste, and A. Aubouy (2019). “Changes in monocyte subsets are associated with clinical outcomes in severe malarial anaemia and cerebral malaria”. In: *Scientific Reports* 9.1, p. 17545.

RTS, S Clinical Trials Partnership, G. B. S. (July 2015). “Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: Final results of a phase 3, individually randomised, controlled trial”. In: *The Lancet* 386.9988, pp. 31–45.

Saavedra-Langer, R., J. Marapara, A. Valle-Campos, S. Durand, M. E. Vásquez-Chasnamote, H. Silva, and V. Pinedo-Cancino (Sept. 2018). “IgG subclass responses to excreted-secreted antigens of Plasmodium falciparum in a low-transmission malaria area of the Peruvian Amazon.” In: *Malaria journal* 17.1, p. 328.

Saeed, M., W. Roeffen, N. Alexander, C. J. Drakeley, G. A. T. Targett, and C. J. Sutherland (May 2008). “Plasmodium falciparum Antigens on the Surface of the Gametocyte-Infected Erythrocyte”. In: *PLOS ONE* 3.5, e2280.

Saito, F., K. Hirayasu, T. Satoh, C. W. Wang, J. Lusingu, T. Arimori, K. Shida, N. M. Q. Palacpac, S. Itagaki, S. Iwanaga, E. Takashima, T. Tsuboi, M. Kohyama, T. Suenaga, M. Colonna, J. Takagi, T. Lavstsen, T. Horii, and H. Arase (2017). "Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors". In: *Nature* 552.7683, pp. 101–105.

Sakamoto, H., S. Takeo, E. Takashima, K. Miura, B. N. Kanoi, T. Kaneko, E.-T. Han, M. Tachibana, K. Matsuoka, J. Sattabongkot, R. Udomsangpetch, T. Ishino, and T. Tsuboi (Apr. 2018). "Identification of target proteins of clinical immunity to Plasmodium falciparum in a region of low malaria transmission." In: *Parasitology international* 67.2, pp. 203–208.

Salanti, A., T. Staalsoe, T. Lavstsen, A. T. R. Jensen, M. P. K. Sowa, D. E. Arnot, L. Hviid, and T. G. Theander (May 2003). "Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering Plasmodium falciparum involved in pregnancy-associated malaria". In: *Molecular Microbiology* 49.1, pp. 179–191.

Salmon, D., J. Vilde, B. Andrieu, R. Simonovic, and J. Lebras (1986). "Role of immune serum and complement in stimulation of the metabolic burst of human neutrophils by Plasmodium falciparum". In: *Infection and immunity* 51.3, pp. 801–806.

Salmon, J., J. Edberg, R. Kimberly, et al. (1990). "Fc gamma receptor III on human neutrophils. Allelic variants have functionally distinct capacities." In: *The Journal of clinical investigation* 85.4, pp. 1287–1295.

Sarfo, J. O., M. Amoadu, P. Y. Kordorwu, A. K. Adams, T. B. Gyan, A.-G. Osman, I. Asiedu, and E. W. Ansah (2023). "Malaria amongst children under five in sub-Saharan Africa: a scoping review of prevalence, risk factors and preventive interventions". In: *European Journal of Medical Research* 28.1, pp. 1–14.

Sarthou, J. L., G. Angel, G. Aribot, C. Rogier, A. Dieye, A. Toure Balde, B. Diatta, P. Seignot, and C. Roussilhon (Aug. 1997). "Prognostic value of anti-Plasmodium falciparum-specific immunoglobulin G3, cytokines, and their soluble receptors in West African patients with severe malaria." In: *Infection and immunity* 65.8, pp. 3271–3276.

Saxena, A. and D. Wu (2016). "Advances in Therapeutic Fc Engineering – Modulation of IgG-Associated Effector Functions and Serum Half-life". In: 7.

Schieck, E., E. J. Poole, A. Rippert, J. Peshu, P. Sasi, S. Borrmann, and P. C. Bull (2017). "Plasmodium falciparum variant erythrocyte surface antigens: a pilot study of antibody acquisition in recurrent natural infections". In: *Malaria Journal* 16.1, pp. 1–10.

Schreiber, N., N. Brattig, J. Evans, T. Agbenyega, R. D. Horstmann, J. May, and M.-Q. Klinkert (Apr. 2006). "Cerebral malaria is associated with IgG2 and IgG4 antibody responses to recombinant Plasmodium falciparum RIFIN antigen." In: *Microbes and infection* 8.5, pp. 1269–1276.

Schroeder, H. W. J. and L. Cavacini (Feb. 2010). "Structure and function of immunoglobulins." In: *The Journal of allergy and clinical immunology* 125.2 Suppl 2, S41–52.

Schroeder Jr, H. W. and L. Cavacini (2012). "Structure and Function of Immunoglobulins". In: *J Allergy Clin Immunology* 125.2 0 2, S41–S52.

Schwarzer, E., F. Turrini, D. Ulliers, G. Giribaldi, H. Ginsburg, and P. Arese (Oct. 1992). "Impairment of macrophage functions after ingestion of Plasmodium falciparum-infected erythrocytes or isolated malarial pigment." In: *The Journal of experimental medicine* 176.4, pp. 1033–1041.

Seaton, K. E., R. L. Spreng, M. Abraha, M. Reichartz, M. Rojas, F. Feely, R. H. C. Huntwork, S. Dutta, S. V. Mudrak, S. M. Alam, S. Gregory, E. Jongert, M. Coccia, F. Ulloa-Montoya,

U. Wille-Reece, G. D. Tomaras, and S. M. Dennison (2021). "Subclass and avidity of circumsporozoite protein specific antibodies associate with protection status against malaria infection". In: *npj Vaccines* 6.1, p. 110.

Sedegah, M., B. K. Sim, C. Mason, T. Nutman, A. Malik, C. Roberts, A. Johnson, J. Ochola, D. Koech, and B. Were (Aug. 1992). "Naturally acquired CD8+ cytotoxic T lymphocytes against the Plasmodium falciparum circumsporozoite protein." In: *Journal of immunology (Baltimore, Md. : 1950)* 149.3, pp. 966–971.

Seidahmed, O., S. Kurumop, S. Jamea, D. Timbi, M. Hetzel, and W. Pomat (2021). "Papua New Guinea Malaria Indicator Survey 2019-2020 : Final Report on Malaria Prevention , Infection Prevalence , and Treatment- Seeking". In: August.

Selva, K. J., C. E. van de Sandt, M. M. Lemke, C. Y. Lee, S. K. Shoffner, B. Y. Chua, S. K. Davis, T. H. O. Nguyen, L. C. Rowntree, L. Hensen, M. Koutsakos, C. Y. Wong, F. Mordant, D. C. Jackson, K. L. Flanagan, J. Crowe, S. Tosif, M. R. Neeland, P. Sutton, P. V. Licciardi, N. W. Crawford, A. C. Cheng, D. L. Doolan, F. Amanat, F. Krammer, K. Chappell, N. Modhiran, D. Watterson, P. Young, W. S. Lee, B. D. Wines, P. Mark Hogarth, R. Esterbauer, H. G. Kelly, H.-X. Tan, J. A. Juno, A. K. Wheatley, S. J. Kent, K. B. Arnold, K. Kedzierska, and A. W. Chung (Apr. 2021). "Systems serology detects functionally distinct coronavirus antibody features in children and elderly." In: *Nature communications* 12.1, p. 2037.

Seydel, K. B., S. D. Kampondeni, C. Valim, M. J. Potchen, D. A. Milner, F. W. Muwalo, G. L. Birbeck, W. G. Bradley, L. L. Fox, S. J. Glover, C. A. Hammond, R. S. Heyderman, C. A. Chilingulo, M. E. Molyneux, and T. E. Taylor (Mar. 2015). "Brain Swelling and Death in Children with Cerebral Malaria". In: *New England Journal of Medicine* 372.12, pp. 1126–1137.

Shabani, E., B. Hanisch, R. O. Opoka, T. Lavstsen, and C. C. John (Oct. 2017). "Plasmodium falciparum EPCR-binding PfEMP1 expression increases with malaria disease severity and is elevated in retinopathy negative cerebral malaria". In: *BMC Medicine* 15.1, p. 183.

Shalmiev, G., M. Krugliak, F. Turrini, and H. Ginsburg (1996). "Antimalarial drugs inhibit the phagocytosis of erythrocytes infected with Plasmodium falciparum." In: *Transactions of the Royal Society of Tropical Medicine and Hygiene* 90.5, pp. 558–562.

Sharma, A., I. Sharma, D. Kogkasuriyachai, and N. Kumar (2003). "Structure of a gametocyte protein essential for sexual development in Plasmodium falciparum". In: *Nature Structural & Molecular Biology* 10.3, pp. 197–203.

Sherratt, S., A. Patel, D. A. Baker, E. M. Riley, and M. R. Goodier (2020). "Differential IL-18 Dependence of Canonical and Adaptive NK Cells for Antibody Dependent Responses to P. falciparum." In: *Frontiers in immunology* 11, p. 533.

Silver, K. L., S. J. Higgins, C. R. McDonald, and K. C. Kain (Aug. 2010a). "Complement driven innate immune response to malaria: fuelling severe malarial diseases." In: *Cellular microbiology* 12.8, pp. 1036–1045.

Silver, K. L., K. Zhong, R. G. F. Leke, D. W. Taylor, and K. C. Kain (Mar. 2010b). "Dysregulation of angiopoietins is associated with placental malaria and low birth weight." In: *PloS one* 5.3, e9481.

Sinden, R. E. (Apr. 2015). "The cell biology of malaria infection of mosquito: advances and opportunities". In: *Cellular microbiology* 17.4, pp. 451–466.

Sirima, S. B., L. Richert, A. Chêne, A. T. Konate, C. Campion, S. Dechavanne, J.-P. Semblat, N. Benhamouda, M. Bahuaud, P. Loulergue, A. Ouédraogo, I. Nébié, M. Kabore, D. Kargougou, A. Barry, S. M. Ouattara, V. Boilet, F. Allais, G. Roguet, N. Havelange,

E. Lopez-Perez, A. Kuppers, E. Konaté, C. Roussillon, M. Kanté, L. Belarbi, A. Diarra, N. Henry, I. Soulama, A. Ouédraogo, H. Esperou, O. Leroy, F. Batteux, E. Tartour, N. K. Viebig, R. Thiebaut, O. Launay, and B. Gamain (May 2020). "PRIMVAC vaccine adjuvanted with Alhydrogel or GLA-SE to prevent placental malaria: a first-in-human, randomised, double-blind, placebo-controlled study." In: *The Lancet. Infectious diseases* 20.5, pp. 585–597.

Smith, J. D., A. G. Craig, N. Kriek, D. Hudson-Taylor, S. Kyes, T. Fagan, T. Fagen, R. Pinches, D. I. Baruch, C. I. Newbold, and L. H. Miller (Feb. 2000a). "Identification of a Plasmodium falciparum intercellular adhesion molecule-1 binding domain: a parasite adhesion trait implicated in cerebral malaria." In: *Proceedings of the National Academy of Sciences of the United States of America* 97.4, pp. 1766–71.

Smith, J. D., J. A. Rowe, M. K. Higgins, and T. Lavstsen (Dec. 2013). "Malaria's deadly grip: cytoadhesion of Plasmodium falciparum-infected erythrocytes". In: *Cellular Microbiology* 15.12, pp. 1976–1983.

Smith, J. D., G. Subramanian, B. Gamain, D. I. Baruch, and L. H. Miller (2000b). "Classification of adhesive domains in the Plasmodium falciparum Erythrocyte Membrane Protein 1 family". In: *Molecular and Biochemical Parasitology* 110.2, pp. 293–310.

Spiegel, H., A. Boes, R. Kastilan, S. Kapelski, G. Edgue, V. Beiss, I. Chubodova, M. Scheuermayer, G. Pradel, S. Schillberg, et al. (2015). "The stage-specific in vitro efficacy of a malaria antigen cocktail provides valuable insights into the development of effective multistage vaccines". In: *Biotechnology Journal* 10.10, pp. 1651–1659.

Staalsoe, T., C. E. Shulman, J. N. Bulmer, K. Kawuondo, K. Marsh, and L. Hviid (Jan. 2004). "Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated Plasmodium falciparum malaria". In: *The Lancet* 363.9405, pp. 283–289.

Stanisic, D. I., J. Cutts, E. Eriksson, F. J. Fowkes, A. Rosanas-Urgell, P. Siba, M. Laman, T. M. Davis, L. Manning, I. Mueller, et al. (2014). "$\gamma\delta$ T cells and CD14+ monocytes are predominant cellular sources of cytokines and chemokines associated with severe malaria". In: *The Journal of infectious diseases* 210.2, pp. 295–305.

Stanisic, D. I., F. J. I. Fowkes, M. Koinari, S. Javati, E. Lin, B. Kiniboro, J. S. Richards, L. J. Robinson, L. Schofield, J. W. Kazura, C. L. King, P. Zimmerman, I. Felger, P. M. Siba, I. Mueller, and J. G. Beeson (Feb. 2015). "Acquisition of antibodies against Plasmodium falciparum merozoites and malaria immunity in young children and the influence of age, force of infection, and magnitude of response." In: *Infection and immunity* 83.2, pp. 646–660.

Stanisic, D. I., J. Gerrard, J. Fink, P. M. Griffin, X. Q. Liu, L. Sundac, S. Sekuloski, I. B. Rodriguez, J. Pingnet, Y. Yang, Y. Zhou, K. R. Trenholme, C. Y. T. Wang, H. Hackett, J.-A. A. Chan, C. Langer, E. Hanssen, S. L. Hoffman, J. G. Beeson, J. S. McCarthy, and M. F. Good (Aug. 2016). "Infectivity of Plasmodium falciparum in Malaria-Naive Individuals Is Related to Knob Expression and Cytoadherence of the Parasite". In: *Infection and immunity* 84.9, pp. 2689–2696.

Stanisic, D. I., J. S. Richards, F. J. McCallum, P. Michon, C. L. King, S. Schoepflin, P. R. Gilson, V. J. Murphy, R. F. Anders, I. Mueller, and J. G. Beeson (Mar. 2009). "Immunoglobulin G subclass-specific responses against Plasmodium falciparum merozoite antigens are associated with control of parasitemia and protection from symptomatic illness." In: *Infection and immunity* 77.3, pp. 1165–1174.

Stanley, H. A., J. T. Mayes, N. R. Cooper, and R. T. Reese (Feb. 1984). “Complement activation by the surface of Plasmodium falciparum infected erythrocytes.” In: *Molecular immunology* 21.2, pp. 145–150.

Stapleton, N. M., J. T. Andersen, A. M. Stemerding, S. P. Bjarnarson, R. C. Verheul, J. Gerritsen, Y. Zhao, M. Kleijer, I. Sandlie, M. De Haas, et al. (2011). “Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential”. In: *Nature communications* 2.1, p. 599.

Steel, R. W. J., B. K. Sack, M. Tsuji, M. J. L. Navarro, W. Betz, M. E. Fishbaugher, E. L. Flannery, and S. H. I. Kappe (Feb. 2017). “An Opsonic Phagocytosis Assay for Plasmodium falciparum Sporozoites.” In: *Clinical and vaccine immunology : CVI* 24.2.

Stevens, T. L., A. Bossie, V. M. Sanders, R. Fernandez-Botran, R. L. Coffman, T. R. Mosmann, and E. S. Vitetta (1988). “Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells”. In: *Nature* 334, pp. 255–258.

Stevenson, L., P. Huda, A. Jeppesen, E. Laursen, J. A. Rowe, A. Craig, W. Streicher, L. Barfod, and L. Hviid (June 2015a). “Investigating the function of Fc-specific binding of IgM to Plasmodium falciparum erythrocyte membrane protein 1 mediating erythrocyte rosetting.” In: *Cellular microbiology* 17.6, pp. 819–831.

Stevenson, L., E. Laursen, G. J. Cowan, B. Bandoh, L. Barfod, D. R. Cavanagh, G. R. Andersen, and L. Hviid (July 2015b). “alpha-2-Macroglobulin Can Crosslink Multiple Plasmodium falciparum Erythrocyte Membrane Protein 1 (PfEMP1) Molecules and May Facilitate Adhesion of Parasitized Erythrocytes.” In: *PLoS pathogens* 11.7, e1005022.

Stöckl, J., O. Majdic, W. F. Pickl, A. Rosenkranz, E. Prager, E. Gschwantler, and W. Knapp (May 1995). “Granulocyte activation via a binding site near the C-terminal region of complement receptor type 3 alpha-chain (CD11b) potentially involved in intramembrane complex formation with glycosylphosphatidylinositol-anchored Fc gamma RIIIB (CD16) molecules.” In: *Journal of immunology (Baltimore, Md. : 1950)* 154.10, pp. 5452–5463.

Stone, W. J. R., J. J. Campo, A. L. Ouédraogo, L. Meerstein-Kessel, I. Morlais, D. Da, A. Cohuet, S. Nsango, C. J. Sutherland, M. van de Vegte-Bolmer, R. Siebelink-Stoter, G.-J. van Gemert, W. Graumans, K. Lanke, A. D. Shandling, J. V. Pablo, A. A. Teng, S. Jones, R. M. de Jong, A. Fabra-García, J. Bradley, W. Roeffen, E. Lasonder, G. Gremo, E. Schwarzer, C. J. Janse, S. K. Singh, M. Theisen, P. Felgner, M. Marti, C. Drakeley, R. Sauerwein, T. Bousema, and M. M. Jore (2018). “Unravelling the immune signature of Plasmodium falciparum transmission-reducing immunity”. In: *Nature Communications* 9.1, p. 558.

Storm, J., J. S. Jespersen, K. B. Seydel, T. Szestak, M. Mbewe, N. V. Chisala, P. Phula, C. W. Wang, T. E. Taylor, C. A. Moxon, T. Lavstsen, and A. G. Craig (2019). “Cerebral malaria is associated with differential cytoadherence to brain endothelial cells”. In: *EMBO Molecular Medicine* 11.2, pp. 1–15.

Suau, R., M. Vidal, R. Aguilar, G. Ruiz-Olalla, M. Vázquez-Santiago, C. Jairoce, A. J. Nhabomba, B. Gyan, D. Dosoo, K. P. Asante, S. Owusu-Agyei, J. J. Campo, L. Izquierdo, D. Cavanagh, R. L. Coppel, V. Chauhan, E. Angov, S. Dutta, D. Gaur, J. G. Beeson, G. Moncunill, and C. Dobaño (2021). “RTS,S/AS01E malaria vaccine induces IgA responses against CSP and vaccine-unrelated antigens in African children in the phase 3 trial”. In: *Vaccine* 39.4, pp. 687–698.

Subedi, G. P. and A. W. Barb (2016). “The immunoglobulin G1 N-glycan composition affects binding to each low affinity Fc gamma receptor.” In: *mAbs* 8.8, pp. 1512–1524.

Suscovich, T. J., J. K. Fallon, J. Das, A. R. Demas, J. Crain, C. H. Linde, A. Michell, H. Natarajan, C. Arevalo, T. Broge, T. Linnekin, V. Kulkarni, R. Lu, M. D. Slein, C. Luedemann, M. Marquette, S. March, J. Weiner, S. Gregory, M. Coccia, Y. Flores-Garcia, F. Zavala, M. E. Ackerman, E. Bergmann-Leitner, J. Hendriks, J. Sadoff, S. Dutta, S. N. Bhatia, D. A. Lauffenburger, E. Jongert, U. Wille-Reece, and G. Alter (July 2020). "Mapping functional humoral correlates of protection against malaria challenge following RTS,S/AS01 vaccination." In: *Science translational medicine* 12.553.

Swanson, P. A. 2., G. T. Hart, M. V. Russo, D. Nayak, T. Yazew, M. Peña, S. M. Khan, C. J. Janse, S. K. Pierce, and D. B. McGavern (Dec. 2016). "CD8+ T Cells Induce Fatal Brainstem Pathology during Cerebral Malaria via Luminal Antigen-Specific Engagement of Brain Vasculature." In: *PLoS pathogens* 12.12, e1006022.

Tatem, A. J., P. W. Gething, D. L. Smith, and S. I. Hay (2013). "Urbanization and the global malaria recession". In: *Malaria Journal* 12.1, p. 133.

Taylor, T. E., W. J. Fu, R. A. Carr, R. O. Whitten, J. S. Mueller, N. G. Fosiko, S. Lewallen, N. G. Liomba, and M. E. Molyneux (Feb. 2004). "Differentiating the pathologies of cerebral malaria by postmortem parasite counts." In: *Nature medicine* 10.2, pp. 143–145.

Tebo, A. E., P. G. Kremsner, and A. J. F. Luty (2002). "Fcgamma receptor-mediated phagocytosis of Plasmodium falciparum-infected erythrocytes in vitro". In: *Clinical and Experimental Immunology* 130.2, pp. 300–306.

Teo, A., W. Hasang, P. Boeuf, and S. Rogerson (2015). "A Robust Phagocytosis Assay to Evaluate the Opsonic Activity of Antibodies against Plasmodium falciparum-Infected Erythrocytes BT - Malaria Vaccines: Methods and Protocols". In: ed. by A. Vaughan, pp. 145–152.

Teo, A., W. Hasang, L. M. Randall, G. Feng, L. Bell, H. Unger, C. Langer, J. G. Beeson, P. M. Siba, I. Mueller, M. E. Molyneux, G. V. Brown, and S. J. Rogerson (2014). "Decreasing malaria prevalence and its potential consequences for immunity in pregnant women". In: *Journal of Infectious Diseases* 210.9, pp. 1444–1455.

Tessema, S. K., R. Nakajima, A. Jasinskas, S. L. Monk, L. Lekieffre, E. Lin, B. Kiniboro, C. Proietti, P. Siba, P. L. Felgner, D. L. Doolan, I. Mueller, and A. E. Barry (Nov. 2019). "Protective Immunity against Severe Malaria in Children Is Associated with a Limited Repertoire of Antibodies to Conserved PfEMP1 Variants". In: *Cell Host and Microbe* 26.5, 579–590.e5.

Tessema, S. K., D. Utama, O. Chesnokov, A. N. Hodder, C. S. Lin, G. L. A. Harrison, J. S. Jespersen, B. Petersen, L. Tavul, P. Siba, D. Kwiatkowski, T. Lavstsen, D. S. Hansen, A. V. Oleinikov, I. Mueller, and A. E. Barry (2018). " Antibodies to Intercellular Adhesion Molecule 1-Binding Plasmodium falciparum Erythrocyte Membrane Protein 1-DBLβ Are Biomarkers of Protective Immunity to Malaria in a Cohort of Young Children from Papua New Guinea ". In: *Infection and Immunity* 86.8, pp. 1–14.

Thawani, N., M. Tam, M.-J. Bellemare, D. S. Bohle, M. Olivier, J. B. de Souza, and M. M. Stevenson (Jan. 2014). "Plasmodium products contribute to severe malarial anemia by inhibiting erythropoietin-induced proliferation of erythroid precursors." In: *The Journal of infectious diseases* 209.1, pp. 140–149.

Tibúrcio, M., M. W. Dixon, O. Looker, S. Y. Younis, L. Tilley, and P. Alano (2015). "Specific expression and export of the Plasmodium falciparum Gametocyte EXported Protein-5 marks the gametocyte ring stage". In: *Malaria journal* 14, pp. 1–12.

Tibúrcio, M., F. Silvestrini, L. Bertuccini, A. F. Sander, L. Turner, T. Lavstsen, and P. Alano (Apr. 2013). "Early gametocytes of the malaria parasite Plasmodium falciparum specifically remodel the adhesive properties of infected erythrocyte surface." In: *Cellular microbiology* 15.4, pp. 647–659.

Tongren, J. E., C. J. Drakeley, S. L. R. McDonald, H. G. Reyburn, A. Manjurano, W. M. M. Nkya, M. M. Lemnge, C. D. Gowda, J. E. Todd, P. H. Corran, and E. M. Riley (Jan. 2006). "Target antigen, age, and duration of antigen exposure independently regulate immunoglobulin G subclass switching in malaria." In: *Infection and immunity* 74.1, pp. 257–264.

Tonkin-Hill, G. Q., L. Trianty, R. Noviyanti, H. H. Nguyen, B. F. Sebayang, D. A. Lampah, J. Marfurt, S. A. Cobbold, J. S. Rambhatla, M. J. McConville, S. J. Rogerson, G. V. Brown, K. P. Day, R. N. Price, N. M. Anstey, A. T. Papenfuss, and M. F. Duffy (2018). "The Plasmodium falciparum transcriptome in severe malaria reveals altered expression of genes involved in important processes including surface antigen–encoding var genes". In: *PLoS Biology* 16.3, e2004328.

Tonwong, N., J. Sattabongkot, T. Tsuboi, H. Iriko, S. Takeo, J. Sirichaisinthop, and R. Udomsangpetch (2012). "Natural infection of Plasmodium falciparum induces inhibitory antibodies against gametocyte development in human hosts". In: *Japanese journal of infectious diseases* 65.2, pp. 152–156.

Tornyigah, B., T. D'almeida, G. Escriou, F. Viwami, N. Fievet, A. J. Luty, A. Massougbodji, M. A. Nielsen, P. Deloron, and N. Tuikue Ndam (2021). "Plasmodium falciparum VAR2CSA-specific IgG subclass responses reflect protection against low birth weight and pregnancy-associated malaria". In: *Frontiers in Immunology* 12, p. 610305.

Torres, M. and A. Casadevall (2008). "The immunoglobulin constant region contributes to affinity and specificity". In: *Trends in Immunology* 29.2, pp. 91–97.

Travassos, M. A., A. Niangaly, J. A. Bailey, A. Ouattara, D. Coulibaly, K. E. Lyke, M. B. Laurens, J. Pablo, A. Jasinskas, R. Nakajima, A. A. Berry, M. Adams, C. G. Jacob, A. Pike, S. Takala-Harrison, L. Liang, B. Kouriba, A. K. Kone, J. A. Rowe, J. Moulds, D. A. Diallo, O. K. Doumbo, M. A. Thera, P. L. Felgner, and C. V. Plowe (2018). "Children with cerebral malaria or severe malarial anaemia lack immunity to distinct variant surface antigen subsets". In: *Scientific Reports* 8, p. 6281.

Treutiger, C. J., A. Heddini, V. Fernandez, W. A. Muller, and M. Wahlgren (Dec. 1997). "PECAM-1/CD31, an endothelial receptor for binding Plasmodium falciparum-infected erythrocytes." In: *Nature medicine* 3.12, pp. 1405–8.

Trubowitz, S. and B. Masek (1968). "Plasmodium falciparum: phagocytosis by polymorphonuclear leukocytes". In: *Science* 162.3850, pp. 273–274.

Tsuboi, T., S. Takeo, T. U. Arumugam, H. Otsuki, and M. Torii (2010). "The wheat germ cell-free protein synthesis system: a key tool for novel malaria vaccine candidate discovery". In: *Acta tropica* 114.3, pp. 171–176.

Tuikue Ndam, N., A. Moussiliou, T. Lavstsen, C. Kamaliddin, A. T. Jensen, A. Mama, R. Tahar, C. W. Wang, J. S. Jespersen, J. M. Alao, B. Gamain, T. G. Theander, and P. Deloron (2017). "Parasites Causing Cerebral Falciparum Malaria Bind Multiple Endothelial Receptors and Express EPCR and ICAM-1-Binding PfEMP1". In: *Journal of Infectious Diseases* 215.12, pp. 1918–1925.

Tuikue Ndam, N. G., A. Salanti, G. Bertin, M. Dahlbäck, N. Fievet, L. Turner, A. Gaye, T. Theander, and P. Deloron (2005). "High level of var2csa transcription by Plasmodium falciparum isolated from the placenta". In: *The Journal of infectious diseases* 192.2, pp. 331–335.

Turner, G. D. H., H. Morrison, M. Jones, T. M. E. Davis, S. Looareesuwan, I. D. Buley, K. C. Gatter, C. I. Newbold, S. Pukritayakamee, B. Nagachinta, N. J. White, and A. R. Berendt (1994). "Immunohistochemical Study of the Pathology of Fatal Malaria". In: *American Journal of Pathology* 145.5, pp. 1057–1069.

Turner, L., T. Lavstsen, S. S. Berger, C. W. Wang, J. E. Petersen, M. Avril, A. J. Brazier, J. Freeth, J. S. Jespersen, M. A. Nielsen, P. Magistrado, J. Lusingu, J. D. Smith, M. K. Higgins, and T. G. Theander (2013). "Severe malaria is associated with parasite binding to endothelial protein C receptor". In: *Nature* 498.7455, pp. 502–5. arXiv: `NIHMS150003`.

Turner, L., T. Lavstsen, B. P. Mmbando, C. W. Wang, P. A. Magistrado, L. S. Vestergaard, D. S. Ishengoma, D. T. R. Minja, J. P. Lusingu, and T. G. Theander (Aug. 2015). "IgG Antibodies to Endothelial Protein C Receptor-Binding Cysteine-Rich Interdomain Region Domains of Plasmodium falciparum Erythrocyte Membrane Protein 1 Are Acquired Early in Life in Individuals Exposed to Malaria". In: *Infection and Immunity* 83.8. Ed. by J. H. Adams, pp. 3096–3103.

Turner, L., T. G. Theander, and T. Lavstsen (2018). "Immunization with Recombinant Plasmodium falciparum Erythrocyte Membrane Protein 1 CIDR1 Domains Induces Domain Subtype Inhibitory Antibodies". In: *Infection and Immunity* 86.11, pp. 1–11.

Umbers, A. J., E. H. Aitken, and S. J. Rogerson (Apr. 2011). "Malaria in pregnancy: small babies, big problem." In: *Trends in parasitology* 27.4, pp. 168–75.

Uwimana, A., E. Legrand, B. H. Stokes, J.-L. M. Ndikumana, M. Warsame, N. Umulisa, D. Ngamije, T. Munyaneza, J.-B. Mazarati, K. Munguti, P. Campagne, A. Criscuolo, F. Ariey, M. Murindahabi, P. Ringwald, D. A. Fidock, A. Mbituyumuremyi, and D. Menard (Oct. 2020). "Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda." In: *Nature medicine* 26.10, pp. 1602–1608.

Vidarsson, G., G. Dekkers, and T. Rispens (2014). "IgG subclasses and allotypes: From structure to effector functions". In: *Frontiers in Immunology* 5.OCT.

Vigan-Womas, I., A. Lokossou, M. Guillotte, A. Juillerat, G. Bentley, A. Garcia, O. Mercereau-Puijalon, and F. Migot-Nabias (Oct. 2010). "The humoral response to Plasmodium falciparum VarO rosetting variant and its association with protection against malaria in Beninese children." In: *Malaria journal* 9, p. 267.

Wahlgren, M., K. Berzins, P. Perlmann, and M. Persson (Oct. 1983). "Characterization of the humoral immune response in Plasmodium falciparum malaria. II. IgG subclass levels of anti-P. falciparum antibodies in different sera." In: *Clinical and experimental immunology* 54.1, pp. 135–142.

Walker, I. S., A. W. Chung, T. Damelang, and S. J. Rogerson (2022a). "Analysis of Antibody Reactivity to Malaria Antigens by Microsphere-Based Multiplex Immunoassay." In: *Methods in molecular biology* 2470, pp. 309–325.

Walker, I. S. and S. J. Rogerson (Nov. 2022b). "Pathogenicity and virulence of malaria: sticky problems and tricky solutions." In: *Virulence*.

Walker, M. R., A. S. Knudsen, F. D. Partey, M. R. Bassi, A. M. Frank, F. C. Castberg, E. W. Sarbah, M. F. Ofori, L. Hviid, and L. Barfod (Dec. 2020). "Acquisition and decay of IgM and IgG responses to merozoite antigens after Plasmodium falciparum malaria in Ghanaian children". In: *PLOS ONE* 15.12, e0243943.

Wang, C. W. and L. Hviid (July 2015). "Rifins, rosetting, and red blood cells." In: *Trends in parasitology* 31.7, pp. 285–286.

Warimwe, G. M., T. M. Keane, G. Fegan, J. N. Musyoki, C. R. J. C. Newton, A. Pain, M. Berriman, K. Marsh, and P. C. Bull (2009). "Plasmodium falciparum var gene expression is modified by host immunity". In: *Proceedings of the National Academy of Sciences* 106.51, pp. 21801–6.

Warncke, J. D. and H.-P. Beck (Sept. 2019). "Host Cytoskeleton Remodeling throughout the Blood Stages of Plasmodium falciparum". In: *Microbiology and molecular biology reviews : MMBR* 83.4, e00013–19.

Weaver, R., L. Reiling, G. Feng, D. R. Drew, I. Mueller, P. M. Siba, T. Tsuboi, J. S. Richards, F. J. I. Fowkes, and J. G. Beeson (Sept. 2016). "The association between naturally acquired IgG subclass specific antibodies to the PfRH5 invasion complex and protection from Plasmodium falciparum malaria". In: *Scientific reports* 6, p. 33094.

Webster, R., S. Sekuloski, A. Odedra, S. Woolley, H. Jennings, F. Amante, K. R. Trenholme, J. Healer, A. F. Cowman, E. M. Eriksson, P. Sathe, J. Penington, A. J. Blanch, M. W. A. Dixon, L. Tilley, M. F. Duffy, A. Craig, J. Storm, J.-A. Chan, K. Evans, A. T. Papenfuss, L. Schofield, P. Griffin, B. E. Barber, D. Andrew, M. J. Boyle, F. de Labastida Rivera, C. Engwerda, and J. S. McCarthy (Nov. 2021). "Safety, infectivity and immunogenicity of a genetically attenuated blood-stage malaria vaccine." In: *BMC medicine* 19.1, p. 293.

Weil, G. J., W. Kastens, M. Susapu, S. J. Laney, S. A. Williams, C. L. King, J. W. Kazura, and M. J. Bockarie (2008). "The impact of repeated rounds of mass drug administration with diethylcarbamazine plus albendazole on bancroftian filariasis in Papua New Guinea." In: *PLoS neglected tropical diseases* 2.12, e344.

Weiss, D. J., T. C. D. Lucas, M. Nguyen, A. K. Nandi, D. Bisanzio, K. E. Battle, E. Cameron, K. A. Twohig, D. A. Pfeffer, J. A. Rozier, H. S. Gibson, P. C. Rao, D. Casey, A. Bertozzi-Villa, E. L. Collins, U. Dalrymple, N. Gray, J. R. Harris, R. E. Howes, S. Y. Kang, S. H. Keddie, D. May, S. Rumisha, M. P. Thorn, R. Barber, N. Fullman, C. K. Huynh, X. Kulikoff, M. J. Kutz, A. D. Lopez, A. H. Mokdad, M. Naghavi, G. Nguyen, K. A. Shackelford, T. Vos, H. Wang, D. L. Smith, S. S. Lim, C. J. L. Murray, S. Bhatt, S. I. Hay, and P. W. Gething (July 2019). "Mapping the global prevalence, incidence, and mortality of Plasmodium falciparum, 2000–17: a spatial and temporal modelling study". In: *The Lancet* 394.10195, pp. 322–331.

Wenisch, C., S. Spitzauer, K. Florris-Linau, H. Rumpold, S. Vannaphan, B. Parschalk, W. Graninger, and S. Looareesuwan (Nov. 1997). "Complement activation in severe Plasmodium falciparum malaria." In: *Clinical immunology and immunopathology* 85.2, pp. 166–171.

White, M. T., J. T. Griffin, O. Akpogheneta, D. J. Conway, K. A. Koram, E. M. Riley, and A. C. Ghani (Oct. 2014). "Dynamics of the antibody response to Plasmodium falciparum infection in African children." In: *The Journal of infectious diseases* 210.7, pp. 1115–1122.

White, N. J. (2018). "Anaemia and malaria". In: *Malaria Journal* 17.1, pp. 1–17.

Wiesner, J., H. Jomaa, M. Wilhelm, H. P. Tony, P. G. Kremsner, P. Horrocks, and M. Lanzer (Oct. 1997). "Host cell factor CD59 restricts complement lysis of Plasmodium falciparum-infected erythrocytes." In: *European journal of immunology* 27.10, pp. 2708–2713.

Willcocks, L. C., E. J. Carr, H. A. Niederer, T. F. Rayner, T. N. Williams, W. Yang, J. A. G. Scott, B. C. Urban, N. Peshu, T. J. Vyse, et al. (2010). "A defunctioning polymorphism in FCGR2B is associated with protection against malaria but susceptibility to systemic lupus erythematosus". In: *Proceedings of the National Academy of Sciences* 107.17, pp. 7881–7885.

Wines, B. D., H. A. Vanderven, S. E. Esparon, A. B. Kristensen, S. J. Kent, and P. M. Hogarth (2016). “Dimeric FcgammaR Ectodomains as Probes of the Fc Receptor Function of Anti-Influenza Virus IgG.” In: *Journal of immunology (Baltimore, Md. : 1950)* 197.4, pp. 1507–16.

Winograd, E., J. G. Prudhomme, and I. W. Sherman (2005). “Band 3 clustering promotes the exposure of neoantigens in Plasmodium falciparum-infected erythrocytes”. In: *Molecular and biochemical parasitology* 142.1, pp. 98–105.

World Health Organization (Sept. 2014a). “Severe malaria.” In: *Tropical medicine international health : TM IH* 19 Suppl 1, pp. 7–131.

— (2014b). “WHO Severe malaria 2014”. In: *Tropical Medicine and International Health* 19 (Suppl. Pp. 7–131.

— (2020). “World Malaria Report: 20 years of global progress and challenges”. In: WHO/HTM/GM.December, p. 299.

— (2021a). “WHO Guidelines for Malaria”. In.

— (2021b). “WHO recommends groundbreaking malaria vaccine for children at risk”. In.

— (2021c). “Word Malaria Report 2021”. In.

— (2022). “Malaria vaccine: WHO position paper – March 2022”. In: *Weekly epidemiological record* 9, p. 76.

Worley, M. J., K. Fei, A. J. Lopez-Denman, A. D. Kelleher, S. J. Kent, and A. W. Chung (2018). “Neutrophils mediate HIV-specific antibody-dependent phagocytosis and ADCC”. In: *Journal of Immunological Methods* 457, pp. 41–52.

Wu, Y., R. D. Ellis, D. Shaffer, E. Fontes, E. M. Malkin, S. Mahanty, M. P. Fay, D. Narum, K. Rausch, A. P. Miles, et al. (2008). “Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51”. In: *PloS one* 3.7, e2636.

Al-Yaman, F., B. Genton, D. Mokela, A. Raiko, S. Kati, S. Rogerson, J. Reeder, and M. Alpers (1995). “Human cerebral malaria: lack of significant association between erythrocyte rosetting and disease severity.” In: *Transactions of the Royal Society of Tropical Medicine and Hygiene* 89.1, pp. 55–58.

Yeap, W. H., K. L. Wong, N. Shimasaki, E. C. Y. Teo, J. K. S. Quek, H. X. Yong, C. P. Diong, A. Bertoletti, Y. C. Linn, and S. C. Wong (Sept. 2016). “CD16 is indispensable for antibodydependent cellular cytotoxicity by human monocytes”. In: *Scientific Reports* 6.

Yenchitsomanus, P., K. M. Summers, P. G. Board, K. K. Bhatia, G. L. Jones, K. Johnston, and G. T. Nurse (Dec. 1986). “Alpha-thalassemia in Papua New Guinea.” In: *Human genetics* 74.4, pp. 432–437.

Yenchitsomanus, P. T., K. M. Summers, K. K. Bhatia, J. Cattani, and P. G. Board (July 1985). “Extremely high frequencies of alpha-globin gene deletion in Madang and on Kar Kar Island, Papua New Guinea.” In: *American journal of human genetics* 37.4, pp. 778–784.

Yman, V., J. Tuju, M. T. White, G. Kamuyu, K. Mwai, N. Kibinge, M. Asghar, C. Sundling, K. Sondén, L. Murungi, D. Kiboi, R. Kimathi, T. Chege, E. Chepsat, P. Kiyuka, L. Nyamako, F. H. A. Osier, and A. Färnert (Jan. 2022). “Distinct kinetics of antibodies to 111 Plasmodium falciparum proteins identifies markers of recent malaria exposure.” In: *Nature communications* 13.1, p. 331.

Yone, C. L. R. P., P. G. Kremsner, and A. J. F. Luty (Apr. 2005). “Immunoglobulin G isotype responses to erythrocyte surface-expressed variant antigens of Plasmodium falciparum predict protection from malaria in African children”. In: *Infection and immunity* 73.4, pp. 2281–2287.

Zelter, T., J. Strahilevitz, K. Simantov, O. Yajuk, Y. Adams, A. Ramstedt Jensen, R. Dzikowski, and Z. Granot (Apr. 2022). "Neutrophils impose strong immune pressure against PfEMP1 variants implicated in cerebral malaria." In: *EMBO reports*, e53641.

Zhao, J., L. Ma, S. Chen, Y. Xie, L. Xie, Y. Deng, Y. He, T. Li, J. Wang, S. Li, and X. Qin (Apr. 2014). "Association between Fc-gamma receptor IIa (CD32) gene polymorphism and malaria susceptibility: a meta-analysis based on 6928 subjects." In: *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases* 23, pp. 169–75.

Zhou, J., G. Feng, J. Beeson, P. M. Hogarth, S. J. Rogerson, Y. Yan, and A. Jaworowski (2015). "CD14hiCD16+ monocytes phagocytose antibody-opsonised Plasmodium falciparum infected erythrocytes more efficiently than other monocyte subsets, and require CD16 and complement to do so". In: *BMC Medicine* 13.1, p. 154.

Zhou, J., L. E. Ludlow, W. Hasang, S. J. Rogerson, and A. Jaworowski (2012). "Opsonization of malaria-infected erythrocytes activates the inflammasome and enhances inflammatory cytokine secretion by human macrophages". In: *Malaria Journal* 11.1, p. 1.

Zou, H. and T. Hastie (Apr. 2005). "Regularization and variable selection via the elastic net". In: *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* 67.2, pp. 301–320.