Summary

Members of the LSHTM Malaria Centre conduct fundamental research into the cellular and molecular basis of immunity to malaria and the contribution of immune responses to malarial pathology as well as translational research on vaccines and on the use of serology for mapping malaria transmission. Work encompasses epidemiological studies, *ex vivo* and *in vitro* studies of human cells and work in experimental model systems.

Here, we summarise recent studies designed to understand the complex immunomodulatory consequences of infections, such as the role of T cell regulatory receptors in inhibiting T cell effector function and ameliorating severe malarial pathology. A particular highlight is the demonstration that the heme-oxygenase pathway of heme detoxification (which is essential for preventing oxidative tissue damage during malaria infections) acts to impair the normal maturation of bone marrow neutrophils. This neutrophil dysfunction underlies the increased susceptibility of malaria-infected hosts to gram-negative (and particularly non-typhoid Salmonella) bacteraemia and provides the first mechanistic explanation for the well described clinical association between severe malarial anaemia (and other forms of haemolytic anaemia) and bacteraemia.

Other studies include recent epidemiological studies in Africa to investigate the capacity of naturally acquired antibodies to neutralise the inflammatory activity of malarial "toxins", to characterise the dynamics of acquisition and retention of malaria specific memory B cells in an area of very low and seasonal malaria endemicity, and to better understand the relationship between asymptomatic infections and the maintenance and boosting of anti-malarial antibodies (with a particular emphasis on the longevity of antibody responses that reduce transmission of malaria between humans to mosquitoes). Results are also presented of a study in Thailand designed to estimate the longevity of malaria specific T cell-mediated immunity. This type of data is essential for improving our understanding of naturally acquired immunity and for setting benchmarks by which we can assess vaccine-induced immunity.

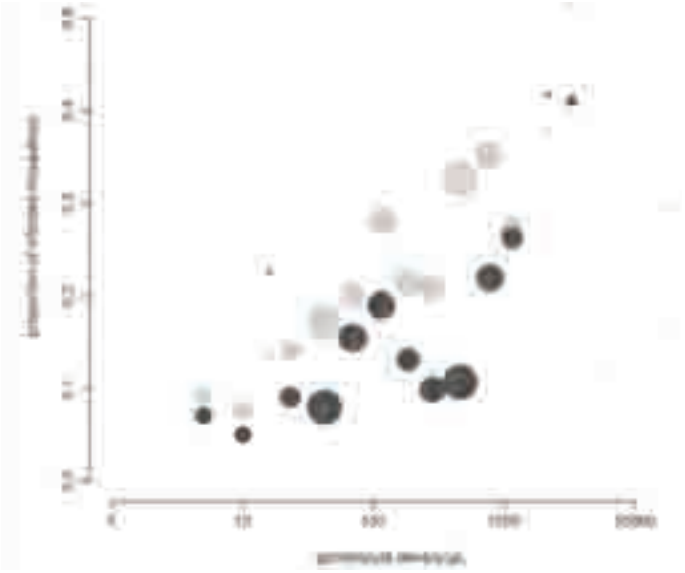
Finally, members continue to provide intellectual, technical and logistic support to the very large Phase 3 clinical trial of the RTS,S vaccine, the very first phase 3 trial of a malaria vaccine, and were delighted that the initial analysis of the trial outcomes confirmed the results of the numerous phase 2 trials, with the vaccine conferring approximately 50% protection against clinical malaria episodes. Importantly, our analysis of cellular immune responses induced by the vaccine identified several new and highly sensitive markers of antigen-specific T cell responses that may be useful in future studies to determine the magnitude and duration of these responses and assist in the search for immunological correlates of protection.

Human immune responses that reduce the transmission of Plasmodium falciparum in African populations.

***LSHTM Investigators**: Teun Bousema, Colin Sutherland, Eleanor Riley, Geoffrey Targett & Chris Drakeley.*

***External Investigators/Collaborators:** Thomas Churcher (Imperial College London, UK); Bert Mulder (Microbiology Laboratory Twente, The Netherlands); Louis Gouagna (L'Institut de recherche pour le développement, France).*

***Funding Body:** Wellcome Trust and European Union.*



The relationship between gametocyte density by microscopy and the proportion of infected mosquitoes. Light grey circles indicate the proportion of infected mosquitoes after feeding on blood samples with control serum; dark grey circles indicate autologous plasma. The sizes of the circles are proportional to the number of mosquitoes dissected for a given range of gametocyte densities.

Malaria-infected individuals can develop antibodies which reduce the infectiousness of *Plasmodium* gametocytes to biting *Anopheles* mosquitoes. When ingested in a bloodmeal together with gametocytes, these antibodies reduce or prevent subsequent parasite maturation in the insect host. This transmission blocking immunity is usually measured in human sera by testing its effect on the infectivity of gametocytes grown *in vitro*. Here we evaluate evidence of transmission-blocking immunity in eight studies conducted in three African countries. *Plasmodium falciparum* gametocytes isolated from each individual were fed to mosquitoes in both autologous plasma collected with the parasites, and permissive serum from non-exposed donors. Evidence of transmission reducing effects of autologous plasma was found in all countries. Experiments involving 116 Gambian children (aged 0.5–15 years) were combined to determine which factors were associated with transmission reducing immune responses. The chances of infecting at least one mosquito and the average proportion of infected mosquitoes were negatively associated with recent exposure to gametocytes and sampling late in the season. These results suggest that effective malaria transmission-reducing antibodies do not commonly circulate in African children, and that recent gametocyte carriage is required to initiate and/or boost such responses.

The dynamics of malaria-specific antibody responses in relation to microscopic and submicroscopic Plasmodium falciparum infections.

***LSHTM Investigators**: Carla Proietti, Federica Verra, Michael Bretscher, Patrick Corran, Eleanor Riley, Teun Bousema & Chris Drakeley.*

***External Investigators/Collaborators:** Andrea Crisanti (Imperial College London, UK); Raffaele Ronca & Bruno Arca (Federico II University, Italy); Bernard Kanoi, Betty Balikagala & Tomas Egwang (Medical Biotech Laboratories, Uganda).*

***Funding Body:** European Comission-FP7 Marie Curie Actions.*

Understanding of the dynamics of antibody responses against malaria infection, and in particular, the effect of immune boosting on the acquisition and maintenance of naturally acquired immunity, is essential for the interpretation of findings from cross-sectional and even longitudinal studies.

We examined the effect of microscopic and sub-microscopic malaria infections on malaria-specific antibody acquisition and explore the dynamics of antibody responses in relation to malaria infections in longitudinal cohorts from high endemic area of Apac, Uganda.

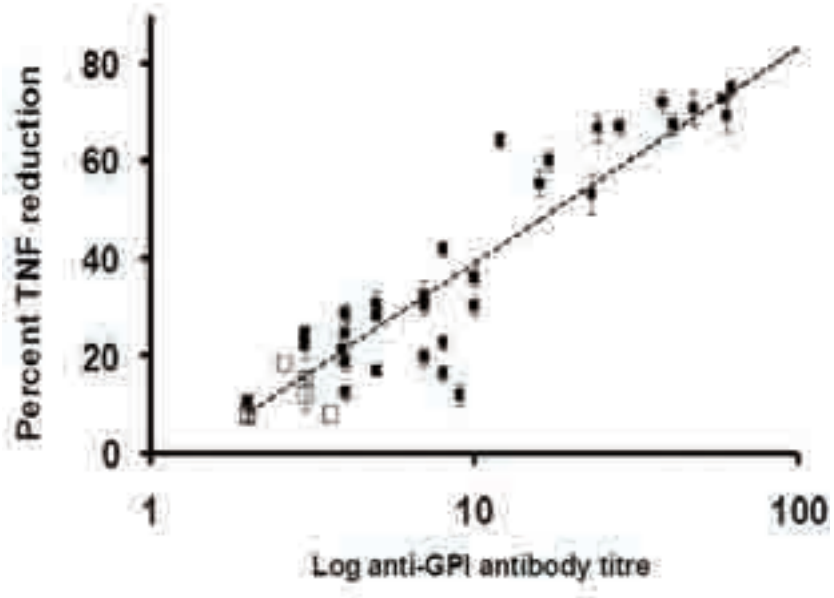
500 individuals were selected (250 <5years, 125 <10years, and 125 adults) and followed throughout the transmission season. At three time-points, plasma was collected and antibody responses against *Plasmodium falciparum* AMA1, MSP-1, MSP-2 and CSP and Anopheles-specific gSG6 were determined. Antibody responses against all antigens were associated with current submicroscopic infections with *Plasmodium falciparum*. Individuals with no parasites had the lowest antibody levels but no difference was seen in antibody levels between individuals with patent or submicroscopic infections. This suggest that in this endemic setting, low density infections are important for maintaining antibody levels.

Neutralization of malaria glycosylphosphatidylinositol in vitro by serum IgG from malaria exposed individuals.

***LSHTM Investigators**: Brian de Souza, Kevin Couper, Patrick Corran, Lucy Okell, Tom Doherty, Colin Sutherland & Eleanor Riley.*

***External Investigators/Collaborators:** Channe Gowda (Pennsylvania State University, USA); Geoff Pasvol (Imperial College London, UK).*

***Funding Body:** Wellcome Trust.*



IgG from malaria immune individuals neutralises GPI-induced TNF production. Neutralization of GPI-induced TNF release from macrophages after incubation with endotoxin-free IgG. Each point represents the mean percentage TNF reduction (±SD) of IgG samples from control (open symbols) and immune subjects (solid symbols) from at least three separate experiments. The horizontal dotted line shows the cut-off level for neutralizing activity; values below this cut-off are classified as "non-neutralizers". The regression line shows a significant ($p<0.001$) association between neutralization and anti-GPI antibody titre.

Parasite-derived glycosylphosphatidylinositol (GPI) is believed to be a major inducer of the pathways leading to pathology and morbidity during malaria infection and has been termed a malaria "toxin". The generation of neutralising anti-GPI ("anti-toxic") antibodies has, therefore, been hypothesised to be an important step in the acquisition of anti-disease immunity to malaria. However to date the GPI-neutralising capacity of antibodies induced during natural malaria infection has not been evaluated. We have developed an in vitro macrophage-based assay to assess the neutralising capacity of malarial GPI-specific IgG. We demonstrate that IgG from *Plasmodium falciparum*-exposed individuals can significantly inhibit GPI-induced activation of macrophages *in vitro*, as shown by reduced TNF production and attenuation of CD40 expression. The GPI neutralising capacity of individual IgG samples was directly correlated with anti-GPI antibody titre. IgG from malaria-exposed individuals also neutralised the macrophage activating effects of *Plasmodium falciparum* schizont extract (PfSE) but there was only a poor correlation between PfSE neutralising activity and anti-GPI antibody titre, suggesting that PfSE contains other macrophage activating moieties in addition to GPI. In conclusion, we have established an *in vitro* assay to test the "toxin" neutralising activity of anti-malarial antibodies and have shown that anti-GPI antibodies from malaria immune individuals are able to neutralise GPI- induced macrophage activation; however the clinical relevance of anti-GPI antibodies remains to be proven given that malarial schizonts contain other pro-inflammatory moieties in addition to GPI.

Dynamics of the breadth and magnitude of circulating memory B cell responses to Plasmodium falciparum in an area of low malaria transmission.

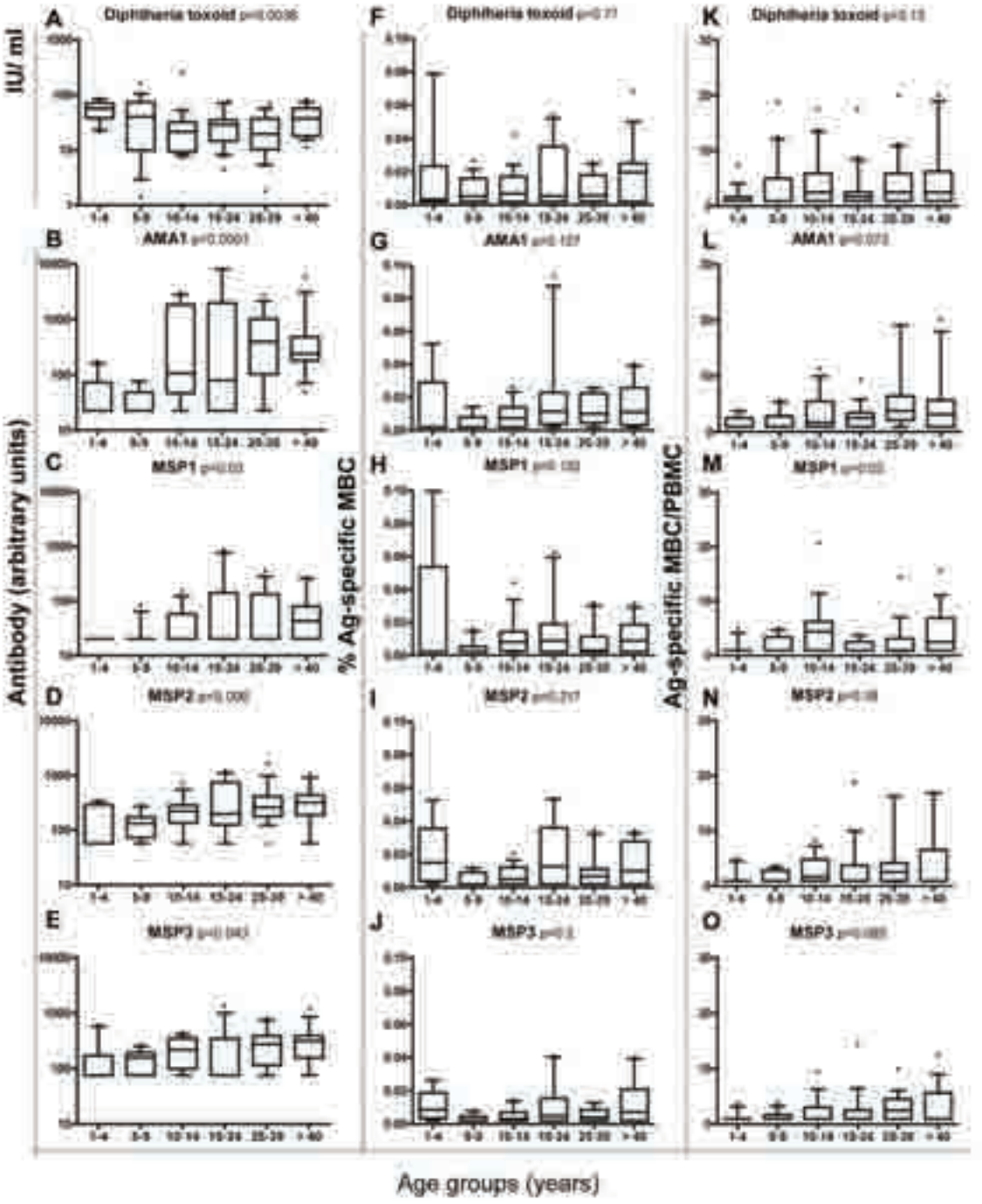
***LSHTM Investigators**: Sarah Nogaro, Eleanor Riley, Julius Hafalla, David Conway & Kevin Tetteh.*

***External Investigators/Collaborators:** Michael Walther & Brigitte Walther (Medical Research Council, The Gambia); Edmond Remarque (Biomedical Primate Research Centre, The Netherlands).*

***Funding Body:** Medical Research Council, UK.*

Immunity against symptoms of malaria requires repeated exposure, suggesting either that the parasite is poorly immunogenic or that the development of effective immune responses to malaria may be impaired. To begin to test these two hypotheses, we carried out two age-stratified cross-sectional surveys of anti-malarial humoral immune responses in a Gambian village where *Plasmodium falciparum* malaria transmission is low and sporadic. Circulating antibodies and memory B cells (MBC) to four malarial antigens were measured using ELISA and cultured B cell ELISpot. The proportion of individuals with malaria-specific MBC and antibodies, and the average number of antigens recognised by each individual, increased with age but the magnitude of these

responses did not. Malaria-specific antibody levels did not correlate with either the prevalence or median number of MBC, indicating that these two assays are measuring different aspects of the humoral immune response. Among those with immunological evidence of malaria exposure (defined as a positive response to at least one malarial antigen either by ELISA or ELISpot), the median number of malaria-specific MBC was similar to median numbers of diphtheria-specific MBC, suggesting that the circulating memory cell pool for malaria antigens is of similar size to that for other antigens.



The magnitude of the malaria antigen-specific MBC response does not increase with age. The magnitude of Ag-specific serum IgG (A–E) and of Ag-specific MBC responses [expressed as %MBC (F–J) or expressed as MBC/PBMC (K–O)] are shown for individuals who showed evidence of previous malaria exposure. Box plots indicate the 25th, 50st and 75th percentile, with whiskers representing the 10th and 90th percentiles. Outliers are denoted by a spot. P values are given for Kruskal-Wallis tests.

Duration of effector and memory T cell responses to malaria in an area of low malaria endemicity.

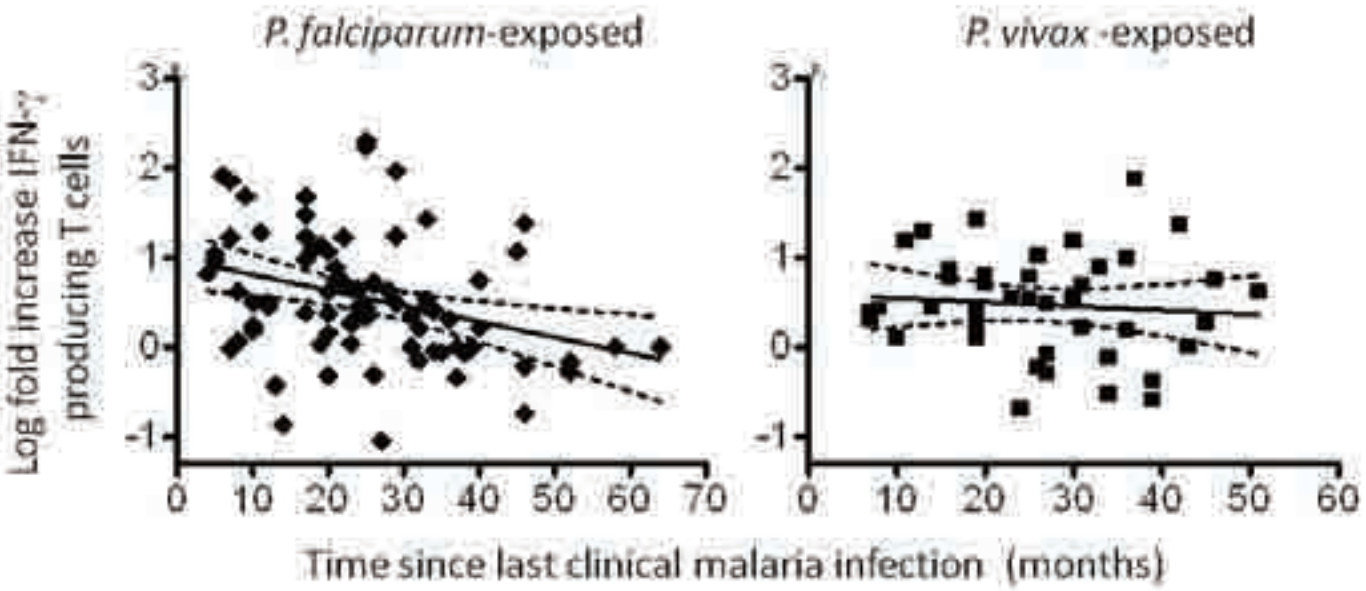
***LSHTM Investigators**: Eleanor Riley, Julius Hafalla & Lucy Okell.*

***External Investigators/Collaborators:** Jiraprapa Wipasa, Supachai Sakkhachornphop, Chaisuree Suphavilai, Kriangkrai Chawansuntati. (Research Institute for Health Sciences, Thailand); Witaya Liewsaree (Office Disease Prevention and Control, Thailand).*

***Funding Body:** Wellcome Trust.*

Immunity to malaria is widely believed to wane in the absence of reinfection, but direct evidence for the presence or absence of durable immunological memory to malaria is limited. To test this assumption, we have analysed malaria-specific CD4+ T cell responses of individuals living in an area of low malaria transmission in northern Thailand, who had had a documented clinical attack of *Plasmodium falciparum* and/or *Plasmodium vivax* in the past 6 years.

CD4+ T cell effector memory (CD45RO+) IFN-γ (24 hours *ex vivo* restimulation) and cultured IL-10 (6 day secretion into culture supernatant) responses to malaria schizont antigens were detected only in malaria-exposed subjects and were more prominent in subjects with long-lived antibodies or memory B cells specific to malaria antigens. The number of IFN-γ-producing effector memory T cells declined significantly over the 12 months of the study, and with time since last documented malaria infection, with an estimated half life of the response of 3.3 (95% CI 1.9-10.3) years. In sharp contrast, IL-10 responses were sustained for many years after last known malaria infection with no significant decline over at least 6 years. Our observations have clear implications for understanding the immunoepidemiology of naturally acquired malaria infections and for malaria vaccine development.



The magnitude of T cell IFN-γ responses declines with time since last infection for those infected with Plasmodium falciparum, but not those infected with Plasmodium vivax.

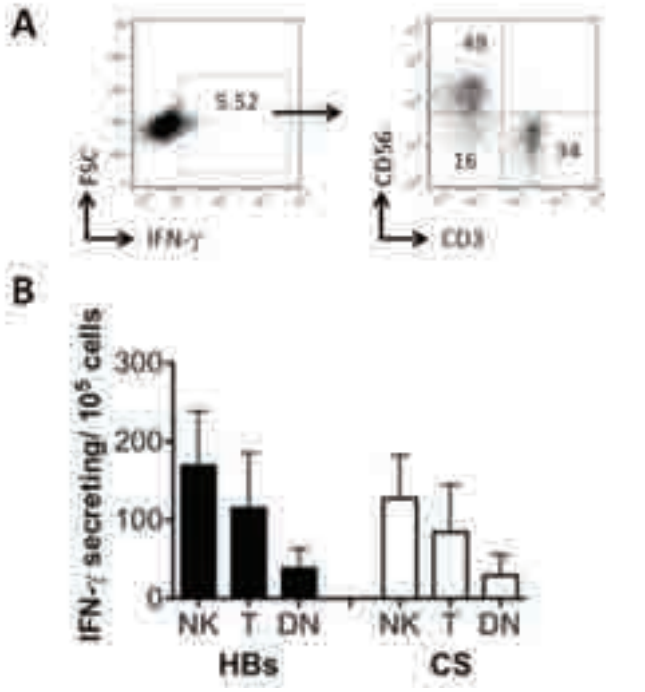
Cellular immune responses induced by immunization of Tanzanian children with the RTS,S/AS01 malaria vaccine.

***LSHTM Investigators**: Amir Horowitz, Julius Hafalla, Elizabeth King, Denise Dekker, Patrick Corran, Chris Drakeley, Lorenz von Seidlein & Eleanor Riley.*

***External Investigators/Collaborators:** John Lusingu (National Institute for Medical Research, Tanzania); Amanda Leach, Philippe Moris, Joe Cohen & Johan Vekemans, (GlaxoSmithKline Biologicals, Belgium); Tonya Villafana (PATH Malaria Vaccine Initiative, USA); Philip Bejon (Kenya Medical Research Institute/ Wellcome Trust Programme, Kenya).*

***Funding Body:** PATH Malaria Vaccine Initiative.*

RTS,S/AS01, a vaccine targeting pre-erythrocytic stages of *Plasmodium falciparum*, is undergoing clinical trials. We conducted an analysis of cellular immune response to component antigens of RTS,S – hepatitis B surface antigen (HBs) and *Plasmodium falciparum* circumsporozoite (CS) protein – among Tanzanian children in a Phase IIb RTS,S/AS01E trial. We observed that RTS,S/AS01 E vaccinees make stronger T cell IFN-γ, CD69 and CD25 responses to HBs peptides than do controls, indicating that RTS,S boosts pre-existing HBs responses. T cell CD69 and CD25 responses to CS and CS-specific secreted IL-2, were augmented by RTS,S vaccination. Importantly, more than 50% of peptide-induced IFN-γ+ lymphocytes were NK cells and the magnitude of the NK cell CD69 response to HBs peptides correlated with secreted IL-2 concentration. CD69 and CD25 expression and IL-2 secretion may represent sensitive markers of RTS,S-induced, CS-specific T cells. The potential for T cell-derived IL-2 to augment NK cell activation in RTS,S-vaccinated individuals, and the relevance of this for protection, needs to be explored further.



Relative contribution of T cells and NK cells to the total IFN-g response to HBs and CS peptides in RTS,S/AS01E vaccinated subjects. PBMCs from RTS,S/AS01E vaccinees (n = 80) were cultured with HBs or CS peptides for 24 h and analysed by flow cytometry. (Top) An example of the gating strategy to identify IFN-g+ cells and then to determine the proportion of these cells that are either T cells (CD3+), NK cells (CD3-, CD56+) or CD3-CD56- cells for HBs-stimulated cells from one vaccinated subject. (Bottom) Bar charts indicate the absolute number (mean/SD per 105 PBMCs) of IFN-g+ cells that are NK cells, T cells or CD3-CD56- (DN, double negative) lymphocytes among HBs-stimulated (filled bars) or CS-stimulated (open bars) PBMC.

Phase III Trial of RTS,S/AS01 Malaria Vaccine Candidate in African Children.

***LSHTM Investigators**: Daniel Chandramohan, Chris Drakeley, Brian Greenwood, Seth Owusu-Agyei & David Schellenberg.*

***External Investigators/Collaborators:** The RTS,S Clinical Trials Partnership.*

***Funding Body:** PATH Malaria Vaccine Initiative.*

RTS,S/AS01 is the most advanced malaria vaccine candidate which has progressed through phase II clinical trials to a multi-centre phase III study. This is being conducted by a broad partnership involving researchers from 11 research centres in 7 countries in sub-Saharan Africa, their associated northern partners, GSK and the Malaria Vaccine Initiative at PATH. The study has recruited 15,460 children in two age categories – 6-12 weeks and 5-17 months old at the time of dose 1 – and randomised them to receive either RTS,S or a comparator vaccine. The primary endpoint is efficacy against clinical malaria and secondary endpoints include efficacy against severe disease, efficacy in different transmission settings and the role of a booster dose.

Results of the primary endpoint analysis during 12 months of follow-up in the 5-17 month age category were published in 2011. RTS,S efficacy against clinical malaria was 55.8% (97.5% CI, 50.6 to 60.4), with 0.55 episodes of clinical malaria per person-year in the control group and 0.32 episodes per person-year in RTS,S recipients). Efficacy against severe malaria was 45.1% (95% CI, 23.8 to 60.5). Overall, serious adverse events occurred with a similar frequency in the two study groups. The rate of generalized convulsive seizures after RTS,S/AS01 was 1.04 per 1000 doses (95% CI, 0.62 to 1.64). The primary endpoint for children in the younger age category will be evaluated towards the end of 2012, and complete trial results are expected to be available for review by policy makers in 2015.

The CTLA-4 and PD-1/PD-L1 inhibitory pathways independently regulate host resistance to acute Plasmodium-induced immune pathology.

***LSHTM Investigators**: Julius Hafalla, Kevin Couper & Eleanor Riley.*

***External Investigators/Collaborators:** Brian de Souza (University College, London); Carla Claser & Laurent Renia (Singapore Immunology Network, Singapore); Georges Grau (The University of Sydney, Australia).*

***Funding Body:** Wellcome Trust and The Royal Society.*

The balance between pro-inflammatory and regulatory immune responses in determining optimal T cell activation is vital for the successful resolution of malaria infections. During chronic infections, this balance is maintained in part by the negative regulators of T cell activation, CTLA-4 and PD-1/PD-L1, which dampen effector responses. However, their role in acute infections, such as malaria, remains less clear. Thus, we determined the contribution of CTLA-4 and PD-1/PD-L to the regulation of T cell responses during *Plasmodium berghei* ANKA (PbA)-induced experimental cerebral malaria (ECM) in susceptible (C57BL/6) and resistant (BALB/c) mice.

We found that the expression of CTLA-4 and PD-1 on T cells correlates with the extent of pro-inflammatory responses induced during PbA infection, being higher in C57BL/6 than in BALB/c mice. However, antibody-mediated blockade of either the CTLA-4 or PD-1/PD-L1, but not the PD-1/PD-L2, pathways during PbA-infection in ECM-resistant BALB/c mice resulted in higher levels of T cell activation, enhanced IFN-γ production, increased intravascular arrest of both parasitised erythrocytes and CD8 T cells to the brain, and augmented incidence of ECM. Thus, in ECM-resistant BALB/c mice, CTLA-4 and PD-1/PD-L1 represent essential, independent and non-redundant pathways for maintaining T cell homeostasis during a virulent malaria infection. Moreover, neutralisation of IFN-γ or depletion of CD8+ T cells during PbA infection was shown to reverse the pathologic effects of regulatory pathway blockade, highlighting that the aetiology of ECM in the BALB/c mice is similar to that in C57BL/6 mice. In summary, our results underscore the differential and complex regulation that governs immune responses to malaria parasites.

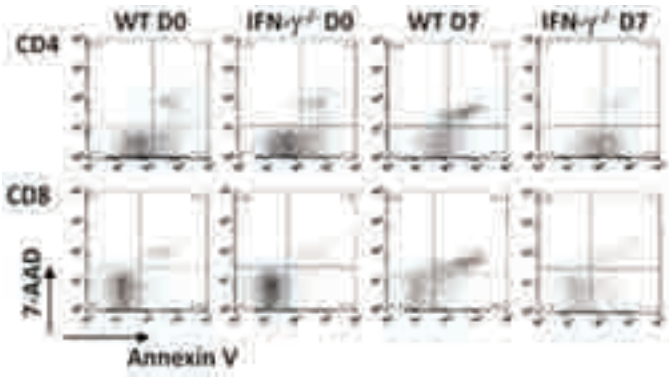
Regulation of effector T cell responses by IFN-γ during Plasmodium berghei ANKA infection.

***LSHTM Investigators**: Ana Villegas-Mendez, Brian de Souza, Tovah Shaw, Rachel Greig, Eleanor Riley & Kevin Couper.*

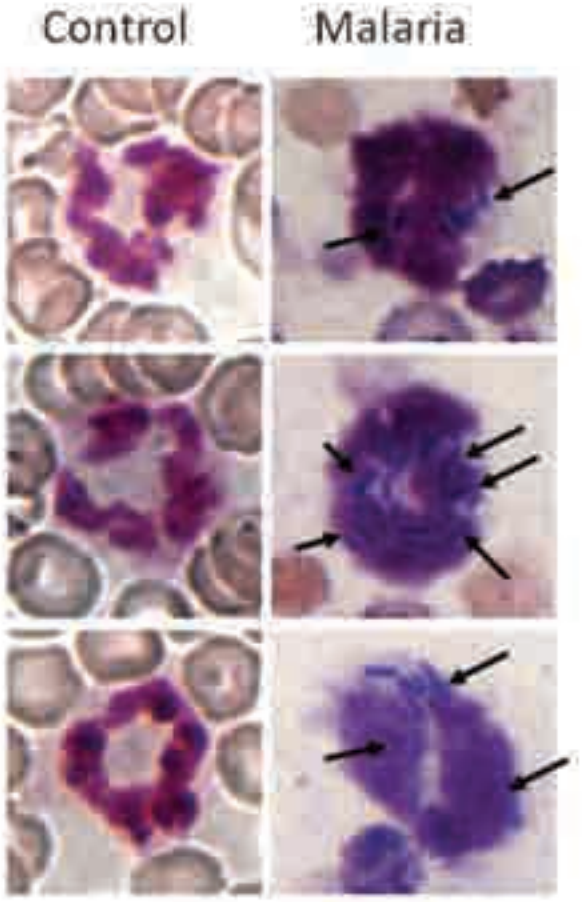
***Funding Body:** Biotechnology and Biological Sciences Research Council.*

IFN-γ and T cells are both required for the development of experimental cerebral malaria during *Plasmodium berghei* ANKA infection but the role of IFN-γ in shaping the effector CD4+ and CD8+ T cell response has not been examined in detail. To address this, we compared the effector T cell responses in wild-type and IFN-γ-/- mice during *Plasmodium berghei* ANKA infection.

The expansion of splenic CD4+ and CD8+ T cells was not affected by the absence of IFN-γ but the contraction phase of the T cell response was significantly attenuated. Splenic T cell activation and effector function were essentially normal in IFN-γ-/- mice, however, the migration to, and accumulation of, effector CD4+ and CD8+ T cells in the lung, liver and brain was altered in IFN-γ-/- mice. Regulation of splenic T cell numbers depended upon active IFN-γ-dependent environmental signals, leading to T cell apoptosis. In summary, this study to reveals a novel role for IFN-γ during malaria infection, being required for efficient contraction of the pool of activated T cells upon resolution of the infection.



IFN-γ promotes apoptosis of splenic T cells during P. berghei ANKA infection. WT and IFN-γ−/− mice were infected i.v. with 10^4 P. berghei ANKA pRBC. Representative dot plots showing the surface expression of annexin V and 7-AAD on naive and infection-derived (D7) CD4+ and CD8+ T cells.



Neutrophils in blood of control mice and malaria infected mice. Both groups were also infected with *Salmonella typhimurium*. The neutrophils from malaria infected mice contain numerous *Salmonella typhimurium* (arrows) which are not visible in those from control mice.

Mechanisms of impaired resistance to Salmonella in malaria patients.

***LSHTM Investigators**: Aubrey Cunnnington & Eleanor Riley.*

***External Investigators/Collaborators:** Michael Walther (Medical Research Council, The Gambia) & Brian de Souza (University College London, UK).*

***Funding Body:** Medical Research Council, UK.*

In sub-Saharan Africa, invasive nontyphoid Salmonella (NTS) infection is a common and often fatal complication of *Plasmodium falciparum* infection. Induction of heme oxygenase-1 (HO-1) mediates tolerance to the cytotoxic effects of heme during malarial hemolysis. We hypothesised that HO-1 might impair resistance to NTS by limiting production of bactericidal reactive oxygen species. We have shown that co-infection of mice with *Plasmodium yoelii* 17XNL (Py17XNL) and Salmonella enterica serovar Typhimurium 12023 (*Salmonella typhimurium*) causes acute, fatal bacteremia with high bacterial load. *Salmonella typhimurium* localized predominantly in granulocytes. We found that malaria infection caused premature mobilization of granulocytes from bone marrow with a quantitative defect in the oxidative burst. Inhibition of HO by tin protoporphyrin abrogated the impairment of resistance to *Salmonella typhimurium*. Since our data suggest that HO inhibitors might be useful adjunctive therapy for NTS infection in the context of hemolysis, studies were undertaken in The Gambia to determine whether similar defects in neutrophil function occur in malaria patients.

Acute, uncomplicated malaria caused the appearance of a population of neutrophils with reduced oxidative burst activity, which gradually normalized over 8 weeks of follow-up. The degree of oxidative burst impairment correlated significantly with markers of hemolysis. HO-1 expression was increased in blood during an acute malaria episode. Together these data suggest that neutrophil dysfunction also occurs in children with *Plasmodium falciparum* malaria and may explain the associated susceptibility to NTS infection.