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ABSTRACTS


African water storage pots for the delivery of the entomopathogenic fungus Metarhizium anisopliae to the malaria vectors Anopheles gambiae s.s. and Anopheles funestus.


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We studied the use of African water storage pots for point source application of Metarhizium anisopliae against the malaria vectors Anopheles gambiae s.s. and An. funestus. Clay pots were shown to be attractive resting sites for male and female An. gambiae s.s. and were not repellent after impregnation with fungus. M. anisopliae was highly infective and virulent after spray application inside pots. At a dosage of 4 x 10(10) conidia/m(2), an average of 95 +/- 1.2% of An. gambiae s.s. obtained a fungal infection. A lower dosage of 1 x 10(10) conidia/m(2) infected an average of 91.5 +/- 0.6% of An. gambiae s.s. and 91.8 +/- 1.2% of An. funestus mosquitoes. Fungal infection significantly reduced mosquito longevity, as shown by differences between survival curves and LT(50) values. These pots are suitable for application of entomopathogenic fungi against malaria vectors and their potential for sustainable field implementation is discussed.


Modeling the financial and clinical implications of malaria rapid diagnostic tests in the case-management of older children and adults in Kenya.

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Using data on clinical practices for outpatients 5 years and older, test accuracy, and malaria prevalence, we model financial and clinical implications of malaria rapid diagnostic tests (RDTs) under the new artemether-lumefantrine (AL) treatment policy in one high and one low malaria prevalence district in Kenya. In the high transmission district, RDTs as actually used would improve malaria treatment (61% less over-treatment but 8% more under-treatment) and lower costs (21% less). Nonetheless, the majority of patients with malaria would not be correctly treated with AL. In the low transmission district, especially because the treatment policy was new and AL was not widely used, RDTs as actually used would yield a minor reduction in under-treatment errors (36% less but the base is small) with 41% higher costs. In both districts, adherence to revised clinical practices with RDTs has the potential to further decrease treatment errors with acceptable costs.


Malaria vectors in areas of Plasmodium falciparum epidemic transmission in the Amazon region, Brazil.

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The main vectors of malaria in Brazil are Anopheles darlingi, An. aquasalis, and some species of the An. albiritarsis complex, whereas others have questionable importance with regard to the disease transmission. To identify these vectors in the State of Pará, Brazil, in a high-prevalence P. falciparum area, 565 anophelines were captured and identified while the seasonal variation and daily biting activity were determined. Of the seven anopheline species (An. strodei, An. albiritarsis s.l., An. rondoni, An. darlingi, An. triannulatus, An. oswaldoi, and An. nuneztovari), the plasmodia circumsporozoite protein (CSP) was detected in three of them, with a total infection rate of 6.2%. An. darlingi was the most prevalent species (22.4%), followed by An. albiritarsis (5.2%) and An. rondoni (3.6%). An. rondoni was found to be infected for the first time, which was also confirmed through PCR. This result possibly represents a new malaria vector based on its highest frequency, biting and seasonal activities in the peak of malaria transmission.


Halting the toll of malaria in Africa.

Campbell CC. Malaria Control and Evaluation Partnership in Africa, PATH, Seattle, Washington 98107, USA. cccampbell@path.org

A renaissance in commitment to malaria control is transforming the perspectives and aspirations of the global community, prompting a consideration of goals for confronting a disease that is responsible for legendary death and suffering in Africa. The results in several countries are producing confidence that current control interventions can result in a dramatic reduction in the burden that malaria causes. However, the complexities of the challenges that must be addressed for comprehensive Africa programming are formidable in terms of the time required and the resources that will have to be mobilized. Progress toward elimination of the malaria burden in the African region in the next 5 years will be the critical benchmark for the feasibility of a comprehensive global campaign to eliminate and potentially eradicate malaria.


Malaria and poverty.

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Malaria is one of the most important challenges to global public health. African countries south of the Sahara bear today the heaviest burden of malaria. The relationship between poverty and malaria has long been recognized but its paths are multiple and complex. Recent studies suggest that causality works both ways, trapping communities in reinforcing cycles of poverty and disease. If malaria is to be controlled or eventually eliminated, the social and economic conditions that fuel malaria transmission need to be addressed. At the same time, malaria control should be seen as a poverty reduction strategy.

Antimicrob Agents Chemother. 2008 Jun 16

First-Time-In-Humans Safety, Tolerability, Pharmacokinetics and Ex Vivo Pharmacodynamic Antimalarial Activity of the New Artemisinin Derivative, Artemisone.

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Rieckmann KH, Edstein MD.

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In preclinical studies, artemisone (BAY 44-9585), a new artemisinin derivative, has been shown to possess enhanced efficacy over artesunate and does not possess the neurotoxicity characteristic of the current artemisinins. In a phase I program with double-blind, randomized, placebo-controlled, single- and multiple-ascending oral dose studies we evaluated the safety, tolerability, pharmacokinetics and ex vivo pharmacodynamic antimalarial activity of artemisone. Single-doses (10, 20, 30, 40 and 80 mg), and multiple-doses (40 and 80 mg daily x 3 days) of artemisone were administered orally to healthy subjects. Plasma concentrations of artemisone and its metabolites were measured by liquid chromatography/tandem mass spectrometry. Artemisone was well tolerated, with no serious adverse events and no clinical relevant changes in laboratory and vital parameters. The pharmacokinetics of artemisone over the 10 to 80 mg range demonstrated dose linearity. After the single 80 mg dose, artemisone had a geometric mean (range) maximum concentration of 140.2 ng/ml (80.6 - 391.0), a short elimination half-life (t(1/2)) of 2.79 h (1.56 - 4.88), a high oral clearance (CL/F) of 284.1 liters/h (106.7 - 546.7) and a large volume of distribution (V/F) of 14.50 liters/kg (3.21 - 51.58). Due to artemisone's short t(1/2), its pharmacokinetics was comparable after single- and multiple-dosing. Plasma samples taken after multiple-dosing showed marked ex vivo pharmacodynamic antimalarial activity against two multidrug-resistant Plasmodium falciparum lines. Artemisone equivalent concentrations measured by bioassay revealed higher activity than artemisone measured by LC/MS/MS, confirming the presence of active metabolites. Comparable to other artemisinin's, artemisone's t(1/2) is well suited for artemisinin-based combination therapy for the treatment of falciparum malaria.


Retention of Plasmodium falciparum ring-infected erythrocytes in the slow, open micro-circulation of the human spleen.


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The current paradigm in Plasmodium falciparum malaria pathogenesis states that young, ring-infected red blood cells (rings) circulate in peripheral blood whereas mature stages are sequestered in the vasculature, thereby avoiding clearance by the spleen. Through the ex-vivo perfusion of intact human spleens we were able to examine the interaction of this unique blood-filtering organ with P. falciparum-infected red blood cells. As predicted, mature stages were rapidly retained. However, >50% rings were also retained, and accumulated upstream from endothelial sinus wall slits of the open, slow red pulp microcirculation. Ten percent of rings were retained at each spleen passage, a rate matching the proportion of blood flowing through the slow circulatory compartment established in parallel using spleen contrast-enhanced ultrasonography in healthy volunteers. Rings displayed a mildly but significantly reduced elongation index, consistent with a retention process due to their altered mechanical properties. This raises the new paradigm of a heterogeneous ring population, the less deformable subset being retained in the spleen thereby reducing the parasite biomass that will
subsequently sequester in vital organs, influencing the risk of severe complications such as cerebral malaria or severe anaemia. Cryptic ring retention uncovers a new role for the spleen in the control of parasite density, opening novel intervention opportunities.


**Vouchers for scaling up insecticide-treated nets in Tanzania: Methods for monitoring and evaluation of a national health system intervention.**


**ABSTRACT:** BACKGROUND: The Tanzania National Voucher Scheme (TNVS) uses the public health system and the commercial sector to deliver subsidised insecticide-treated nets (ITNs) to pregnant women. The system began operation in October 2004 and by May 2006 was operating in all districts in the country. Evaluating complex public health interventions which operate at national level requires a multidisciplinary approach, novel methods, and collaboration with implementers to support the timely translation of findings into programme changes. This paper describes this novel approach to delivering ITNs and the design of the monitoring and evaluation (M&E). METHODS: A comprehensive and multidisciplinary M&E design was developed collaboratively between researchers and the National Malaria Control Programme. Five main domains of investigation were identified: (1) ITN coverage among target groups, (2) provision and use of reproductive and child health services, (3) "leakage" of vouchers, (4) the commercial ITN market, and (5) cost and cost-effectiveness of the scheme. RESULTS: The evaluation plan combined quantitative (household and facility surveys, voucher tracking, retail census and cost analysis) and qualitative (focus groups and in-depth interviews) methods. This plan was defined in collaboration with implementing partners but undertaken independently. Findings were reported regularly to the national malaria control programme and partners, and used to modify the implementation strategy over time. CONCLUSIONS: The M&E of the TNVS is a potential model for generating information to guide national and international programmers about options for delivering priority interventions. It is independent, comprehensive, provides timely results, includes information on intermediate processes to allow implementation to be modified, measures leakage as well as coverage, and measures progress over time.


**Antimalarial responses in Anopheles gambiae: from a complement-like protein to a complement-like pathway.**

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Malaria transmission between humans depends on the ability of Anopheles mosquitoes to support Plasmodium development. New perspectives in vector control are emerging from understanding the mosquito immune system, which plays critical roles in parasite recognition and killing. A number of factors controlling this process have been recently identified, and key among them is TEP1, a homolog of human complement factor C3 whose binding to the parasite surface targets it for subsequent killing. Here, we review our current knowledge of mosquito factors that respond to Plasmodium infection and elaborate on the activity and mode of action of the TEP1 complement-like pathway.

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SUMO (Small Ubiquitin-like MOdifier) conjugation is a post-translational modification implicated in a variety of cellular functions including transcriptional regulation, nuclear location and signal transduction. Sumoylation, although conserved and vital in eukaryotes, has not been studied in malaria parasites. Here we identify SUMO conjugation of blood stage parasites of P. falciparum. Antibodies raised against synthetic peptides of the plasmodial SUMO orthologue PfSUMO, a 100 amino acid protein, reacted with distinctive sub-cellular compartments of the parasitized erythrocyte during blood stage development. Anti-PfSUMO stains the nucleus and parasite cytoplasm. We also found antibody reactivity in the host cell cytoplasm with the parasite-derived structures called Maurer's clefts. Anti-PfSUMO reacts in Western blot with a number of blood stage proteins ranging from approx. 40 to 250 kDa. Parasites expressing FLAG-tagged PfSUMO gave similar results in Immunofluorescence assay (IFA) and Western blots. In addition, we show that anti-PfSUMO identified PfSir2, a telomere-associated nuclear protein involved in var gene silencing, as a target for sumoylation. Furthermore, LC-MS/MS analysis of a two-step Immunoprecipitation with anti-FLAG and anti-PfSUMO antibodies reveals a number of putative P. falciparum sumoylated proteins. Our results imply that SUMO conjugation has an essential function in a number of different biological processes in P. falciparum.

PbCap380, a novel oocyst capsule protein, is essential for malaria parasite survival in the mosquito.

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An essential requisite for transmission of Plasmodium, the causative agent of malaria, is the successful completion of a complex developmental cycle in its mosquito vector. Of hundreds of ookinetes that form in the mosquito midgut, only few transform into oocysts, a loss attributed to the action of the mosquito immune system. However, once oocysts form, they appear to be resistant to mosquito defences. During oocyst development, a thick capsule forms around the parasite and appears to function as a protective cover. Little information is available about the composition of this capsule. Here we report on the identification and partial characterization of the first Plasmodium oocyst capsule protein (PbCap380). Genetic analysis indicates that the gene is essential and that PbCap380(-) mutant parasites form oocysts in normal numbers but are gradually eliminated. As a result, mosquitoes infected with PbCap380(-) parasites do not transmit malaria. Targeting of the oocyst capsule may provide a new strategy for malaria control.
Activities of Artemether-Lumefantrine and Amodiaquine-Sulfalene-Pyrimethamine against Sexual-Stage Parasites in Falciparum Malaria in Children.

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The activities of artemether-lumefantrine and amodiaquine-sulfalene-pyrimethamine against sexual-stage parasites were evaluated in 42 of 181 Nigerian children with uncomplicated Plasmodium falciparum malaria who had gametocytaemia before, during or after treatment with the two combination therapies. The children were randomized to the standard dose regimens. Clinical recovery from illness occurred in all children who carried gametocytes. Gametocytaemia was detected in 20 patients (11%) before treatment and in another 22 patients (12.2%) after treatment. Game- tocyte carriage rates were similar in both combination treatment groups, but the area under the curve of gametocytaemia plotted against time was 8-fold higher in the amodiaquine-sulfalene-pyrimethamine-treated than in the artemether-lumefantrine-treated children. The pretreatment gametocyte sex ratio was female biased in both treatment groups. During follow-up, there was a short-lived but significant increase in the gametocyte sex ratio in children treated with amodiaquine-sulfalene-pyrimethamine but not in those treated with artemether-lumefantrine. These results indicate that both combination therapies had moderate effects on gametocyte carriage, but artemether-lumefantrine may be more potent at reducing transmissibility in P. falciparum malaria by exerting greater effects on post-treatment gametocyte density and gametocyte sex ratio. Copyright © 2008 S. Karger AG, Basel.

Comparative testing of six Plasmodium falciparum merozoite stage antigen-based malaria vaccine candidates.

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Immunogenicity testing of Plasmodium falciparum antigens being considered as malaria vaccine candidates was undertaken in rabbits. The antigens compared were recombinant baculovirus MSP-119 and five Pichia pastoris candidates, including two versions of MSP-119, AMA-1 (domains I-II), AMA-1+MSP-119, and fused AMA-1-MSP-119. Animals were immunized with equimolar amounts of each antigen, formulated in Montanide ISA720. Specificities and titers of antibodies were compared using immuno-fluorescence assays (IFA) and ELISA. Anti-parasite activity of IgG in in vitro cultures was determined by growth inhibition assays (GIA), flow cytometry, lactate dehydrogenase assay and microscopy. Baculovirus MSP-119 immunizations produced the highest parasite-specific antibody titers in IFA assays. In ELISA assays, baculovirus-produced MSP-119 induced more antibodies than any other single MSP-119 immunogen, and three times more MSP-119 specific
antibodies than the AMA-1-MSP-119 fusion. Antibodies induced by baculovirus MSP-119 gave the highest levels of growth inhibition in HB3 and 3D7 parasite cultures, followed by AMA-1+MSP-119 and the AMA-1/MSP-119 fusion. With the FCR3 isolate (homologous to the AMA-1 construct), antibodies to the three AMA-1-containing candidates gave the highest levels of growth inhibition at high IgG concentrations, but antibodies to baculovirus MSP-119 inhibited as well or better at lower IgG concentrations. The two Pichia pastoris- produced MSP-119-induced IgGs conferred the lowest growth inhibition. Comparative analysis of immunogenicity of vaccine antigens can be used to prioritize candidates before moving to expensive GMP production and clinical testing. The assays used have given discriminating readouts but it is not known whether any of them accurately reflect clinical protection.


Enzyme-linked immunosorbent assay for detection of Plasmodium falciparum histidine-rich protein 2 in blood, plasma, and serum.

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Microscopy, the gold standard for the detection and quantification of malaria parasites in blood, is in many aspects deficient for this purpose. The method is poorly reproducible and can be inaccurate because Plasmodium falciparum parasites sequester for a portion of each asexual cycle. Due to these deficiencies, biomarkers such as P. falciparum histidine-rich protein 2 (PfHRP2) are increasingly being used. In this study, we evaluated the use of a commercial PfHRP2 enzyme-linked immunosorbent assay (ELISA) kit with some procedural modifications. We determined the linear range of the assay, including the lower limits of detection and quantitation, using recombinant PfHRP2 (rPfHRP2). In 10 repeat experiments, the linear range of optical densities (ODs) at 450 to 650 nm was from 0.05 +/- 0.002 to 2.28 +/- 0.042, corresponding to 3.91 to 250 ng/ml of rPfHRP2. The coefficient of variation (CV) at each target concentration ranged from 1.93 to 8.07%. Using cultured parasites, we confirmed the linear range of ODs as well as the association between the PfHRP2 ELISA results and the microscopic parasite densities. For whole-blood samples spiked with cultured, washed, ring-stage-infected red blood cells (iRBCs), the linear range was 11.7 to 750 iRBCs/microl, with CVs of 0.29 to 7.56%. The same spiked samples evaluated by microscopists had similar sensitivities, but the CVs were unacceptably high (20.7 to 161.6%). Stock rPfHRP2 was stable through four freeze-thaw cycles (P < 0.05; paired t test). When different patient sample types at different concentrations within the linear range of the assay are compared, the recoveries of PfHRP2 from blood and serum were within +/-20%, whereas the recoveries from plasma ranged between +35 and -41%. We conclude that PfHRP2 ELISA using whole-blood and serum samples is a suitable adjunct to microscopy and could ultimately benefit malaria intervention trials.


Monitoring Plasmodium falciparum growth and development by UV flow cytometry using an optimized Hoechst-thiazole orange staining strategy.

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The complex life cycle of Plasmodium falciparum (Pf) makes it difficult to limit infections and reduce the risk of severe malaria. Improved understanding of Pf
blood-stage growth and development would provide new opportunities to evaluate and interfere with successful completion of the parasite's life cycle. Cultured blood stage Pf was incubated with Hoechst 33342 (HO) and thiazole orange (TO) to stain DNA and total nucleic acids, respectively. Correlated HO and TO fluorescence emissions were then measured by flow cytometry. Complex bivariate data patterns were analyzed by manual cluster gating to quantify parasite life cycle stages. The permutations of viable staining with both reagents were tested for optimal detection of parasitized RBC (pRBC). Pf cultures were exposed to HO and TO simultaneously to achieve optimal staining of pRBC and consistent quantification of early and late stages of the replicative cycle (rings through schizonts). Staining of Pf nucleic acids allows for analysis of parasite development in the absence of fixatives, lysis, or radioactivity to enable examination of erythrocytes from parasite invasion through schizont rupture using sensitive and rapid assay procedures. Investigation of the mechanisms by which anti-malarial drugs and antibodies act against different Pf lifecycle stages will be aided by this cytometric strategy. (c) 2008 International Society for Advancement of Cytometry.

Eval Health Prof. 2008 Jun 10.

**Sociocultural Barriers and Malaria Health Care in Tanzania.**

Nsimba SE, Kayombo EJ.

In Tanzania, since the time of its ancestors, cultural beliefs have existed which influence the treatment and management of diseases. This article focuses on malaria as a current major cause of morbidity and mortality in Tanzania. Patients and caretakers have tended to rely on traditional sociocultural practices as a means of treating the convulsions associated with severe malaria in children and often do not seek care at health facilities, therefore, delaying prompt management of the disease.


**The skin stage of malaria infection: biology and relevance to the malaria vaccine effort.**

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Plasmodium sporozoites, the infective stage of the malaria parasite, are injected into the mammalian host by mosquitoes and travel to the liver where they invade hepatocytes. Recent studies demonstrate that sporozoites are inoculated into the skin, remain there for hours before exiting and that 20% of the inoculum goes to the lymph node draining the inoculation site, suggest that there is a 'skin stage' to malaria infection that may set the stage for subsequent host responses to the parasite. Here, we present an overview of what is currently known about sporozoite-host interactions at the inoculation site and the draining lymph node, and discuss the impact of the skin stage of malaria on immunity to pre-erythrocytic stages and malaria vaccine design.


**Alterations of splenic architecture in malaria are induced independently of Toll-like receptors 2, 4, 9 or MyD88, and may affect antibody affinity.**


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Splenic microarchitecture is substantially altered during acute malaria infections, which may affect development and regulation of immune responses. Here we have investigated whether engagement of host Toll-like receptors TLR 2, 4 and 9 and the adaptor protein MyD88 are required for the induction of these changes, and whether antibody responses are modified when immunisation takes place during the period of splenic disruption. Alterations in splenic microarchitecture were maximal shortly after the peak of parasitaemia, and were not dependent on engagement of TLR receptors 2, 4, or 9, and were only minimally affected by the absence of the MyD88 adaptor molecule. Although germinal centres were formed in infected mice, they did not contain the usual light and dark zones. Immunisation of mice with chicken gamma globulin 2 weeks prior to acute P. chabaudi infection did not affect the quantity or avidity of the IgG antibody response to that antigen. However, immunisation given at the same time as the primary P. chabaudi infection resulted in a clear transient reduction in antibody avidity in the month following immunisation. These data suggest that the alterations in splenic structure, particularly the germinal centres, may affect the quality of an antibody response during a malaria infection and could impact on development of immunity to malaria or other to infections or immunisations occurring during a malaria infection.


**Effector CD8+ T lymphocytes against malaria liver stages do not require IFN-{gamma} for anti-parasite activity.**

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The protective immune response against liver stages of the malaria parasite critically requires CD8(+) T cells. Although the nature of the effector mechanism utilized by these cells to repress parasite development remains unclear, a critical role for IFN-gamma has been widely assumed based on circumstantial evidence. However, the requirement for CD8(+) T cell-mediated IFN-gamma production in protective immunity to this pathogen has not been directly tested. In this report, we use an adoptive transfer strategy with circumsporozoite (CS) protein-specific transgenic T cells to examine the role of CD8(+) T cell-derived IFN-gamma production in Plasmodium yoelii-infected mice. We show that despite a marginal reduction in expansion of naïve IFN-gamma deficient CS-specific transgenic T cells, their anti-parasite activity remains intact. Further, adoptively transferred IFN-gamma deficient CD8(+) T cells were as efficient as their wild type counterparts in limiting parasite growth in naïve mice. Taken together, these studies demonstrate that IFN-gamma-secretion by CS-specific CD8(+) T cells is not essential to protect mice against live sporozoite challenge.


**A diversity-covering approach to immunization with Plasmodium falciparum apical membrane antigen 1 induces broader allelic recognition and growth inhibition responses in rabbits.**

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Plasmodium falciparum apical membrane antigen 1 (PfAMA1), a candidate malaria vaccine, is polymorphic. This polymorphism is believed to be generated predominantly under immune selection pressure and, as a result, may compromise attempts at vaccination. Alignment of 355 PfAMA1 sequences shows that around 10% of the 622 amino acid residues can vary between alleles and that linkages between polymorphic residues occur. Using this analysis, we have designed three diversity-covering (DiCo) PfAMA1 sequences that take account of these linkages and, when taken together, on average incorporate 97% of amino acid variability observed. For each of the three DiCo sequences, a synthetic gene was constructed and used to transform the methylotrophic yeast Pichia pastoris, allowing recombinant expression. All three DiCo proteins were reactive with the reduction-sensitive monoclonal antibody 4G2, suggesting the DiCo sequences had conformations similar to those of naturally occurring PfAMA1. Rabbits were immunized with FVO strain PfAMA1 or with the DiCo proteins either individually or as a mixture. Antibody titers and the ability to inhibit parasite growth in vitro were determined. Animals immunized with the DiCo mix performed similarly to animals immunized with FVO AMA1 when measured against FCR3 strain parasites but outperformed animals immunized with FVO AMA1 when assessed against other strains. The levels of growth inhibition (approximately 70%) induced by the mix of three DiCo proteins were comparable for FVO, 3D7, and HB3, suggesting that a considerable degree of diversity in AMA1 is adequately covered. This suggests that vaccines based upon the DiCo mix approach provide a broader functional immunity than immunization with a single allele.


**In vitro evaluations of antimalarial drugs and their relevance to clinical outcomes.**

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Plasmodium falciparum resistance to the former first-line antimalarials chloroquine and sulfadoxine/pyrimethamine has reached critically high levels in many malaria-endemic regions. This has spurred the introduction of several new artemisinin-based combination therapies (ACTs) that display excellent potency in treating drug-resistant malaria. Monitoring for the emergence of drug resistant P. falciparum is important for maximising the clinically effective lifespan of ACTs. Here, we provide a commentary on the article by Kaddouri et al., published in this issue of the International Journal of Parasitology, which documents the levels of susceptibility to ACT drugs and chloroquine in P. falciparum isolates from Mali. These authors report that some isolates approached a proposed in vitro threshold of resistance to monodesethyl-amodiaquine (the principal effective metabolite of amodiaquine, an important ACT partner drug), and establish baseline levels of susceptibility to the ACT drugs dihydroartemisinin and lumefantrine. The majority of clinical isolates manifested in vitro resistance to chloroquine. The authors also show good concordance between field-based assays employing a non-radioactive lactate dehydrogenase-based method of determining in vitro drug IC(50) values and the well-established [(3)H]hypoxanthine-based radioactive method. This work illustrates a good example of drug resistance surveillance, whose global coordination is being championed by the World Antimalarial Resistance Network. Our current opinion also more generally discusses the complexities inherent to conducting in vitro investigations with P. falciparum patient isolates and correlating these findings with treatment outcome data.
Vaccination with a Plasmodium chabaudi adami multivalent DNA vaccine cross-protects A/J mice against challenge with P. c. adami DK and virulent Plasmodium chabaudi chabaudi AS parasites.

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A current goal of malaria vaccine research is the development of vaccines that will cross-protect against multiple strains of malaria. In the present study, the breadth of cross-reactivity induced by a 30K multivalent DNA vaccine has been evaluated in susceptible A/J mice (H-2a) against infection with the Plasmodium chabaudi adami DK strain and a virulent parasite subspecies, Plasmodium chabaudi chabaudi AS. Immunized A/J mice were significantly protected against infection with both P. c. adami DK (31-40% reduction in cumulative parasitemia) and P. c. chabaudi AS parasites, where a 30-39% reduction in cumulative parasitemia as well as enhanced survival was observed. The 30K vaccine-induced specific IFN-gamma production by splenocytes in response to native antigens from both P. c. chabaudi AS and P. c. adami DK. Specific antibodies reacting with surface antigens expressed on P. c. adami DS and P. c. chabaudi AS infected red blood cells, and with opsonizing properties, were detected. These results suggest that multivalent vaccines encoding conserved antigens can feasibly induce immune cross-reactivity that span Plasmodium strains and subspecies and can protect hosts of distinct major histocompatibility complex haplotypes.


The structure of a chondroitin sulphate-binding domain important in placental malaria.

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Adhesive PfEMP1 proteins are displayed on the surface of malaria-infected red blood cells. They play a critical role in the disease, tethering infected cells away from destruction by the spleen and causing many severe symptoms. A molecular understanding of how these domains maintain their binding properties while evading immune detection will be important in developing therapeutics. In malaria of pregnancy, domains from the var2csa encoded PfEMP1 protein interact with chondroitin sulphate on the placenta surface. This causes accumulation of infected red blood cells, leading to placental inflammation and block of blood flow to the developing foetus. This is associated with maternal anemia, low birth weight and premature delivery and can lead to the death of mother and child. Here we present the structure of the chondroitin sulphate-binding DBL3X domain from a var2csa encoded PfEMP1 protein. The domain adopts a fold similar to malarial invasion proteins, with extensive loop insertions. One loop is flexible in the unliganded structure but observed in the presence of sulphate or disaccharide where it completes a sulphate-binding site. This loop, and others surrounding this putative carbohydrate-binding site, are flexible and polymorphic, perhaps protecting the binding site from immune detection. This suggests a model for how the domain maintains ligand binding while evading the immune response and will guide future drug and vaccine development.
HEALTH-SEEKING BEHAVIOUR FOR CHILDHOOD MALARIA: HOUSEHOLD DYNAMICS IN RURAL SENEGAL.

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Research on health care behaviour in sub-Saharan Africa usually considers the mother as the reference in the household when a child is sick. The study of health care management within the family is a key issue for understanding therapeutic rationales. This study was conducted in the region of Fatick in Senegal among 902 children with malaria-related fever. The data were taken from a retrospective quantitative survey conducted in all compounds of the DSS (Demographic Surveillance Site) of Niakhar. The results show that child care-taking is fundamentally a collective process: in 70.9% of out-of-home resorts, the treatment decision was collective. The health care process of 68.1% of morbid episodes involved several individuals. The involvement of the mother, the father and other relatives in the collective management of health care followed different logics. Each care-giver had a specific and complementary function depending on gender norms, intergenerational relations and characteristics of the family unit. Family management of illness aims at optimizing financial and human resources given the economic, logistical and social constraints on health care. Nevertheless, collective management also favoured home-based care, prevented good treatment compliance and delayed the resort to health facilities. These results suggest that health education campaigns should focus on an early involvement of fathers in health care-giving and also on the strengthening of the autonomy of mothers. Mothers' empowerment should give women more autonomy in their child's treatment choice. Lastly, there is a need to develop community health facilities and establish shared funding at the community level.

Plasmodium falciparum possesses two GRASP proteins that are differentially targeted to the Golgi complex via a higher- and lower-eukaryote-like mechanism.


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Plasmodium falciparum, the causative agent of malaria, relies on a complex protein-secretion system for protein targeting into numerous subcellular destinations. Recently, a homologue of the Golgi re-assembly stacking protein (GRASP) was identified and used to characterise the Golgi organisation in this parasite. Here, we report on the presence of a splice variant that leads to the expression of a GRASP isoform. Although the first GRASP protein (GRASP1) relies on a well-conserved myristoylation motif, the variant (GRASP2) displays a different N-terminus, similar to GRASPs found in fungi. Phylogenetic analyses between GRASP proteins of numerous taxa point to an independent evolution of the unusual N-terminus that could reflect unique requirements for Golgi-dependent protein sorting and organelle biogenesis in P. falciparum. Golgi association of GRASP2 depends on the hydrophobic N-terminus that resembles a signal anchor, leading to a unique mode of Golgi targeting and membrane attachment.
A new model for hemoglobin ingestion and transport by the human malaria parasite Plasmodium falciparum.

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The current model for hemoglobin ingestion and transport by intraerythrocytic Plasmodium falciparum malaria parasites shares similarities with endocytosis. However, the model is largely hypothetical, and the mechanisms responsible for the ingestion and transport of host cell hemoglobin to the lysosome-like food vacuole (FV) of the parasite are poorly understood. Because actin dynamics play key roles in vesicle formation and transport in endocytosis, we used the actin-perturbing agents jasplakinolide and cytochalasin D to investigate the role of parasite actin in hemoglobin ingestion and transport to the FV. In addition, we tested the current hemoglobin trafficking model through extensive analysis of serial thin sections of parasitized erythrocytes (PE) by electron microscopy. We find that actin dynamics play multiple, important roles in the hemoglobin transport pathway, and that hemoglobin delivery to the FV via the cytostomes might be required for parasite survival. Evidence is provided for a new model, in which hemoglobin transport to the FV occurs by a vesicle-independent process.

Effects of artesunate-cotrimoxazole and amodiaquine-артесуate against asexual and sexual stages of Plasmodium falciparum malaria in Nigerian children.

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The activities of artesunate-cotrimoxazole and artesunate-amodiaquine combinations against asexual- and sexual-stage parasites were evaluated in 182 Nigerian children with uncomplicated Plasmodium falciparum malaria. One hundred and twenty-one children received artesunate-cotrimoxazole and 61 received artesunate-amodiaquine and all were followed up for 28 days. Clinical recovery from illness occurred in all children. There was no significant difference in fever clearance time (P = 0.35). Both treatment groups achieved a parasite clearance time of less than 2 days (1.84 +/- 0.66 days and 1.31 +/- 0.48 days); gametocyte carriage rates were comparable in the two treatment groups prior to and following treatment; both treatments appeared to reduce gametocyte carriage. The pretreatment gametocyte sex ratio, which was female-biased, was maintained throughout the period of follow up in both treatment groups. Reduction of gametocyte carriage by these two treatment regimens may reduce transmissibility in P. falciparum malaria, and this reduction is presumed to be related to the accelerated clearance of the asexual forms of the parasite.

alpha(+)–Thalassemia Protects against Anemia Associated with Asymptomatic Malaria: Evidence from Community-Based Surveys in Tanzania and Kenya.

Veenemans J, Andang'o PE, Mbugi EV, Krayaijenhagen RJ, Mwaniki DL, Mockenhaupt FP, Roewer S, Olomi RM, Shao JF, Meer JW, Savelkoul HF, Verhoef H.

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Environmental Health at USAID – Malaria Bulletin, July 2008
University, Wageningen, 3Meander Medical Centre, Amersfoort, and 4University Medical Centre Nijmegen, Nijmegen, The Netherlands; 5Institute of Tropical Medicine and International Health, Charité–University Medicine Berlin, Berlin, Germany; 6Kenyan Institute of Medical Research, Nairobi, Kenya; 7Kilimanjaro Christian Medical Centre, Moshi, Tanzania.

Background. In hospital-based studies, alpha(+)–thalassemia has been found to protect against severe, life-threatening falciparum malaria. alpha(+)–Thalassemia does not seem to prevent infection or high parasite densities but rather limits progression to severe disease—in particular, severe malarial anemia. We assessed to what extent alpha(+)–thalassemia influences the association between mild, asymptomatic Plasmodium falciparum infection and hemoglobin concentration. Methods. The study was based on 2 community-based surveys conducted among afebrile children (0.5–8 years old; [Formula: see text]) in Kenya and Tanzania. Results. Among children without inflammation (whole-blood C-reactive protein concentration \( \leq 10 \text{ mg/L} \)), P. falciparum infection was associated with only small reductions in hemoglobin concentration, and effects were similar across alpha-globin genotypes. By contrast, the reduction in hemoglobin concentration associated with P. falciparum infection accompanied by inflammation was larger and strongly depended on genotype (normal, \(-21.8 \text{ g/L}\); heterozygous, \(-16.7 \text{ g/L}\); and homozygous, \(-4.6 \text{ g/L}\)). Relative to children with a normal genotype, this difference in effect was 5.1 g/L (95% confidence interval [CI], \(-1.0 \text{ to } 11.1 \text{ g/L}\)) for heterozygotes and 17.2 g/L (95% CI, 8.3 to 26.2 g/L) for homozygotes (estimates are adjusted for study site, age, height-for-age z score, and iron deficiency). Conclusions. alpha(+)–Thalassemia limits the decline in hemoglobin concentration that is associated with afebrile infections, particularly those that are accompanied by inflammation.


Molecular markers of resistance to sulfadoxine-pyrimethamine during intermittent preventive treatment for malaria in Mozambican infants.


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BACKGROUND: Intermittent preventive treatment in infants (IPTi) with sulfadoxine-pyrimethamine (SP) is a potential malaria control strategy. There is concern about the impact that increasing in vivo resistance to SP has on the efficacy of IPTi, as well as about the potential contribution of IPTi to increases in resistance. METHODS: We compared the frequency of clinical episodes of malaria caused by P. falciparum parasites with mutations in dhfr and dhps among sick children who received SP or placebo in the context of a randomized, double-blind, placebo-controlled IPTi trial in Mozambique. RESULTS: Half of the children who received placebo harbored quintuple-pure mutant parasites. Nevertheless, the protective efficacy of IPTi within the 35 days after the third dose was 70.8% (95% confidence interval [CI], 40.7%–85.6%). Between month 2 after the third IPTi dose and the end of the follow-up period, children receiving SP harbored more dhps codon 437 mixed infections (odds ratio [OR], 10.56 [95% CI, 1.30–86.14]) and fewer dhps double-pure mutant parasites (OR, 0.43 [95% CI, 0.22–0.84]) than did placebo recipients. CONCLUSIONS: IPTi appears to be associated with some changes in the prevalence of genotypes involved in SP resistance. In the face of a high prevalence of quintuple-mutant parasites, SP exhibited a high level of efficacy in the prevention of new episodes of malaria in infants.
**Triazolopyrimidine-Based Dihydroorotate Dehydrogenase Inhibitors with Potent and Selective Activity against the Malaria Parasite Plasmodium falciparum.**

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A Plasmodium falciparum dihydroorotate dehydrogenase (PfDHODH) inhibitor that is potent (K_i = 15 nM) and species-selective (>5000-fold over the human enzyme) was identified by high-throughput screening. The substituted triazolopyrimidine and its structural analogues were produced by an inexpensive three-step synthesis, and the series showed good association between PfDHODH inhibition and parasite toxicity. This study has identified the first nanomolar PfDHODH inhibitor with potent antimalarial activity in whole cells (EC_50 = 79 nM).

**A Prodomain Peptide of Plasmodium falciparum Cysteine Protease (Falcipain-2) Inhibits Malaria Parasite Development.**

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Falcipain-2 (FP-2), a papain family cysteine protease of Plasmodium falciparum, is a promising target for antimalarial chemotherapy. Designing inhibitors that are highly selective for falcipain-2 has been difficult because of broad specificity of different cysteine proteinases. Because propeptide regions of cysteine proteases have been shown to inhibit their cognate enzymes specifically and selectively, in the present study, we evaluated the inhibitory potential of few falcipain-2 proregion peptides. A 15 residue peptide (PP1) inhibited falcipain-2 enzyme activity in vitro. Studies on the uptake of PP1 into the parasitized erythrocytes showed access of peptide into the infected RBCs. PP1 fused with Antennapedia homeoprotein internalization domain blocked hemoglobin hydrolysis, merozoite release and markedly inhibited Plasmodium falciparum growth and maturation. Together, our results identify a peptide derived from the proregion of falcipain-2 that blocks late-stage malaria parasite development in RBCs, suggesting the development of peptide and peptidometric drugs against the human malaria parasite.

**Median knock-down time as a new method for evaluating insecticide-treated textiles for mosquito control.**

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ABSTRACT: BACKGROUND: Insecticide treated bed nets are major tools for the Roll Back Malaria campaign. There are two types of Long-Lasting Insecticide-treated Nets (LNts) on the market: coated nets and insecticide-incorporated nets. Nets provided to this market need a recommendation from the World Health Organization to be purchased by donors and NGOs. During laboratory study (phase I), the first step consists in evaluating the wash resistance of a new LN product. When insecticide-incorporated nets are washed, it takes time to regenerate the insecticidal activity, i.e. insecticide must migrate to the net surface to be accessible to mosquitoes. The interval of time required for regeneration must be carefully determined to ensure the accuracy of further results. WHOPES procedures currently recommend the determination of the regeneration time by using mortality.
data. However, as mortality cannot exceed 100%, a LN that regenerates a surface concentration exceeding the dosage for 100% mortality, will have its regeneration time underestimated. METHODS: The Median Knock Down Time (MKDT) was determined as function of insecticide dosage on an inert surface, glass, and on polyester nettings using an acetone solution or a simple emulsion. Dosage response was also established for mortality data. The same method was then applied to a commercially polyethylene netting, currently under WHOPES evaluation, to determine the dynamics of regeneration as function of repeated washings. The deltamethrin content of these nets was estimated by Capillary Gas Chromatography (GC-ECD). RESULTS: MKDT was a linear function of log insecticide dosage on glass as on nettings. Mortality data were either 0 or 100% for most concentrations except for a narrow range. MKDT was log linear function of total deltamethrin content in a commercial polyethylene net exposed to washings. The regeneration time of this net increased with the number of washes and MKDT became higher. A new, easy and rapid method to determine MKDT is suggested. DISCUSSION: The MKDT is linearly correlated to log dosage on a given substrate and shows no saturation as mortality data do. It is suited to determine regeneration time of a product that is exposed to a stress, like washing or heating, where the process impacts on the bio-availability of the insecticide. Mortality data are useful for measuring product efficacy, whereas MKDT are better to measure dynamics of surface concentration like regeneration after a stressing process. Change in MKDT can be used to illustrate the loss of insecticide due to washing, but the slope of the curve is product and surface-dependent.


Polymorphism patterns in Duffy-binding protein among Thai Plasmodium vivax isolates.


ABSTRACT: BACKGROUND: The Duffy-binding protein II of Plasmodium vivax (PvDBPII) has been considered as an attractive target for vaccine-mediated immunity despite a possible highly polymorphic nature. Among seven PvDBP domains, domain II has been shown to exhibit a high rate of nonsynonymous polymorphism, which has been suggested to be a potential immune (antibody binding) evasion mechanism. This study aimed to determine the extent of genetic polymorphisms and positive natural selection at domain II of the PvDBP gene among a sampling of Thai P. vivax isolates. METHODS: The PvDBPII gene was PCR amplified and the patterns of polymorphisms were characterized from 30 Thai P. vivax isolates using DNA cloning and sequencing. Phylogenetic analysis of the sequences and positive selection were done using DnaSP ver 4.0 and MEGA ver 4.0 packages. RESULTS: This study demonstrated a high rate of nonsynonymous polymorphism. Using Sal I as the reference strain, a total of 30 point-mutations were observed in the PvDBPII gene among the set of Thai P. vivax isolates, of which 25 nonsynonymous and five synonymous were found. The highest frequency of polymorphism was found in five variant amino acids (residues D384G, R390H, L424I, W437R, I503K) with the variant L424I having the highest frequency. The difference between the rates of nonsynonymous and synonymous mutations estimated by the Nei and Gojobori's method suggested that PvDBPII antigen appears to be under selective pressure. Phylogenetic analysis of PvDBPII Thai P. vivax isolates to others found internationally demonstrated six distinct allele groups. Allele groups 4 and 6 were unique to Thailand. CONCLUSIONS: Polymorphisms within PvDBPII indicated that Thai vivax malaria parasites are genetically diverse. Phylogenetic analysis of DNA sequences using the Neighbour-Joining method demonstrated that Thai isolates shared distinct alleles with P. vivax isolates from different geographical areas. The study reported here will be valuable for the development of PvDBPII-based malaria vaccine.
Spatially-explicit risk profiling of Plasmodium falciparum infections at a small scale: a geostatistical modelling approach.

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ABSTRACT: BACKGROUND: There is a renewed political will and financial support to eradicate malaria. Spatially-explicit risk profiling will play an important role in this endeavour. Patterns of Plasmodium falciparum infection prevalence were examined among schoolchildren in a highly malaria-endemic area. METHODS: A questionnaire was administered and finger prick blood samples collected from 3,962 children, aged six to 16 years, attending 55 schools in a rural part of western Cote d'Ivoire. Information was gathered from the questionnaire on children's socioeconomic status and the use of bed nets for the prevention of malaria. Blood samples were processed with standardized, quality-controlled methods for diagnosis of Plasmodium spp. infections. Environmental data were obtained from satellite images and digitized maps. Bayesian variogram models for spatially-explicit risk modelling of P. falciparum infection prevalence were employed, assuming for stationary and non-stationary spatial processes. FINDINGS: The overall prevalence of P. falciparum infection was 64.9%, ranging between 34.0% and 91.9% at the unit of the school. Risk factors for a P. falciparum infection included age, socioeconomic status, not sleeping under a bed net, distance to health care facilities and a number of environmental features (i.e. normalized difference vegetation index, rainfall and distance to rivers). After taking into account spatial correlation only age remained significant. Non-stationary models performed better than stationary models. CONCLUSIONS: Spatial risk profiling of P. falciparum prevalence data provides a useful tool for targeting malaria control intervention, and hence will play a role in the quest of local elimination and ultimate eradication of the disease.

One-year delayed effect of fog on malaria transmission: a time-series analysis in the rain forest area of Mengla County, south-west China.


ABSTRACT: BACKGROUND: Malaria is a major public health burden in the tropics with the potential to significantly increase in response to climate change. Analyses of data from the recent past can elucidate how short-term variations in weather factors affect malaria transmission. This study explored the impact of climate variability on the transmission of malaria in the tropical rain forest area of Mengla County, south-west China. METHODS: Ecological time-series analysis was performed on data collected between 1971 and 1999. Auto-regressive integrated moving average (ARIMA) models were used to evaluate the relationship between weather factors and malaria incidence. RESULTS: At the time scale of months, the predictors for malaria incidence included: minimum temperature, maximum temperature, and fog day frequency. The effect of minimum temperature on malaria incidence was greater in the cool months than in the hot months. The fog day frequency in October had a positive effect on malaria incidence in May of the following year. At the time scale of years, the annual fog day frequency was the only weather predictor of the annual incidence of malaria. CONCLUSIONS: Fog day frequency was for the first time found to be a predictor of malaria incidence in a rain forest area. The one-year delayed effect of fog on malaria transmission may involve providing water input and maintaining aquatic breeding sites for mosquitoes in vulnerable times when there is little rainfall in the 6-month dry seasons. These findings should be considered in the prediction of future patterns of malaria for similar tropical rain forest areas worldwide.
Full blood count and haemoglobin-containing leukocytes in children with malaria: diagnostic value and association with disease severity.

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BACKGROUND: Diligent and correct laboratory diagnosis and up-front identification of risk factors for progression to severe disease are the basis for optimal management of malaria. METHODS: Febrile children presenting to the Medical Research Unit at the Albert Schweitzer Hospital (HAS) in Lambaréné, Gabon, were assessed for malaria. Giemsa-stained thick films for qualitative and quantitative diagnosis and enumeration of malaria pigment, or haemoglobin (Hb)-containing leukocytes (PCL) were performed, and full blood counts (FBC) were generated with a Cell Dyn 3000 instrument. RESULTS: Compared to standard light microscopy of Giemsa-stained thick films, diagnosis by platelet count only, by malaria pigment-containing monocytes (PCM) only, or by pigment-containing granulocytes (PCN) only yielded sensitivities/specificities of 92%/93%; 96%/96%; and 85%/96%, respectively. The platelet count was significantly lower in children with malaria compared to those without (p < 0.001), and values showed little overlap between groups. Compared to microscopy, scatter flow cytometry as applied in the Cell-Dyn 3000(R) instrument detected significantly more patients with PCL (p < 0.01). Both PCM and PCN numbers were higher in severe versus non-severe malaria yet reached statistical significance only for PCN (p < 0.0001; PCM: p = 0.14). Of note was the presence of another, so far ill-defined pigment-containing group of phagocytic cells, identified by laser-flow cytometry as lymphocyte-like gated events, and predominantly found in children with malaria-associated anaemia. CONCLUSION: In the age group examined in the Lambaréné area, platelets are an excellent adjuvant tool to diagnose malaria. Pigment-containing leukocytes (PCL) are more readily detected by automated scatter flow cytometry than by microscopy. Automated Hb detection by an instrument as used here is a reliable diagnostic tool and correlates with disease severity. However, clinical usefulness as a prognostic tool is limited due to an overlap of PCL numbers recorded in severe versus non-severe malaria. However, this is possibly because of the instrument detection algorithm was not geared towards this task, and data lost during processing; and thus adjusting the instrument's algorithm may allow to establish a meaningful cut-off value.

Monitoring antimalarial safety and tolerability in clinical trials: a case study from Uganda.


ABSTRACT: BACKGROUND: New antimalarial regimens, including artemisinin-based combination therapies (ACTs), have been adopted widely as first-line treatment for uncomplicated malaria. Although these drugs appear to be safe and well-tolerated, experience with their use in Africa is limited and continued assessment of safety is a priority. However, no standardized guidelines for evaluating drug safety and tolerability in malaria studies exist. A system for monitoring adverse events in antimalarial trials conducted in Uganda was developed. Here the reporting system is described, and difficulties faced in analysing and interpreting the safety results are illustrated, using data from the trials. Case description Between 2002 and 2007, eleven randomized, controlled
Clinical trials were conducted to compare the efficacy, safety, and tolerability of different antimalarial regimens for treatment of uncomplicated malaria in Uganda. The approach to adverse event monitoring was similar in all studies. A total of 5,614 treatments were evaluated in 4,876 patients. Differences in baseline characteristics and patterns of adverse event reporting were noted between the sites, which limited the ability to pool and analyse data. Clinical failure following antimalarial treatment confounded associations between treatment and adverse events that were also common symptoms of malaria, particularly in areas of lower transmission intensity. Discussion and evaluation Despite prospectively evaluating for adverse events, limitations in the monitoring system were identified. New standardized guidelines for monitoring safety and tolerability in antimalarial trials are needed, which should address how to detect events of greatest importance, including serious events, those with a causal relationship to the treatment, those which impact on adherence, and events not previously reported. CONCLUSIONS: Although the World Health Organization has supported the development of pharmacovigilance systems in African countries deploying ACTs, additional guidance on adverse events monitoring in antimalarial clinical trials is needed, similar to the standardized recommendations available for assessment of drug efficacy.


Safety and tolerability of combination antimalarial therapies for uncomplicated falciparum malaria in Ugandan children.


ABSTRACT: BACKGROUND: Combination antimalarial therapy is recommended for the treatment of uncomplicated falciparum malaria in Africa; however, some concerns about the safety and tolerability of new regimens remain. This study compared the safety and tolerability of three combination antimalarial regimens in a cohort of Ugandan children. METHODS: A longitudinal, single-blind, randomized clinical trial of children was conducted between November 2004 and May 2007 in Kampala, Uganda. Upon diagnosis of the first episode of uncomplicated malaria, participants were randomized to treatment with amodiaquine + sulphadoxine-pyrimethamine (AQ+SP), artesunate + amodiaquine (AS+AQ), or artemether-lumefantrine (AL). Once randomized, participants received the same regimen for all subsequent episodes of uncomplicated malaria. Participants were actively monitored for adverse events for the first 14 days after each treatment, and then passively followed until their next study medication treatment, or withdrawal from study. Outcome measures included the risk of adverse events at 14 and 42 days after treatment. RESULTS: Of 601 enrolled children, 382 were diagnosed with at least one episode of uncomplicated malaria and were treated with study medications. The median age at treatment was 6.3 years (range 1.1 - 12.3 years). At 14 days of follow-up, AQ+SP treatment was associated with a higher risk of anorexia, weakness, and subjective fever than treatment with AL, and a higher risk of weakness, and subjective fever than treatment with AS+AQ. Treatment with AL was associated with a higher risk of elevated temperature. Repeated episodes of neutropaenia associated with AS+AQ were detected in one participant. Considering only children less than five years, those who received AQ+SP were at higher risk of developing moderate or severe anorexia and weakness than those treated with AL (anorexia: RR 3.82, 95% CI 1.59 - 9.17; weakness: RR 5.40, 95% CI 1.86 - 15.7), or AS+AQ (anorexia: RR 2.10, 95% CI 1.04 - 4.23; weakness: RR 2.26, 95% CI 1.01 - 5.05). Extending the analysis to 42 days of follow-up had little impact on the findings. CONCLUSIONS: This study confirms the safety and tolerability of AS+AQ and AL in Ugandan children, and suggests that AQ+SP is safe, but less well-tolerated, particularly in younger children. As newer antimalarial regimens are deployed, collecting data on their safety and tolerability will be essential.
The resting sites and blood-meal sources of Anopheles minimus in Taiwan.

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BACKGROUND: The WHO declared Taiwan free from malaria in 1965, but in 2003 the reporting of two introduced cases in a rural area suggested a possible local transmission of this disease. Therefore, understanding the resting sites and the blood sources of Anopheles minimus is crucial in order to provide information for implementing vector control strategies. METHODS: During a two-year survey, mosquitoes were collected in houses and their surrounding areas and at the bank of larval habitats by backpack aspirators in 17 villages in rural areas of southern and eastern Taiwan for 1 hr. On the same day, blacklight traps were hung downward overnight. Blood-fed mosquito samples were analysed by PCR. RESULTS: Of the 195 total households surveyed by backpack aspirators, no Anopheles adults were collected inside the houses, while a single Anopheles minimus and a single Anopheles maculatus were collected outside of the houses. On the same day, 23 An. minimus, two An. maculatus, two Anopheles ludlowae, two Anopheles sinensis, and one Anopheles tesselatus were collected along the bank of larval habitats. In blacklight traps hung outside of the houses in the villages, 69 An. minimus, 62 An. ludlowae, 31 An. sinensis, and 19 An. maculatus were collected. In larval habitats, 98 An. ludlowae, 64 An. minimus, 49 An. sinensis, and 14 An. maculatus were collected. Of a total of 10 blood-fed samples, An. minimus fed on four animals including bovine (60%), dogs (20%), pig (10%), and non-chicken avian (10%). CONCLUSION: Anopheles minimus, an opportunist feeder in Taiwan, was not collected inside the houses, but was found outside of the houses in villages and surrounding larval habitats. Therefore, an outdoor transmission of malaria is likely to occur and, thus, the bed nets, which are favoured for controlling the late biting of An. minimus, should be a very efficient and effective method for those local residents who sleep outdoors. Additionally, space spray of insecticides for Anopheles at night, as well as residual spray inside animal huts and selective larval habitats, are also helpful to control female adults.

Identification of glycosaminoglycan binding regions in the Plasmodium falciparum encoded placental sequestration ligand, VAR2CSA.

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BACKGROUND: Pregnancy malaria is caused by Plasmodium falciparum-infected erythrocytes binding the placental receptor chondroitin sulfate A (CSA). This results in accumulation of parasites in the placenta with severe clinical consequences for the mother and her unborn child. Women become resistant to placental malaria as antibodies are acquired which specifically target the surface of infected erythrocytes binding in the placenta. VAR2CSA is most likely the parasite-encoded protein which mediates binding to the placental receptor CSA. Several domains have been shown to bind CSA in vitro; and it is apparent that a VAR2CSA-based vaccine cannot accommodate all the CSA binding domains and serovariants. It is thus of high priority to define minimal ligand binding regions throughout the VAR2CSA molecule. METHODS: To define minimal CSA-binding
regions/peptides of VAR2CSA, a phage display library based on the entire var2csa coding region was constructed. This library was screened on immobilized CSA and cells expressing CSA resulting in a limited number of CSA-binding phages. Antibodies against these peptides were affinity purified and tested for reactivity against CSA-binding infected erythrocytes. RESULTS: The most frequently identified phages expressed peptides residing in the parts of VAR2CSA previously defined as CSA binding. In addition, most of the binding regions mapped to surface-exposed parts of VAR2CSA. The binding of a DBL2X peptide to CSA was confirmed with a synthetic peptide. Antibodies against a CSA-binding DBL2X peptide reacted with the surface of infected erythrocytes indicating that this epitope is accessible for antibodies on native VAR2CSA on infected erythrocytes. CONCLUSION: Short continuous regions of VAR2CSA with affinity for multiple types of CSA were defined. A number of these regions localize to CSA-binding domains and to surface-exposed regions within these domains and a synthetic peptide corresponding to a peptide sequence in DBL2 was shown to bind to CSA and not to CSC. It is likely that some of these epitopes are involved in native parasite CSA adhesion. However, antibodies directed against single epitopes did not inhibit parasite adhesion. This study supports phage display as a technique to identify CSA-binding regions of large proteins such as VAR2CSA.


Comparison of male reproductive success in malaria-refractory and susceptible strains of Anopheles gambiae.

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BACKGROUND: In female mosquitoes that transmit malaria, the benefits of being refractory to the Plasmodium parasite are balanced by the immunity costs in the absence of infection. Male mosquitoes, however, gain no advantage from being refractory to blood-transmitted parasites, so that any costs associated with an enhanced immune system in the males limit the evolution of female refractoriness and has practical implications for the release of transgenic males. METHODS: Aspects of the male cost of carrying Plasmodium-refractory genes were estimated by comparing the males' immune response and reproductive success among strains of Anopheles gambiae that had been selected for refractoriness or extreme susceptibility to the rodent malaria parasite, Plasmodium yoelii nigeriensis. The refractory males had a stronger melanization response than males from the susceptible line. Four traits were used as correlates of a male's reproductive success: the proportion of females that were inseminated by a fixed number of males in a cage within a fixed time frame, the proportion of females with motile sperm in their spermathecae, the proportion of ovipositing females, and the mean number of eggs per batch. RESULTS: Although there were significant differences among groups of males in sperm motility and oviposition success, these differences in male reproductive success were not associated with the refractory or susceptible male genotypes. Contrary to expectation, females mated to early emerging refractory males laid significantly more eggs per batch than females mated to later emerging susceptible males. Sperm motility and oviposition success were strongly correlated suggesting that variation in sperm motility influences female oviposition and ultimately male reproductive success. CONCLUSION: An increased melanization response in male A. gambiae does not diminish male reproductive success under the experimental protocol used in this study. That refractory males induced ovipositing females to lay more eggs than susceptible males is an interesting result for any strategy considering the release of transgenic males. That sperm motility influences female oviposition is also important for the release of transgenic males.
The insecticide resistance status of malaria vectors in the Mekong region.


ABSTRACT: BACKGROUND: Knowledge on insecticide resistance in target species is a basic requirement to guide insecticide use in malaria control programmes. Malaria transmission in the Mekong region is mainly concentrated in forested areas along the country borders, so that decisions on insecticide use should ideally be made at regional level. Consequently, cross-country monitoring of insecticide resistance is indispensable to acquire comparable baseline data on insecticide resistance. METHODS: A network for the monitoring of insecticide resistance, MALVECASIA, was set up in the Mekong region in order to assess the insecticide resistance status of the major malaria vectors in Cambodia, Laos, Thailand, and Vietnam. From 2003 till 2005, bioassays were performed on adult mosquitoes using the standard WHO susceptibility test with diagnostic concentrations of permethrin 0.75% and DDT 4%. Additional tests were done with pyrethroid insecticides applied by the different national malaria control programmes. RESULTS: Anopheles dirus s.s., the main vector in forested malaria foci, was susceptible to permethrin. However, in central Vietnam, it showed possible resistance to type II pyrethroids. In the Mekong delta, Anopheles epiroticus was highly resistant to all pyrethroid insecticides tested. It was susceptible to DDT, except near Ho Chi Minh City where it showed possible DDT resistance. In Vietnam, pyrethroid susceptible and tolerant Anopheles minimus s.l. populations were found, whereas An. minimus s.l. from Cambodia, Laos and Thailand were susceptible. Only two An. minimus s.l. populations showed DDT tolerance. Anopheles vagus was found resistant to DDT and to several pyrethroids in Vietnam and Cambodia. CONCLUSION: This is the first large scale, cross-country survey of insecticide resistance in Anopheles species in the Mekong region. A unique baseline data on insecticide resistance for the Mekong is now available, which enables the follow-up of trends in susceptibility status in the region and which will serve as the basis for further resistance management. Large differences in insecticide resistance status were observed among species and countries. In Vietnam, insecticide resistance was mainly observed in low or transmission-free areas, hence an immediate change of malaria vector control strategy is not required. Though, resistance management is important because the risk of migration of mosquitoes carrying resistance genes from non-endemic to endemic areas. Moreover, trends in resistance status should be carefully monitored and the impact of existing vector control tools on resistant populations should be assessed.

Malaria transmission pattern resilience to climatic variability is mediated by insecticide-treated nets.

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ABSTRACT: BACKGROUND: Malaria is an important public-health problem in the archipelago of Vanuatu and climate has been hypothesized as important influence on transmission risk. Beginning in 1988, a major intervention using insecticide-treated bed nets (ITNs) was implemented in the country in an attempt to reduce Plasmodium transmission. To date, no study has addressed the impact of ITN intervention in Vanuatu, how it may have modified the burden of disease, and whether there were any changes in malaria incidence that might be related to climatic drivers. Methods and findings Monthly time series (January 1983 through December 1999) of confirmed Plasmodium falciparum and Plasmodium vivax infections in the archipelago were analysed. During this 17 year period, malaria dynamics underwent a major regime shift around May 1991, following the introduction of bed
nets as a control strategy in the country. By February of 1994 disease incidence from both parasites was reduced by at least 50%, when at most 20% of the population at risk was covered by ITNs. Seasonal cycles, as expected, were strongly correlated with temperature patterns, while inter-annual cycles were associated with changes in precipitation. Following the bed net intervention, the influence of environmental drivers of malaria dynamics was reduced by 30-80% for climatic forces, and 33-54 % for other factors. A time lag of about five months was observed for the qualitative change ("regime shift") between the two parasites, the change occurring first for P. falciparum. The latter might be explained by interspecific interactions between the two parasites within the human hosts and their distinct biology, since P. vivax can relapse after a primary infection. CONCLUSION: The Vanuatu ITN programme represents an excellent example of implementing an infectious disease control programme. The distribution was undertaken to cover a large, local proportion (~80%) of people in villages where malaria was present. The successful coverage was possible because of the strategy for distribution of ITNs by prioritizing the free distribution to groups with restricted means for their acquisition, making the access to this resource equitable across the population. These results emphasize the need to implement infectious disease control programmes focusing on the most vulnerable populations.


Population analysis using the nuclear white gene detects Pliocene/Pleistocene lineage divergence within Anopheles nuneztovari in South America.

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Anopheles (Nyssorhynchus) nuneztovari Gabaldón (Diptera: Culicidae), a locally important malaria vector in some regions of South America, has been hypothesized to consist of at least two cryptic incipient species. We investigated its phylogeographic structure in several South American localities to determine the number of lineages and levels of divergence using the nuclear white gene, a marker that detected two recently diverged genotypes in the primary Neotropical malaria vector Anopheles darlingi Root. In An. nuneztovari, five distinct lineages (1-5) were elucidated: (1) populations from northeastern and central Amazonia; (2) populations from Venezuela east and west of the Andes; (3) populations from Colombia and Venezuela west of the Andes; (4) southeastern and western Amazonian Brazil populations, and (5) southeastern and western Amazonian Brazil and Bolivian populations. There was a large amount of genetic differentiation among these lineages. The deepest and earliest divergence was found between lineage 3 and lineages 1, 2 and 4, which probably accounts for the detection of lineage 3 in some earlier studies. The multiple lineages within Amazonia are partially congruent with previous mtDNA and ITS2 data, but were undetected in many earlier studies, probably because of their recent (Pliocene) divergence and the differential mutation rates of the markers. The estimates for the five lineages, interpreted as recently evolved or incipient species, date to the Pleistocene and Pliocene. We hypothesize that the diversification in An. nuneztovari is the result of an interaction between the Miocene/Pliocene marine incursion and Pleistocene climatic changes leading to refugial isolation. The identification of cryptic lineages in An. nuneztovari could have a significant impact on local vector control measures.
**Altered cord blood gammadelta T cell repertoire in Nigeria: Possible impacts of environmental factors on neonatal immunity.**


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Infectious diseases during pregnancy can impact the development of fetal immunity, leading to reduced neonatal resistance to infection and decreased responses to pediatric vaccines. Plasmodium falciparum causes placental infection in low parity pregnant women and is among the pathogens that affect fetal immunity. Recognizing the relationship between malaria and gammadelta T lymphocytes in adults, we asked whether neonatal gammadelta T cells would be altered in malaria-endemic regions as a marker for changes in fetal immunity. Our initial studies compared cord blood gammadelta T cells from deliveries to HIV-mothers in Jos (Nigeria) where malaria is endemic, or in Rome (Italy). We noted substantial differences in the Vgamma2 repertoire for cord blood collected in Jos or Rome; differences were consistent with a negative selection mechanism operating on the fetal Vgamma2 chain repertoire in neonates from Jos. A specific disruption affected the fraction of gammadelta T cells that we expect will respond to Bacille Calmette-Guerin (BCG). Fetal gammadelta T cell depletion might be a mechanism for impaired neonatal immunity and lowered responses to pediatric vaccines.

**Plasmodium falciparum origin recognition complex subunit 5: functional characterization and role in DNA replication foci formation.**

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The mechanism of DNA replication initiation and progression is poorly understood in the parasites, including human malaria parasite Plasmodium falciparum. Using bioinformatics tools and yeast complementation assay, we identified a putative homologue of Saccharomyces cerevisiae origin recognition complex subunit 5 in P. falciparum (PfORC5). PfORC5 forms distinct nuclear foci colocalized with the replication foci marker proliferating cell nuclear antigen (PfPCNA) and co-immunoprecipitates with PfPCNA during early-to-mid trophozoite stage replicating parasites. Interestingly, these proteins separate from each other at the non-replicating late schizont stage, citing the evidence of the presence of both PCNA and ORC components in replication foci during eukaryotic DNA replication. PfORC1, another ORC subunit, colocalizes with PfPCNA and PfORC5 at the beginning of DNA replication, but gets degraded at the late schizont stage, ensuring the regulation of DNA replication in the parasites. Further, we have identified putative PCNA-interacting protein box in PfORC1 that may explain in part the colocalization of PfORC and PfPCNA. Additionally, use of specific DNA replication inhibitor hydroxyurea affects ORC5/PCNA foci formation and parasitic growth. These results strongly favour replication factory model in the parasites and confer great potential to understand the co-ordination between ORC and PCNA during eukaryotic DNA replication in general.
Targeted mutagenesis of the ring exported protein-1 of Plasmodium falciparum disrupts the architecture of Maurer's cleft organelles.


Mature red blood cells have no internal trafficking machinery so the intraerythrocytic malaria parasite, Plasmodium falciparum, establishes its own transport system to export virulence factors to the red blood cell surface. Maurer's clefts are parasite-derived membranous structures that form an important component of this exported secretory system. A protein with sequence similarity to a Golgi tethering protein, referred to as ring-exported protein-1 (REX1), is associated with Maurer's clefts. A REX1-GFP chimera is trafficked to the Maurer's clefts and preferentially associates with the edges of these structures, as well as with vesicle-like structures and with stalk-like extensions that are involved in tethering the Maurer's clefts to other membranes. We have generated transfected P. falciparum expressing REX1 truncations or deletion. Electron microscopy reveals that the Maurer's clefts of REX1 truncation mutants have stacked cisternae, while the 3D7 parent line has unstacked Maurer's clefts. D10 parasites, which have lost the right end of chromosome 9, including the rex1 gene, also display Maurer's clefts with stacked cisternae. Expression of full-length REX1-GFP in D10 parasites restores the 3D7-type unstacked Maurer's cleft phenotype. These studies reveal the importance of the REX1 protein in determining the ultrastructure of the Maurer's cleft system.

PbSR is synthesized in macrogametocytes and involved in formation of the malaria crystalloids.

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Crystalloids are transient organelles that form in developing malaria ookinete and disappear after oocinete-to-oocyst transition. Their origins and functions remain poorly understood. The Plasmodium berghei scavenger receptor-like protein PbSR is essential for mosquito-to-host transmission of the parasite: PbSR knockout parasites produce normal numbers of oocysts that fail to form sporozoites, pointing to a role for PbSR in the oocyst during sporogony. Here, using fluorescent protein tagging and targeted gene disruption, we show that PbSR is synthesized in macrogametocytes, gets targeted to the crystalloids of developing ookinete and is involved in crystalloid formation. While oocyst sporulation rates of PbSR knockout parasites are highly reduced in parasite-infected mosquitoes, sporulation rates in vitro are not adversely affected, supporting the view that mosquito factors could be involved in the PbSR loss-of-function phenotype. These findings are the first to identify a parasite protein involved with the crystalloid organelle, and suggest a novel protein-trafficking mechanism to deliver PbSR to the oocysts.

Plasmodium falciparum Sec24 marks transitional ER that exports a model cargo via a diacidic motif.

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Exit from the endoplasmic reticulum (ER) often occurs at distinct sites of vesicle formation known as transitional ER (tER) that are enriched for COPII vesicle coat proteins. We have characterized the organization of ER export in the malaria parasite, Plasmodium falciparum, by examining the localization of two components of the COPII machinery, PfSec12 and PfSec24a. PfSec12 was found throughout the ER, whereas the COPII cargo adaptor, PfSec24a, was concentrated at distinct foci that likely correspond to tER sites. These foci were closely apposed to cis-Golgi sites marked by PfGRASP-GFP, and upon treatment with brefeldin A they accumulated a model cargo protein via a process dependent on the presence of an intact diacidic export motif. Our data suggest that the cargo-binding function of PfSec24a is conserved and that accumulation of cargo in discrete tER sites depends upon positive sorting signals. Furthermore, the number and position of tER sites with respect to the cis-Golgi suggests a co-ordinated biogenesis of these domains.


Investigation of the Redox Behavior of Ferroquine, a New Antimalarial.

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Ferroquine (FQ or SR97193) is a unique ferrocene antimalarial drug candidate which just entered phase IIb clinical trials in autumn 2007. FQ is able to overcome the chloroquine (CQ) resistance problem, an important limit to the control of Plasmodium falciparum, the principal causative agent of malaria. However, as for other therapeutic agents such as chloroquine (CQ) and artemisin, its mechanism of action remains partially unknown. Most investigations have so far focused on comparing the activity of FQ to that of CQ in order to understand how the ferrocene core contributes to a stronger antiplasmodial activity. Studies have already shown that the ferrocene altered the shape, volume, lipophilicity, basicity and also electronic profile of the parent molecule and, hence, its pharmacodynamic behavior. However, few investigations have been undertaken to probe the real contribution of redox properties of the ferrocene (iron(II))/ferricinium (iron(III)) system in FQ as reported in this article. In our experimental and theoretical approach, we considered the redox profile of the ferrocene core of FQ in the specific conditions (acidic and oxidizing) of the parasitic digestive vacuole as a possible discriminating property from CQ in the antimalarial activity.


Processing of the circumsporozoite protein in infected hepatocytes is not dependent on aspartic proteases.

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CD8(+) T cells play a major role in the protective immune response against the liver stage of malaria. It was previously shown that the circumsporozoite protein (CSP) is processed and presented to specific T cells by both traversed and
infected hepatocytes, but their respective antigen processing requirements were not completely defined. In the present study, we show that in vitro processing of the Plasmodium berghei CSP by infected mouse primary hepatocytes is exclusively dependent on proteasomes, while aspartic proteases are also needed in the case of traversed hepatocytes.


**Cellular immune responses to recombinant Plasmodium vivax tryptophan-rich antigen (PvTRAg) among individuals exposed to vivax malaria.**

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Plasmodium vivax, the most widespread species of human malaria parasite responsible for 70-80 million cases each year requires a vaccine. In recent years, many potential vaccine candidate antigens have been identified from P. vivax including PvTRAg. We describe here cellular immune response to recombinant PvTRAg expressed in Escherichia coli. The in vitro stimulation of PBMCs derived from P. vivax-exposed individuals (n = 16) showed strong proliferative response (SI > 2.2) to PvTRAg as compared to PBMCs from normal healthy controls (n = 8). Although both Th1 (IFN-gamma, TNF-alpha and IL-12) and Th2 (IL-4 and IL-10) cytokines were secreted by the PBMCs of the P. vivax-exposed individuals in response to PvTRAg, the overall response was more inclined towards Th2. In conclusion, recombinant PvTRAg was found to elicit strong cellular immune response among the P. vivax-exposed individuals.


**Effect of GPI anchor moiety on the immunogenicity of DNA plasmids encoding the 19-kDa C-terminal portion of Plasmodium falciparum MSP-1.**

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The glycosylphosphatidylinositol (GPI)-anchored Plasmodium falciparum merozoite surface protein 1 (MSP-1) is a widely studied malaria vaccine candidate. The C-terminal 19-kDa portion of MSP-1 (MSP-1(19)) is of particular interest because this polypeptide moiety remains bound to the parasite even after erythrocyte invasion, while the remainder of MSP-1 is shed during invasion. Studies have shown that antibodies against MSP-1(19) inhibit merozoite invasion of erythrocytes efficiently, and that MSP-1(19) produces protective immunity in mice and monkeys. To investigate the efficacy of MSP-1(19) DNA vaccine and role of GPI anchor moiety in the immunogenicity of MSP-1(19), we constructed expression vectors that produce MSP-1(19) as either secretory or GPI-anchored polypeptide. Both constructs efficiently expressed MSP-1(19) in transfected HEK-293 cells. While the recombinant plasmid lacking GPI anchor signal sequence expressed MSP-1(19) mainly as secreted polypeptide, that containing GPI anchor signal sequence produced GPI-anchored MSP-1(19) on cell surface. In immunized mice, both constructs produced substantial levels of MSP-1(19)-specific IgG1, IgG2a, IgG2b, IgG3, IgA and IgM antibodies. In both cases, the IgG1 level was significantly higher than other isotypes. Interestingly, the plasmid containing GPI anchor signal sequence produced significantly higher levels of IgG2a and IgG2b than the plasmid that lacks GPI signal sequence.
Atovaquone-proguanil resistance in imported falciparum malaria in a young child.

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We discuss a case of atovaquone-proguanil treatment failure in a child from Mozambique, recently arrived in North America. Four weeks after completing therapy, symptomatic parasitemia recurred, caused by Plasmodium falciparum parasites bearing a Tyr268Ser mutation in cytochrome b. We review the literature concerning atovaquone-proguanil resistance, and emphasize the importance of follow-up and consideration of resistance where patients have relapsed symptoms.

Cerebral Malaria in Children Is Associated With Long-term Cognitive Impairment.

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OBJECTIVE. Cerebral malaria affects >785,000 African children every year. We previously documented an increased frequency of cognitive impairment in children with cerebral malaria 6 months after their initial malaria episode. This study was conducted to determine the long-term effects of cerebral malaria on the cognitive function of these children. METHODS. Children who were 5 to 12 years of age and presented to Mulago Hospital, Kampala, Uganda, with cerebral malaria (n = 44) or uncomplicated malaria (n = 54), along with healthy, asymptomatic community children (n = 89), were enrolled in a prospective cohort study of cognition. Cognitive testing was performed at enrollment and 2 years later. The primary outcome was presence of a deficit in >/=1 of 3 cognitive areas tested. RESULTS. At 2-year follow-up testing, 26.3% of children with cerebral malaria and 12.5% with uncomplicated malaria had cognitive deficits in >/=1 area, as compared with 7.6% of community children. Deficits in children with cerebral malaria were primarily in the area of attention (cerebral malaria, 18.4%, vs community children, 2.5%). After adjustment for age, gender, nutrition, home environment, and school level, children with cerebral malaria had a 3.67-fold increased risk for a cognitive deficit compared with community children. Cognitive impairment at 2-year follow-up was associated with hyporeflexia on admission and neurologic deficits 3 months after discharge. CONCLUSIONS: Cerebral malaria is associated with long-term cognitive impairments in 1 of 4 child survivors. Future studies should investigate the mechanisms involved so as to develop interventions aimed at prevention and rehabilitation.

Antimalarial activity of anthothecol derived from Khaya anthotheca (Meliaceae).

Lee SE, Kim MR, Kim JH, Takeoka GR, Kim TW, Park BS.

Antimalarial activity of anthothecol, a limonoid of Khaya anthotheca (Meliaceae) against Plasmodium falciparum was tested using a [(3)H]-hypoxanthine and 48h culture assay in vitro. Anthothecol showed potent antimalarial activity against malaria parasites with IC(50) values of 1.4 and 0.17microM using two different
assays. Also, gedunin had antimalarial activity with IC(50) values of 3.1 and 0.14μM. However, the citrus limonoids, limonin and obacunone did not show any antimalarial activity. The antimalarial activities were compared with the three currently used antimalarial medicines quinine, chloroquine and artemisinin.


**Gametogenesis in malaria parasites is mediated by the cGMP-dependent protein kinase.**


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Malaria parasite transmission requires differentiation of male and female gametocytes into gametes within a mosquito following a blood meal. A mosquito-derived molecule, xanthurenic acid (XA), can trigger gametogenesis, but the signalling events controlling this process in the human malaria parasite Plasmodium falciparum remain unknown. A role for cGMP was revealed by our observation that zaprinast (an inhibitor of phosphodiesterases that hydrolyse cGMP) stimulates gametogenesis in the absence of XA. Using cGMP-dependent protein kinase (PKG) inhibitors in conjunction with transgenic parasites expressing an inhibitor-insensitive mutant PKG enzyme, we demonstrate that PKG is essential for XA- and zaprinast-induced gametogenesis. Furthermore, we show that intracellular calcium (Ca2+) is required for differentiation and acts downstream of or in parallel with PKG activation. This work defines a key role for PKG in gametogenesis, elucidates the hierarchy of signalling events governing this process in P. falciparum, and demonstrates the feasibility of selective inhibition of a crucial regulator of the malaria parasite life cycle.


**Multidrug-resistant Plasmodium vivax associated with severe and fatal malaria: a prospective study in Papua, Indonesia.**

Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, Lampah DA, Price RN.


BACKGROUND: Multidrug-resistant Plasmodium vivax (Pv) is widespread in eastern Indonesia, and emerging elsewhere in Asia-Pacific and South America, but is generally regarded as a benign disease. The aim of the study was to review the spectrum of disease associated with malaria due to Pv and P. falciparum (Pf) in patients presenting to a hospital in Timika, southern Papua, Indonesia. METHODS AND FINDINGS: Data were prospectively collected from all patients attending the outpatient and inpatient departments of the only hospital in the region using systematic data forms and hospital computerised records. Between January 2004 and December 2007, clinical malaria was present in 16% (60,226/373,450) of hospital outpatients and 32% (12,171/37,800) of inpatients. Among patients admitted with slide-confirmed malaria, 64% of patients had Pf, 24% Pv, and 10.5% mixed infections. The proportion of malarial admissions attributable to Pv rose to 47% (415/887) in children under 1 y of age. Severe disease was present in 2,634 (22%) inpatients with malaria, with the risk greater among Pv (23% [675/2,937]) infections compared to Pf (20% [1,570/7,817]; odds ratio [OR] = 1.19 [95% confidence interval (CI) 1.08-1.32], p = 0.001), and greatest in patients with mixed infections (31% [389/1,273]); overall p < 0.0001. Severe anaemia (haemoglobin < 5 g/dl) was the major complication associated with Pv, accounting
for 87% (589/675) of severe disease compared to 73% (1,144/1,570) of severe manifestations with Pf (p < 0.001). Pure Pv infection was also present in 78 patients with respiratory distress and 42 patients with coma. In total 242 (2.0%) patients with malaria died during admission: 2.2% (167/7,722) with Pf, 1.6% (46/2,916) with Pv, and 2.3% (29/1260) with mixed infections (p = 0.126).

CONCLUSIONS: In this region with established high-grade chloroquine resistance to both Pv and Pf, Pv is associated with severe and fatal malaria particularly in young children. The epidemiology of P. vivax needs to be re-examined elsewhere where chloroquine resistance is increasing.


Plasmodium vivax and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea.

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BACKGROUND: Severe malaria (SM) is classically associated with Plasmodium falciparum infection. Little information is available on the contribution of P. vivax to severe disease. There are some epidemiological indications that P. vivax or mixed infections protect against complications and deaths. A large morbidity surveillance conducted in an area where the four species coexist allowed us to estimate rates of SM among patients infected with one or several species. METHODS AND FINDINGS: This was a prospective cohort study conducted within the framework of the Malaria Vaccine Epidemiology and Evaluation Project. All presumptive malaria cases presenting at two rural health facilities over an 8-y period were investigated with history taking, clinical examination, and laboratory assessment. Case definition of SM was based on the World Health Organization (WHO) criteria adapted for the setting (i.e., clinical diagnosis of malaria associated with asexual blood stage parasitaemia and recent history of fits, or coma, or respiratory distress, or anaemia [haemoglobin < 5 g/dl]). Out of 17,201 presumptive malaria cases, 9,537 (55%) had a confirmed Plasmodium parasitaemia. Among those, 6.2% (95% confidence interval [CI] 5.7%-6.8%) fulfilled the case definition of SM, most of them in children <5 y. In this age group, the proportion of SM was 11.7% (10.4%-13.2%) for P. falciparum, 8.8% (7.1%-10.7%) for P. vivax, and 17.3% (11.7%-24.2%) for mixed P. falciparum and P. vivax infections. P. vivax SM presented more often with respiratory distress than did P. falciparum (60% versus 41%, p = 0.002), but less often with anaemia (19% versus 41%, p = 0.0001). CONCLUSION: P. vivax monoinfections as well as mixed Plasmodium infections are associated with SM. There is no indication that mixed infections protected against SM. Interventions targeted toward P. falciparum only might be insufficient to eliminate the overall malaria burden, and especially severe disease, in areas where P. falciparum and P. vivax coexist.


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Author SummaryInfectious diseases remain a major health and socioeconomic problem in many low-income countries, particularly in sub-Saharan Africa. For many years,
the three most devastating diseases, HIV/AIDS, malaria, and tuberculosis (TB) have received most of the world's attention. However, in rural and impoverished urban areas, a number of infectious diseases remain neglected and cause massive suffering. It has been calculated that a group of 13 neglected infectious diseases affects over one billion people, corresponding to a sixth of the world's population. These diseases include infections with different types of worms and parasites, cholera, and sleeping sickness, and can cause significant mortality and severe disabilities in low-income countries. For most of these diseases, vaccines are either not available, poorly effective, or too expensive. Moreover, these neglected diseases often occur in individuals who are also affected by HIV/AIDS, malaria, or TB, making the problem even more serious and indicating that co-infections are the rule rather than the exception in many geographical areas. To address the importance of combating co-infections, scientists from 14 different countries in Africa and Europe met in Addis Ababa, Ethiopia, on September 9-11, 2007. The message coming from these scientists is that the only possibility for winning the fight against infections in low-income countries is by studying, in the most global way possible, the complex interaction between different infections and conditions of malnourishment. The new scientific and technical tools of the post-genomic era can allow us to reach this goal. However, a concomitant effort in improving education and social conditions will be needed to make the scientific findings effective.


An open label, randomised trial of artesunate+amodiaquine, artesunate+chlorproguanil-dapsone and artemether-lumefantrine for the treatment of uncomplicated malaria.


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BACKGROUND: Artesunate+amodiaquine (AS+AQ) and artemether-lumefantrine (AL) are now the most frequently recommended first line treatments for uncomplicated malaria in Africa. Artesunate+chlorproguanil-dapsone (AS+CD) was a potential alternative for treatment of uncomplicated malaria. A comparison of the efficacy and safety of these three drug combinations was necessary to make evidence based drug treatment policies. METHODS: Five hundred and thirty-four, glucose-6-phosphate dehydrogenase (G6PD) normal children were randomised in blocks of 15 to the AS+AQ, AL or AS+CD groups. Administration of study drugs was supervised by project staff and the children were followed up at home on days 1,2,3,7,14 and 28 post treatment. Parasitological and clinical failures and adverse events were compared between the study groups. MAIN FINDINGS: In a per-protocol analysis, the parasitological and clinical failure rate at day 28 post treatment (PCF28) was lower in the AS+AQ group compared to the AL or AS+CD groups (corrected for re-infections: 6.6% vs 13.8% and 13.8% respectively, p = 0.08; uncorrected: 14.6% vs 27.6% and 28.1% respectively, p = 0.005). In the intention to treat analysis, the rate of early treatment failure was high in all three groups (AS+AQ 13.3%; AL 15.2%; and AS+CD 9.3%, p = 0.2) primarily due to vomiting. However, the PCF28 corrected for re-infection was lower, though not significantly, in the AS+AQ group compared to the AL or the AS+CD groups (AS+AQ 18.3%; AL 24.2%; AS+CD 20.8%, p = 0.4) The incidence of adverse events was comparable between the groups. CONCLUSIONS: AS+AQ is an appropriate first line treatment for uncomplicated malaria in Ghana and possibly in the neighbouring countries in West Africa. The effectiveness of AL in routine programme conditions needs to be studied further in West Africa. TRIAL REGISTRATION: ClinicalTrials.gov NCT00119145.
Depletion of Plasmodium berghei plasmoredoxin reveals a non-essential role for life cycle progression of the malaria parasite.

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Proliferation of the pathogenic Plasmodium asexual blood stages in host erythrocytes requires an exquisite capacity to protect the malaria parasite against oxidative stress. This function is achieved by a complex antioxidant defence system composed of redox-active proteins and low MW antioxidants. Here, we disrupted the P. berghei plasmoredoxin gene that encodes a parasite-specific 22 kDa member of the thioredoxin superfamily. The successful generation of plasmoredoxin knockout mutants in the rodent model malaria parasite and phenotypic analysis during life cycle progression revealed a non-vital role in vivo. Our findings suggest that plasmoredoxin fulfils a specialized and dispensable role for Plasmodium and highlights the need for target validation to inform drug development strategies.

Novel peptide marker corresponding to salivary protein gSG6 potentially identifies exposure to Anopheles bites.


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BACKGROUND: In order to improve malaria control, and under the aegis of WHO recommendations, many efforts are being devoted to developing new tools for identifying geographic areas with high risk of parasite transmission. Evaluation of the human antibody response to arthropod salivary proteins could be an epidemiological indicator of exposure to vector bites, and therefore to risk of pathogen transmission. In the case of malaria, which is transmitted only by anopheline mosquitoes, maximal specificity could be achieved through identification of immunogenic proteins specific to the Anopheles genus. The objective of the present study was to determine whether the IgG response to the Anopheles gambiae gSG6 protein, from its recombinant form to derived synthetic peptides, could be an immunological marker of exposure specific to Anopheles gambiae bites. METHODOLOGY/PRINCIPAL FINDINGS: Specific IgG antibodies to recombinant gSG6 protein were observed in children living in a Senegalese area exposed to malaria. With the objective of optimizing Anopheles specificity and reproducibility, we designed five gSG6-based peptide sequences using a bioinformatic approach, taking into consideration i) their potential antigenic properties and ii) the absence of cross-reactivity with protein sequences of other arthropods/organisms. The specific anti-peptide IgG antibody response was evaluated in exposed children. The five gSG6 peptides showed differing antigenic properties, with gSG6-P1 and gSG6-P2 exhibiting the highest antigenicity. However, a significant increase in the specific IgG response during the rainy season and a positive association between the IgG level and the level of exposure to Anopheles gambiae bites was significant only for gSG6-P1.

CONCLUSIONS/SIGNIFICANCE: This step-by-step approach suggests that gSG6-P1 could be an optimal candidate marker for evaluating exposure to Anopheles gambiae bites. This marker could be employed as a geographic indicator, like remote sensing techniques, for mapping the risk of malaria. It could also represent a direct criterion of efficacy in evaluation of vector control strategies.
Genome-wide compensatory changes accompany drug-selected mutations in the Plasmodium falciparum crt gene.

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Mutations in PfCRT (Plasmodium falciparum chloroquine-resistant transporter), particularly the substitution at amino acid position 76, confer chloroquine (CQ) resistance in P. falciparum. Point mutations in the homolog of the mammalian multidrug resistance gene (pfmdr1) can also modulate the levels of CQ response. Moreover, parasites with the same pfcrt and pfmdr1 alleles exhibit a wide range of drug sensitivity, suggesting that additional genes contribute to levels of CQ resistance (CQR). Reemergence of CQ sensitive parasites after cessation of CQ use indicates that changes in PfCRT are deleterious to the parasite. Some CQR parasites, however, persist in the field and grow well in culture, which may reflect adaptive changes in the parasite genome to compensate for the mutations in PfCRT. Using three isogenic clones that have different drug resistance profiles corresponding to unique mutations in the pfcrt gene (106/1(K76), 106/1(76I), and 106/(76I-352K)), we investigated changes in gene expression in these parasites grown with and without CQ. We also conducted hybridizations of genomic DNA to identify copy number (CN) changes in parasite genes. RNA transcript levels from 45 genes were significantly altered in one or both mutants relative to the parent line, 106/1(K76). Most of the up-regulated genes are involved in invasion, cell growth and development, signal transduction, and transport activities. Of particular interest are genes encoding proteins involved in transport and/or regulation of cytoplasmic or compartmental pH such as the V-type H(+) pumping pyrophosphatase 2 (PfVP2), Ca(2+)/H(+) antiporter VCX1, a putative drug transporter and CN changes in pfmdr1. These changes may represent adaptations to altered functionality of PfCRT, a predicted member of drug/metabolite transporter superfamily found on the parasite food vacuole (FV) membrane. Further investigation of these genes may shed light on how the parasite compensates for functional changes accompanying drug resistance mutations in a gene coding for a membrane/drug transporter.

Associations between Burkitt lymphoma among children in Malawi and infection with HIV, EBV and malaria: results from a case-control study.


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BACKGROUND: Burkitt lymphoma, a childhood cancer common in parts of sub-Saharan Africa, has been associated with Epstein Barr Virus (EBV) and malaria, but its association with human immunodeficiency virus (HIV) is not clear.

METHODOLOGY/PRINCIPAL FINDINGS: We conducted a case-control study of Burkitt lymphoma among children (aged < or = 15 years) admitted to the pediatric oncology unit in Blantyre, Malawi between July 2005 and July 2006. Cases were 148 children diagnosed with Burkitt lymphoma and controls were 104 children admitted with non-malignant conditions or cancers other than hematological malignancies and Kaposi sarcoma. Interviews were conducted and serological samples tested for antibodies against HIV, EBV and malaria. Odds ratios for Burkitt lymphoma were estimated using unconditional logistic regression adjusting for sex, age, and...
residential district. Cases had a mean age of 7.1 years and 60% were male. Cases were more likely than controls to be HIV positive (Odds ratio (OR) = 12.4, 95% Confidence Interval (CI) 1.3 to 116.2, p = 0.03). ORs for Burkitt lymphoma increased with increasing antibody titers against EBV (p = 0.001) and malaria (p = 0.01). Among HIV negative participants, cases were thirteen times more likely than controls to have raised levels of both EBV and malaria antibodies (OR = 13.2; 95% CI 3.8 to 46.6; p = 0.001). Reported use of mosquito nets was associated with a lower risk of Burkitt lymphoma (OR = 0.2, 95% CI, 0.03 to 0.9, p = 0.04). CONCLUSIONS: Our findings support prior evidence that EBV and malaria act jointly in the pathogenesis of Burkitt lymphoma, suggesting that malaria prevention may decrease the risk of Burkitt lymphoma. HIV may also play a role in the etiology of this childhood tumor.


**Safety profile of L-arginine infusion in moderately severe falciparum malaria.**


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BACKGROUND: L-arginine infusion improves endothelial function in malaria but its safety profile has not been described in detail. We assessed clinical symptoms, hemodynamic status and biochemical parameters before and after a single L-arginine infusion in adults with moderately severe malaria. METHODOLOGY AND FINDINGS: In an ascending dose study, adjunctive intravenous L-arginine hydrochloride was infused over 30 minutes in doses of 3 g, 6 g and 12 g to three separate groups of 10 adults hospitalized with moderately severe Plasmodium falciparum malaria in addition to standard quinine therapy. Symptoms, vital signs and selected biochemical measurements were assessed before, during, and for 24 hours after infusion. No new or worsening symptoms developed apart from mild discomfort at the intravenous cannula site in two patients. There was a dose-response relationship between increasing mg/kg dose and the maximum decrease in systolic (rho = 0.463; Spearman's, p = 0.02) and diastolic blood pressure (r = 0.42; Pearson's, p = 0.02), and with the maximum increment in blood potassium (r = 0.70, p<0.001) and maximum decrement in bicarbonate concentrations (r = 0.53, p = 0.003) and pH (r = 0.48, p = 0.007). At the highest dose (12 g), changes in blood pressure and electrolytes were not clinically significant, with a mean maximum decrease in mean arterial blood pressure of 6 mmHg (range: 0-11; p<0.001), mean maximal increase in potassium of 0.5 mmol/L (range 0.2-0.7 mmol/L; p<0.001), and mean maximal decrease in bicarbonate of 3 mEq/L (range 1-7; p<0.01) without a significant change in pH. There was no significant dose-response relationship with blood phosphate, lactate, anion gap and glucose concentrations. All patients had an uncomplicated clinical recovery. CONCLUSIONS/SIGNIFICANCE: Infusion of up to 12 g of intravenous L-arginine hydrochloride over 30 minutes is well tolerated in adults with moderately severe malaria, with no clinically important changes in hemodynamic or biochemical status. Trials of adjunctive L-arginine can be extended to phase 2 studies in severe malaria. TRIAL REGISTRATION: ClinicalTrials.gov NCT00147368.


**A Plasmodium falciparum host-targeting motif functions in export during blood stage infection of the rodent malarial parasite Plasmodium berghei.**

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Plasmodium falciparum (P. falciparum) secretes hundreds of proteins— including major virulence proteins—into the host erythrocyte. In order to reach the host cytoplasm, most P. falciparum proteins contain an N terminal host-targeting (HT) motif composed of 11 amino acids. In silico analyses have suggested that the HT motif is conserved throughout the Plasmodium species but experimental evidence only exists for P. falciparum. Here, we show that in the rodent malaria parasite Plasmodium berghei (P. berghei) a reporter-like green fluorescent protein expressed by the parasite can be exported to the erythrocyte cytoplasm in a HT-specific manner. This provides the first experimental proof that the HT motif can function as a signal for protein delivery to the erythrocyte across Plasmodium species. Further, it suggests that P. berghei may serve as a model for validation of P. falciparum secretome proteins. We also show that tubovesicular membranes extend from the vacuolar parasite into the erythrocyte cytoplasm and speculate that these structures may facilitate protein export to the erythrocyte.


Marine actinomycetes: a new source of compounds against the human malaria parasite.


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BACKGROUND: Malaria continues to be a devastating parasitic disease that causes the death of 2 million individuals annually. The increase in multi-drug resistance together with the absence of an efficient vaccine hastens the need for speedy and comprehensive antimalarial drug discovery and development. Throughout history, traditional herbal remedies or natural products have been a reliable source of antimalarial agents, e.g. quinine and artemisinin. Today, one emerging source of small molecule drug leads is the world's oceans. Included among the source of marine natural products are marine microorganisms such as the recently described actinomycete. Members of the genus Salinispora have yielded a wealth of new secondary metabolites including salinosporamide A, a molecule currently advancing through clinical trials as an anticancer agent. Because of the biological activity of metabolites being isolated from marine microorganisms, our group became interested in exploring the potential efficacy of these compounds against the malaria parasite. METHODS: We screened 80 bacterial crude extracts for their activity against malaria growth. We established that the pure compound, salinosporamide A, produced by the marine actinomycete, Salinispora tropica, shows strong inhibitory activity against the erythrocytic stages of the parasite cycle. Biochemical experiments support the likely inhibition of the parasite 20S proteasome. Crystal structure modeling of salinosporamide A and the parasite catalytic 20S subunit further confirm this hypothesis. Ultimately we showed that salinosporamide A protected mice against deadly malaria infection when administered at an extremely low dosage. CONCLUSION: These findings underline the potential of secondary metabolites, derived from marine microorganisms, to inhibit Plasmodium growth. More specifically, we highlight the effect of proteasome inhibitors such as salinosporamide A on in vitro and in vivo parasite development. Salinosporamide A (NPI-0052) now being advanced to phase I trials for the treatment of refractory multiple myeloma will need to be further explored to evaluate the safety profile for its use against malaria.


The malaria secretome: from algorithms to essential function in blood stage infection.

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The malaria agent Plasmodium falciparum is predicted to export a "secretome" of several hundred proteins to remodel the host erythrocyte. Prediction of protein export is based on the presence of an ER-type signal sequence and a downstream Host-Targeting (HT) motif (which is similar to, but distinct from, the closely related Plasmodium Export Element [PEXEL]). Previous attempts to determine the entire secretome, using either the HT-motif or the PEXEL, have yielded large sets of proteins, which have not been comprehensively tested. We present here an expanded secretome that is optimized for both P. falciparum signal sequences and the HT-motif. From the most conservative of these three secretome predictions, we identify 11 proteins that are preserved across human- and rodent-infecting Plasmodium species. The conservation of these proteins likely indicates that they perform important functions in the interaction with and remodeling of the host erythrocyte important for all Plasmodium parasites. Using the piggyBac transposition system, we validate their export and find a positive prediction rate of approximately 70%. Even for proteins identified by all secretomes, the positive prediction rate is not likely to exceed approximately 75%. Attempted deletions of the genes encoding the conserved exported proteins were not successful, but additional functional analyses revealed the first conserved secretome function. This gave new insight into mechanisms for the assembly of the parasite-induced tubovesicular network needed for import of nutrients into the infected erythrocyte. Thus, genomic screens combined with functional assays provide unexpected and fundamental insights into host remodeling by this major human pathogen.


A sporozoite asparagine-rich protein controls initiation of Plasmodium liver stage development.

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Plasmodium sporozoites invade host hepatocytes and develop as liver stages (LS) before the onset of erythrocytic infection and malaria symptoms. LS are clinically silent, and constitute ideal targets for causal prophylactic drugs and vaccines. The molecular and cellular mechanisms underlying LS development remain poorly characterized. Here we describe a conserved Plasmodium asparagine-rich protein that is specifically expressed in sporozoites and liver stages. Gene disruption in Plasmodium berghei results in complete loss of sporozoite infectivity to rodents, due to early developmental arrest after invasion of hepatocytes. Mutant sporozoites productively invade host cells by forming a parasitophorous vacuole (PV), but subsequent remodelling of the membrane of the PV (PVM) is impaired as a consequence of dramatic down-regulation of genes encoding PVM-resident proteins. These early arrested mutants confer only limited protective immunity in immunized animals. Our results demonstrate the role of an asparagine-rich protein as a key regulator of Plasmodium sporozoite gene expression and LS development, and suggest a requirement of partial LS maturation to induce optimal protective immune responses against malaria pre-erythrocytic stages. These findings have important implications for the development of genetically attenuated parasites as a vaccine approach.

The dynamics of acute malaria infections. I. Effect of the parasite's red blood cell preference.

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What determines the dynamics of parasite and anaemia during acute primary malaria infections? Why do some strains of malaria reach higher densities and cause greater anaemia than others? The conventional view is that the fastest replicating parasites reach the highest densities and cause the greatest loss of red blood cells (RBCs). Other current hypotheses suggest that the maximum parasite density is achieved by strains that either elicit the weakest immune responses or infect the youngest RBCs (reticulocytes). Yet another hypothesis is a simple resource limitation model where the peak parasite density and the maximum anaemia (percentage loss of RBCs) during the acute phase of infection equal the fraction of RBCs that the malaria parasite can infect. We discriminate between these hypotheses by developing a mathematical model of acute malaria infections and confronting it with experimental data from the rodent malaria parasite Plasmodium chabaudi. We show that the resource limitation model can explain the initial dynamics of infection of mice with different strains of this parasite. We further test the model by showing that without modification it closely reproduces the dynamics of competing strains in mixed infections of mice with these strains of P. chabaudi. Our results suggest that a simple resource limitation is capable of capturing the basic features of the dynamics of both parasite and RBC loss during acute malaria infections of mice with P. chabaudi, suggesting that it might be worth exploring if similar results might hold for other acute malaria infections, including those of humans.


In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen.


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The growing resistance to current first-line antimalarial drugs represents a major health challenge. To facilitate the discovery of new antimalarials, we have implemented an efficient and robust high-throughput cell-based screen (1,536-well format) based on proliferation of Plasmodium falciparum (Pf) in erythrocytes. From a screen of approximately 1.7 million compounds, we identified a diverse collection of approximately 6,000 small molecules comprised of >530 distinct scaffolds, all of which show potent antimalarial activity (<1.25 μM). Most known antimalarials were identified in this screen, thus validating our approach. In addition, we identified many novel chemical scaffolds, which likely act through both known and novel pathways. We further show that in some cases the mechanism of action of these antimalarials can be determined by in silico compound activity profiling. This method uses large datasets from unrelated cellular and biochemical screens and the guilt-by-association principle to predict which cellular pathway and/or protein target is being inhibited by select compounds. In addition, the screening method has the potential to provide the malaria community with many new starting points for the development of biological probes and drugs with novel antiparasitic activities.
Comparative molecular modeling of Anopheles gambiae CYP6Z1, a mosquito P450 capable of metabolizing DDT.

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One of the challenges faced in malarial control is the acquisition of insecticide resistance that has developed in mosquitoes that are vectors for this disease. Anopheles gambiae, which has been the major mosquito vector of the malaria parasite Plasmodium falciparum in Africa, has over the years developed resistance to insecticides including dieldrin, 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane (DDT), and pyrethroids. Previous microarray studies using fragments of 230 An. gambiae genes identified five P450 loci, including CYP4C27, CYP4H15, CYP6Z1, CYP6Z2, and CYP12F1, that showed significantly higher expression in the DDT-resistant ZAN/U strain compared with the DDT-susceptible Kisumu strain. To predict whether either of the CYP6Z1 and CYP6Z2 proteins might potentially metabolize DDT, we generated and compared molecular models of these two proteins with and without DDT docked in their catalytic sites. This comparison indicated that, although these two CYP6Z proteins share high sequence identity, their metabolic profiles were likely to differ dramatically from the larger catalytic site of CYP6Z1, potentially involved in DDT metabolism, and the more constrained catalytic site of CYP6Z2, not likely to metabolize DDT. Heterologous expressions of these proteins have corroborated these predictions: only CYP6Z1 is capable of metabolizing DDT. Overlays of these models indicate that slight differences in the backbone of SRS1 and variations of side chains in SRS2 and SRS4 account for the significant differences in their catalytic site volumes and DDT-metabolic capacities. These data identify CYP6Z1 as one important target for inhibitor design aimed at inactivating insecticide-metabolizing P450s in natural populations of this malarial mosquito.

Specific DNA-binding by apicomplexan AP2 transcription factors.

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Malaria remains one of the most prevalent infectious diseases worldwide, affecting more than half a billion people annually. Despite many years of research, the mechanisms underlying transcriptional regulation in the malaria-causing Plasmodium spp., and in Apicomplexan parasites generally, remain poorly understood. In Plasmodium, few regulatory elements sufficient to drive gene expression have been characterized, and their cognate DNA-binding proteins remain unknown. This study characterizes the DNA-binding specificities of two members of the recently identified Apicomplexan AP2 (ApiAP2) family of putative transcriptional regulators from Plasmodium falciparum. The ApiAP2 proteins contain AP2 domains homologous to the well characterized plant AP2 family of transcriptional regulators, which play key roles in development and environmental stress response pathways. We assayed ApiAP2 protein-DNA interactions using protein-binding microarrays and combined these results with computational predictions of coexpressed target genes to couple these putative trans factors to corresponding cis-regulatory motifs in Plasmodium. Furthermore, we show that protein-DNA sequence specificity is conserved in orthologous proteins between phylogenetically distant Apicomplexan species. The identification of the
DNA-binding specificities for ApiAP2 proteins lays the foundation for the exploration of their role as transcriptional regulators during all stages of parasite development. Because of their origin in the plant lineage, ApiAP2 proteins have no homologues in the human host and may prove to be ideal antimalarial targets.


**Plasmodium lipid rafts contain proteins implicated in vesicular trafficking and signalling as well as members of the PIR superfamily, potentially implicated in host immune system interactions.**


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Plasmodium parasites, the causal agents of malaria, dramatically modify the infected erythrocyte by exporting parasite proteins into one or multiple erythrocyte compartments, the cytoplasm and the plasma membrane or beyond. Despite advances in defining signals and specific cellular compartments implicated in protein trafficking in Plasmodium-infected erythrocytes, the contribution of lipid-mediated sorting to this cellular process has been poorly investigated. In this study, we examined the proteome of cholesterol-rich membrane microdomains or lipid rafts, purified from erythrocytes infected by the rodent parasite Plasmodium berghei. Besides structural proteins associated with invasive forms, we detected chaperones, proteins implicated in vesicular trafficking, membrane fusion events and signalling. Interestingly, the raft proteome of mixed P. berghei blood stages included proteins encoded by members of a large family (bir) of putative variant antigens potentially implicated in host immune system interactions and targeted to the surface of the host erythrocytes. The generation of transgenic parasites expressing BIR/GFP fusions confirmed the dynamic association of members of this protein family with membrane microdomains. Our results indicated that lipid rafts in Plasmodium-infected erythrocytes might constitute a route to sort and fold parasite proteins directed to various host cell compartments including the cell surface.


**Proteomic analysis of zygote and ookinete stages of the avian malaria parasite Plasmodium gallinaceum delineates the homologous proteomes of the lethal human malaria parasite Plasmodium falciparum.**

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Delineation of the complement of proteins comprising the zygote and ookinete, the early developmental stages of Plasmodium within the mosquito midgut, is fundamental to understand initial molecular parasite-vector interactions. The published proteome of Plasmodium falciparum does not include analysis of the zygote/ookinete stages, nor does that of P. berghei include the zygote stage or secreted proteins. P. gallinaceum zygote, ookinete, and ookinete-secreted/released protein samples were prepared and subjected to Multidimensional protein identification technology (MudPIT). Peptides of P. gallinaceum zygote, ookinete, and ookinete-secreted proteins were identified by MS/MS, mapped to ORFs (> 50 amino acids) in the extent P. gallinaceum whole genome sequence, and then matched to homologous ORFs in P. falciparum. A total of 966 P. falciparum ORFs encoding orthologous proteins were identified; just over
40% of these predicted proteins were found to be hypothetical. A majority of putative proteins with predicted secretory signal peptides or transmembrane domains were hypothetical proteins. This analysis provides a more comprehensive view of the hitherto unknown proteome of the early mosquito midgut stages of P. falciparum. The results underpin more robust study of Plasmodium-mosquito midgut interactions, fundamental to the development of novel strategies of blocking malaria transmission.


Potential use of birthweight indicators in rural Tanzania for monitoring malaria control in pregnancy.

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OBJECTIVE: Birthweight outcomes in rural Tanzania were determined in relation to place of delivery (hospital, dispensary or home), parity and maternal age (adolescent or non-adolescent) in order to evaluate the usefulness of birthweight data for monitoring malaria control in pregnancy. STUDY DESIGN: Retrospective descriptive study. METHODS: Birthweight data for the years 1997-2001 were obtained from Kilosa district hospital (n=6269), nine dispensaries (n=3688) and for home deliveries (n=677). RESULTS: The prevalence of low birthweight in singletons was highest in hospital births (primigravidae, 23.4%; multigravidae, 10.0%). Adolescent primigravidae with home deliveries had the lowest mean birthweight (2.611kg; 95% confidence interval 2.546-2.676kg). An excess risk of low birthweight in primigravidae compared with multigravidae was seen with increasing distance from the district hospital. The population attributable risk percent for low birthweight in primigravidae associated with malaria increased with distance from the hospital, from 30% for Kilosa town to 45.7% at distances >50km. Young adolescent primigravidae were at highest risk of poor birthweight outcomes. Dispensary birthweight data were considered to provide the most representative sample for routine birthweight surveillance. CONCLUSIONS: Birthweight indicators show that malaria control in pregnancy is poor in this population, deteriorates with distance of place of birth from the main hospital location, and is worst in adolescent primigravidae. Greater attention should be given to the use of birthweight indicators in rural areas of Tanzania for monitoring malaria control in pregnancy.


Determinants of provider choice for malaria treatment: Experiences from The Gambia.

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Malaria is responsible for an estimated one million deaths per year, the vast majority in sub-Saharan Africa. Many of these deaths are attributed to delays in seeking treatment and poor adherence to drug regimes. While there are a growing number of studies describing the factors influencing treatment seeking for malaria, far less is known about the relative weight given to these factors in different settings. This study estimates two models of demand for malaria treatment in the Farafenni region of The Gambia. The first examines the determinants of seeking malaria treatment outside the home versus no treatment or self-care while the second identifies the determinants of provider choice conditional on having decided to seek malaria treatment outside the home. Providers included hospital; health centre; and 'other' which included
pharmacies, kiosks; petty traders; neighbours; and traditional healers. Results show that older people were more likely to opt for self-care, or no treatment. The longer the time spent ill or the more severe the fever, the more likely a treatment was sought outside the home. Time of the year and availability of community infrastructure played a key role in both models. Poorer households and those from the Fula ethnic group were much more likely to visit an 'other' provider than a hospital. The policy and methodological implications of these findings are discussed.


The pattern of malaria infection in under-fives in Ile-Ife, Nigeria.

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Over 90% of the burden of malaria occurs in sub-Saharan Africa. Children, especially under-fives, are the most vulnerable. In Nigeria, Africa's most populous nation, it accounts for 25 and 30% of infant and childhood deaths, respectively. One hundred and seventy-six children who fulfilled clinical and parasitological criteria for the diagnosis of malaria, 26.4% of all under-fives, who presented to the Seventh Day Adventist Hospital in Ile-Ife during the months of May to September 2005 were studied to identify the factors that were associated with severe malaria in the target population. The proportion of children with severe malaria in the study was 17%, while the case-fatality rate was 3.5%. Of the 17 variables examined, high malaria parasite density, non-use of mosquito-bite preventive measures and poverty remained independently and significantly associated with an increased risk for severe malaria. Progress in stemming the burden of malaria depends on accurate knowledge and understanding of the epidemiology and control of the disease in the affected populations.


The efficacy and tolerability of three different regimens of tafenoquine versus primaquine for post-exposure prophylaxis of Plasmodium vivax malaria in the Southwest Pacific.

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Tafenoquine is being developed for radical cure and post-exposure prophylaxis of Plasmodium vivax malaria. In an open-label study, 1512 Australian Defence Force personnel received one of three tafenoquine 3 d regimens [400mg once daily (od), 200mg twice daily (bid), 200mg od] or daily primaquine (22.5mg) plus doxycycline (100mg) over 14 d in Bougainville and in Timor-Leste for post-exposure prophylaxis. The relapse rate of subjects treated in Bougainville with tafenoquine (n=173) was 1.2% (200mg bidx3 d) and 2.3% (400mg odx3 d), while primaquine plus doxycycline (n=175) was 3.4%. For subjects treated in Timor-Leste with tafenoquine (n=636), the relapse rate was 4.9% (200mg odx3 d), 5.3% (200mg bidx3 d) and 11.0% (400mg odx3d), while primaquine plus doxycycline (n=289) was 10.0%. The most frequent adverse events reported across all groups were nausea, abdominal distress and diarrhoea. There was a dose-dependent reduction in adverse events with a reduced dose of tafenoquine, with the lowest dose (total 600mg over 3 d) producing rates of adverse events equivalent to that of primaquine plus doxycycline. The much shorter dosing regimen of tafenoquine should increase compliance, which is often suboptimal with primaquine after leaving an endemic area. [Australian New Zealand Clinical Trials Registry Number 12607000588493].
Characterisation of DDT, pyrethroid and carbamate resistance in Anopheles funestus from Obuasi, Ghana.

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Indoor-resting anopheline mosquitoes were collected from Obuasi, Ghana, and were identified morphologically and by PCR as Anopheles funestus Giles. Wild-caught females were induced to lay eggs. Samples of F1 progeny from each family were divided into cohorts and were either exposed to DDT and permethrin or were stored for biochemical analysis. Bioassay data by family show evidence of DDT and pyrethroid resistance in the parent An. funestus population. The sodium channel gene of DDT survivors and DDT-susceptible individuals was PCR amplified and sequenced to determine whether any kdr-type mutations were present. Molecular analysis of the IIS5-IIS6 segment of the sodium channel gene gave no indication of any kdr-type mutations associated with resistance phenotypes. Biochemical analysis suggests that DDT and pyrethroid resistance may be metabolically mediated, although there were no clear correlations between enzyme levels/activities and insecticide resistance across families. Furthermore, an altered acetylcholinesterase conferring carbamate resistance was evident. These results can be used to plan an effective malaria control strategy in the Obuasi region.

kdr: can a single mutation produce an entire insecticide resistance phenotype?

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Knockdown resistance (kdr) is closely associated with DDT and pyrethroid resistance in the major malaria vector Anopheles gambiae and is considered to be causative of the resistance phenotype. The same association does not apply to Sudanese populations of the closely related malaria vector A. arabiensis. Speculation on this discrepancy and on the incidence of multiple insecticide resistance and cross-resistance has implications for the manner in which entomological surveys are conducted in the field as well as on the array of technologies employed to produce usable data.

Plasmodium falciparum shows transcriptional versatility within the human host.

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In a recent study published in Nature, Daily et al. profiled parasite gene expression in Plasmodium falciparum infections and identified three in vivo
'states' based on parasite transcription patterns. Despite similar host clinical features, two states displayed highly divergent gene expression, whereas the third was found in individuals with increased inflammatory markers. These findings suggest that parasites exist in different physiological states in vivo, providing an important foundation for future studies investigating how these states might contribute to malaria pathogenesis and outcome.


**Does activation of the blood coagulation cascade have a role in malaria pathogenesis?**

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Plasmodium falciparum infection is often associated with a procoagulant state. Recent identification of tissue factor in the brain endothelium of patients who have died from cerebral malaria casts new light on our understanding of the coagulation disorder found in P. falciparum infection. It has also been revealed that parasitized red blood cells support the assembly of multimolecular coagulation complexes. Tissue factor expression by the endothelium and amplification of the coagulation cascade by parasitized red blood cells and/or activated platelets (particularly at sequestration sites) have crucial roles in mounting and sustaining a coagulation-inflammation cycle which contributes to organ dysfunction and coma in falciparum malaria.


**Apicoplast translation, transcription and genome replication: targets for antimalarial antibiotics.**

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Several antibiotics possess antimalarial properties, although the mechanisms by which they kill malaria parasites have been poorly understood. Recent data suggest that the target for multiple antimalarial antibiotics is the apicoplast, a chloroplast-like organelle of uncertain function. Translation inhibitors (such as tetracyclines, clindamycin and macrolides) and gyrase inhibitors (such as ciprofloxacin) cause modest antimalarial effects initially but are much more potent against the progeny of treated parasites. These progeny inherit nonfunctional apicoplasts, suggesting that blocking production of apicoplast proteins causes the 'delayed-death effect'. Interestingly, the antibiotics thiostrepton and rifampin are fast acting and might target additional processes outside the apicoplast.


**Study protocol for a three-armed randomized controlled trial to assess whether house screening can reduce exposure to malaria vectors and reduce malaria transmission in The Gambia.**

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ABSTRACT: BACKGROUND: Mosquito-proofing homes was one of the principal methods of
environmental management in the early 1900s. House screening provides protection against malaria by reducing exposure to malaria parasites and has the added benefit of protecting everyone sleeping in the house, avoiding issues of inequity within the household. The aim of this study is to determine whether house screening protects people against malaria in Africa. It is hoped that this study will mark the beginning of a series of trials assessing a range of environmental interventions for malaria control in Africa. DESIGN: A 3-armed randomised-controlled trial will be conducted in and around Farafenni town in The Gambia, West Africa, to assess whether screening windows, doors and closing eaves or installing netting ceilings in local houses can substantially reduce malaria transmission and anaemia compared to homes with no screening. Eligible houses will be sorted and stratified by location and the number of children in each house, then randomly allocated to the interventions in blocks of 5 houses (2 with full screening, 2 with screened ceilings and 1 control house without screening). Risk of malaria transmission will be assessed in each house by routine collections of mosquitoes using light traps and an anaemia prevalence study in children at the end of the main transmission period. DISCUSSION: Practical issues concerning intervention implementation, as well as the potential benefits and risks of the study, are discussed. TRIAL REGISTRATION: ISRCTN51184253 – Screening-homes to prevent malaria.


Effects of revised diagnostic recommendations on malaria treatment practices across age groups in Kenya.

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OBJECTIVE: The recent change of treatment policy for uncomplicated malaria from sulfadoxine-pyrimethamine to artemether-lumefantrine (AL) in Kenya was accompanied by revised malaria diagnosis recommendations promoting presumptive antimalarial treatment in young children and parasitological diagnosis in patients 5 years and older. We evaluated the impact of these age-specific recommendations on routine malaria treatment practices 4-6 months after AL treatment was implemented. METHODS: Cross-sectional, cluster sample survey using quality-of-care assessment methods in all government facilities in four Kenyan districts. Analysis was restricted to the 64 facilities with malaria diagnostics and AL available on the survey day. Main outcome measures were antimalarial treatment practices for febrile patients stratified by age, use of malaria diagnostic tests, and test result. RESULTS: Treatment practices for 706 febrile patients (401 young children and 305 patients > or =5 years) were evaluated. 43.0% of patients > or =5 years and 25.9% of children underwent parasitological malaria testing (87% by microscopy). AL was prescribed for 79.7% of patients > or =5 years with positive test results, for 9.7% with negative results and for 10.9% without a test. 84.6% of children with positive tests, 19.2% with negative tests, and 21.6% without tests were treated with AL. At least one antimalarial drug was prescribed for 75.0% of children and for 61.3% of patients > or =5 years with a negative test result. CONCLUSIONS: Despite different recommendations for patients below and above 5 years of age, malaria diagnosis and treatment practices were similar in the two age groups. Parasitological diagnosis was under-used in older children and adults, and young children were still tested. Use of AL was low overall and alternative antimalarials were commonly prescribed; but AL prescribing largely followed the results of malaria tests. Malaria diagnosis recommendations differing between age groups appear complex to implement; further strengthening of diagnosis and treatment practices under AL policy is required.

Preclinical assessment of the receptor-binding domain of Plasmodium vivax Duffy-binding protein as a vaccine candidate in rhesus macaques.

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The receptor-binding domain of Plasmodium vivax Duffy-binding protein, region II (PvRII), is an attractive candidate for a vaccine against P. vivax malaria. Here, we have studied the safety and immunogenicity of recombinant PvRII in Macaca mulatta (rhesus monkeys). Recombinant PvRII with a C-terminal 6-histidine tag was expressed in E. coli, recovered from inclusion bodies, refolded into its functional conformation, purified to homogeneity and formulated with three adjuvants, namely, Alhydrogel, Montanide ISA 720 and the GSK proprietary Adjuvant System AS02A for use in immunogenicity studies. All the PvRII vaccine formulations tested were safe and highly immunogenic. The overall magnitude of the antibody response was significantly higher for both Montanide ISA 720 and AS02A formulations in comparison with Alhydrogel. Furthermore, there was a significant correlation between antibody recognition titers by ELISA and binding inhibition titers in in vitro binding assays. The PvRII vaccine formulations also induced IFN-gamma recall responses that were identified using ex vivo ELISPOT assays. These results provide support for further clinical development of a vaccine for P. vivax malaria based on recombinant PvRII.


Active immunisation with RAMA does not provide protective immunity against Plasmodium yoelii challenge despite its association with protective responses in endemic populations.

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The rhoptry associated membrane antigen (RAMA) of Plasmodium falciparum has been proposed as a potential candidate for inclusion in a multivalent subunit vaccine against malaria. Previous studies have found that the RAMA gene is refractory to genetic deletion in vitro and is conserved in a range of clinical isolates. Importantly, two independent studies demonstrated that antibodies against the C-terminal region of RAMA are associated with immunity in endemic populations of both Asia and Africa. However, there is presently no direct evidence that anti-RAMA immune responses have a demonstrable anti-parasitic effect either in vitro or in vivo. In this study we used an in vitro invasion inhibition assay and the Plasmodium yoelii mouse model of infection to evaluate the potential of RAMA as a vaccine candidate. Our results demonstrate that anti-PfRAMA antibodies have only a weak inhibitory effect on P. falciparum invasion in vitro. Immunisation with recombinant PyRAMA protein did not protect mice against a lethal P. yoelii infection and did not boost the level of protection induced by a known protective antigen, merozoite surface protein 4/5. Taken together, these data do not support RAMA as a priority vaccine candidate.

High immunogenecity and erythrocyte-binding activity in the tryptophan-rich domain (TRD) of the 74-kDa Plasmodium vivax alanine-tryptophan-rich antigen (PvATRAg74).

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Plasmodium vivax is the most widespread species of human malaria parasite affecting 70-80 million people worldwide each year. In recent years, some potential vaccine candidate antigens from P. vivax have been identified including tryptophan-rich antigens PvTRAg and PvTARAg55. We report here the identification and partial characterization of a 74kDa P. vivax alanine-tryptophan-rich antigen (PvATRAg74) which is expressed by all asexual blood stages of the parasite. This protein contains two major domains, i.e. alanine-rich domain (ARD) in N-terminal region and the tryptophan-rich domain (TRD) at C-terminus. PvATRAg74 also contains variable numbers of octa-peptide repeats in the ARD region. The C-terminal PvATRAg74 containing TRD was highly conserved among 32 P. vivax isolates while N-terminal ARD showed genetic polymorphisms. The 36kDa TRD was expressed in E. coli and named here as His(6)-TRD. The purified recombinant His(6)-TRD showed binding with uninfected human erythrocytes. This antigen was also recognized by all 38 P. vivax patients' sera on ELISA thus showing a very high seropositivity rates. In vitro stimulation of lymphocytes with purified His(6)-TRD indicated that it induced T cell immune response in majority (94%, n=16) of P. vivax exposed individuals. The stimulated T cells produced higher amount of IL-4 and IL-10 than IFN-gamma, TNF-alpha, and IL-12 indicating a Th2 type of response bias. Unlike PvTARAg55, this antigen is more immunogenic in humans and possesses the erythrocyte-binding activity. Immunogenecity of PvATRAg74 is similar to PvTRAg whose erythrocyte-binding activity still remains unknown.

Vaccine. 2008 Jun 2;26(23):2818-23.

Adenovirus 5 and 35 vectors expressing Plasmodium falciparum circumsporozoite surface protein elicit potent antigen-specific cellular IFN-gamma and antibody responses in mice.


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Falciparum malaria vaccine candidates have been developed using recombinant, replication-deficient serotype 5 and 35 adenoviruses (Ad5, Ad35) encoding the Plasmodium falciparum circumsporozoite surface protein (CSP) (Ad5.CS, Ad35.CS) (Crucell Holland BV, Leiden, The Netherlands). To evaluate the immunogenicity of these constructs, BALB/cJ mice were immunized twice with either Ad5.CS, Ad35.CS, empty Ad5-vector (eAd5), empty Ad35 vector (eAd35), or saline. Another group received the CSP-based RTS,S malaria vaccine formulated in the proprietary Adjuvant System AS01B (GlaxoSmithKline Biologicals, Rixensart, Belgium). Here we report that Ad5.CS, Ad35.CS, and RTS,S/AS01B, elicited both cellular and serologic CSP antigen-specific responses in mice. These adenoviral vectors induce strong malaria-specific immunity and warrant further evaluation.