Overproduction, purification and crystallization of a chondroitin sulfate A-binding DBL domain from a Plasmodium falciparum var2csa-encoded PfEMP1 protein.

Higgins MK. Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, England. mkh20@cam.ac.uk

The PfEMP1 proteins of the malaria parasite Plasmodium falciparum are inserted into the membrane of infected red blood cells, where they mediate adhesion to a variety of human receptors. The DBL domains of the var2csa-encoded PfEMP1 protein play a critical role in malaria of pregnancy, tethering infected cells to the surface of the placenta through interactions with the glycosaminoglycan carbohydrate chondroitin sulfate A (CSA). A CSA-binding DBL domain has been overproduced in a bacterial expression system, purified and crystallized. Native data sets extending to 1.9 Å resolution have been collected and phasing is underway.
2: Acta Trop. 2008 Feb 15


University Lyon 1, EA 4170, Parasitology & Tropical Medicine, Faculty of Medicine, 8 avenue Rockefeller, 69373 Lyon cedex 08, France.

Cerebral malaria is the most severe and rapidly fatal complication of Plasmodium falciparum infection. Despite appropriate anti-malarial treatment using quinine or artesinin derivatives, 10-20% of mortality still occurs during the acute phase. To improve cerebral malaria outcome, adjunctive therapies are clearly needed. Most experiments in this area have been dedicated to immuno-modulation with various successes. Since erythropoietin has been shown to be highly effective in human ischemic stroke and in murine cerebral malaria, we addressed the issue of cerebral malaria outcome improvement by erythropoietin-artesunate drug combination. Compared to the previous study using erythropoietin high doses at the early beginning of the disease, erythropoietin treatment was decreased by six-fold and delayed to the pre-mortem phase. We studied effects on survival and on clinical recovery of the drug combination given from day 6 to day 8 post-infection to CBA/J mice infected by Plasmodium berghei ANKA. We showed that the artesunate-erythropoietin drug combination led to clinical recovery 24h earlier for surviving mice, and to increase in the global survival rate compared to artesunate monotherapy (p<0.01). Since erythropoietin has no effect on parasite clearance, it could be stated that this drug combination is efficient and that erythropoietin could be a lead for the implementation of a new adjunctive therapy during the acute phase of cerebral malaria.


Specific stimulation of HIV-1 replication in human placental trophoblasts by an antigen of Plasmodium falciparum.


Unité de Régulation des Infections Rétrovirales, Institut Pasteur, 75724 Paris Cedex 15, France. ahidjo.ayouba@mpl.ird.fr

Epidemiological data point to an increased risk of HIV-1 mother-to-child transmission in pregnant women with malaria, by unknown mechanisms. We show here that surface binding of a recombinant Plasmodium falciparum adhesin to chondroitin sulphate A proteoglycans increases HIV-1 replication in the human placental cell line BeWo, probably by a P. falciparum adhesin-induced long-terminal repeat-driven TNF-alpha stimulation. This suggests that placental malaria could increase the risk of HIV-1 transmission in utero.


An Antimalarial Neem Leaf Extract has Both Schizonticidal and Gametocytocidal Activities.

Udeinya JI, Shu EN, Quakyi I, Ajayi FO.

1Currently, the Rocitus Institute of Research, Enugu, Nigeria, formerly, Howard University College of Medicine, Washington, DC; 2College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria; 3Georgetown University, Washington, DC; and 4Food and Drug Administration, Rockville, MD.

A crude acetone/water (50/50) extract of neem leaves (IRAB) was evaluated for
activity against the asexual (trophozoites/schizonts) and the sexual 
gametocytes) forms of the malarial parasite, Plasmodium falciparum, in vitro. In 
separate 72 hour cultures of both asexual parasites and mature gametocytes 
treated with IRAB (0.5 mug/mL), parasite numbers were less than 50% of the 
numbers in control cultures, which had 8.0% and 8.5% parasitemia, respectively. 
In cultures containing 2.5 mug/mL, asexual parasites and mature and immature 
gametocytes were reduced to 0.1%, 0.2%, and 0% parasitemia, respectively. There 
were no parasites in the cultures containing 5.0 mug/mL. This extract, if found 
safe, may provide materials for development of new antimalarial drugs that may be 
useful both in treatment of malaria as well as the control of its transmission 
through gametocytes.


Miniaturization of a High-throughput pLDH-based Plasmodium falciparum Growth 
Inhibition Assay for Small Volume Samples from Preclinical and Clinical Vaccine 
Trials.

Bergmann-Leitner ES, Duncan EH, Burge JR, Spring M, Angov E.

US Military Malaria Vaccine Program, Division of Malaria Vaccine Development, 
Walter Reed Army Institute of Research, Silver Spring, Maryland.

To date, no immune correlates for blood stage-specific immunity against 
Plasmodium falciparum malaria parasites have been identified. Growth and/or 
invasion inhibition assays using sera from Phase 2a/b trials will aid in 
determining whether correlations with protective immunity can be established for 
these assays. A major constraint in the ability to evaluate functional antibody 
activities from populations in endemic areas is the relatively limited 
availability of sufficient sample quantity. For this reason, we developed a 
miniaturized and high-throughput method to measure growth inhibitory activity by 
quantification of parasite lactate dehydrogenase (pLDH) in a 384-microtiter plate 
format. This culture method can be extended beyond the pLDH-based readout to 
other techniques commonly used to determine growth/invasion inhibition.


Geographical Distribution of Amino Acid Mutations in Plasmodium vivax DHFR and 
DHPS from Malaria Endemic Areas of Thailand.

Rungsirunrat K, Sibley CH, Munthhin M, Na-Bangchang K.

Faculty of Allied Health Sciences, Thammasat University, Pathumtani, Thailand; 
Department of Parasitology, Phramongkutklao College of Medicine, Thailand; 
Department of Genome Sciences, University of Washington, Seattle, Washington.

Both malaria treatment and prophylaxis target the parasite dihydropteroate 
synthase (DHPS) and dihydrofolate reductase (DHFR) enzymes. Specific point 
mutations in these genes confer resistance to sulfadoxine-pyrimethamine (SP) in 
both Plasmodium falciparum and P. vivax. We used direct sequencing to examine the 
prevalence of point mutations in pvdhps and pvdhfr in 160 P. vivax isolates 
collected from areas along the international borders of Thailand. Results show 
that the majority of the isolates harbored a quadruple mutant allele of pvdhfr 
and a double mutant allele of pvdhps, but the distribution was not uniform. The 
highly mutant allele combination was especially prevalent along the Thai-Myanmar 
border, whereas the majority of the isolates from areas along the Thai-Cambodian 
and Thai-Malaysian borders carried double mutant alleles of pvdhfr and single 
mutant alleles of pvdhps. Novel mutations that have not been identified 
previously at codon 512 of pvdhps (K512M, K512E, K512T) were also found.
Efficacy, Safety, and Selection of Molecular Markers of Drug Resistance by Two ACTs in Mali.


Malaria Research and Training Center, Department of Epidemiology of Parasitic Diseases, Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Bamako, Mali.

We conducted a randomized single-blinded trial comparing the efficacy and safety of artesunate (AS) + amodiaquine (AQ, 3 days) versus AS (3 days) + sulfadoxine-pyrimethamine (SP, single dose) versus AS monotherapy (5 days) in Southern Mali. Uncomplicated malaria cases were followed for 28 days. Molecular markers of drug resistance were determined. After identification of recrudescences by genotyping, both artemisinin-based combination therapies (ACTs) reached nearly 100% efficacy at Day 14 and Day 28 versus 98.3% and 96.5% for AS, respectively (P > 0.05). AS + SP significantly selected DHFR and DHPS mutations associated with sulfadoxine and pyrimethamine resistance (P < 0.001), and AS + AQ equally selected PfCRT and PfMDR1 point mutations associated with chloroquine and AQ resistance (P < 0.001). No significant adverse event attributable to any of the study drugs was found. The ACTs were efficacious and safe, but the selection of markers for resistance to the partner drugs raises concerns over their lifespan in areas of intense malaria transmission.

Malaria in Pregnancy in the Solomon Islands: Barriers to Prevention and Control.

Appleyard B, Tuni M, Cheng Q, Chen N, Bryan J, McCarthy JS.

The Australian Centre for International and Tropical Health, a joint program of the Queensland Institute of Medical Research and the School of Population Health, The University of Queensland, Queensland, Australia; Solomon Islands Medical Training and Research Institute, Honiara, Solomon Islands; Drug Resistance and Diagnostics, Australian Army Malaria Institute, Brisbane, Australia.

A study of malaria in pregnancy (MIP) was undertaken in Marovo Lagoon, Solomon Islands, to evaluate pregnancy-specific control strategies for malaria. Peripheral parasitemia was present in 18% (19/106) of women: 15 Plasmodium falciparum and 4 P. vivax. Primigravidae were twice as likely to be parasitemic as multigravidae (31% versus 14%; relative risk: 2.24; 95% confidence interval: 1.01-4.96; P = 0.05). Although ante-natal clinic attendance was high, women booked late (mean, 19.7 weeks) and attended irregularly. Free insecticide-treated nets (ITN) were not distributed despite government policy. Primigravidae were less likely to have an ITN in their homes than multigravidae (relative risk: 2.13; 95% confidence interval: 1.03-4.40). Coverage with chloroquine prophylaxis was low. This study revealed barriers to control of MIP at both the health service and client level. To develop an evidence-based malaria control policy in pregnancy for this region, further study of the epidemiology of malaria and its effects, including social and behavioral aspects, is needed.
Nonmalarial acute undifferentiated fever in a rural hospital in central India: diagnostic uncertainty and overtreatment with antimalarial agents.

Joshi R, Colford JM Jr, Reingold AL, Kalantri S.

Division of Epidemiology, School of Public Health, University of California, Berkeley, California; Department of Medicine, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Maharashtra, India.

Nonmalarial acute undifferentiated fever (NMAUF) refers to a febrile illness with no indication of an organ-specific disease after diagnosis of malaria has been excluded. In developing countries, the empirical treatment of NMAUFs with antimalarial drugs continues even in the era of highly specific rapid diagnostic tests (RDTs) for malaria. We carried out a retrospective review of patients with fever admitted to a rural teaching hospital in central India. We categorized patients with NMAUF into different clinical syndromes and determined their demographic profile, inhospital course, and the pattern of antimalarial use. The study sample included 1,197 adult patients who were investigated for malaria; 1,053 (88%) of them had NMAUF, and use of further diagnostics in this group was limited. Despite one or more negative tests for malaria, many patients (39.9%, 95% CI 37.0-43.3) received antimalarial drugs. These results suggest a need for guidelines and training to improve empirical treatment of NMAUF.

The antimicrobial peptide NK-2, the core region of mammalian NK-lysin, kills intraerythrocytic Plasmodium falciparum.

Gelhaus C, Jacobs T, Andrä J, Leippe M.

From the Department of Zoophysiology, Zoological Institute, University of Kiel, Olshausenstr. 40, 24098 Kiel, Germany; the Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Str. 74, 20359 Hamburg, Germany; and the Division of Biophysics, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Borstel, Parkallee 10, 23845 Borstel, Germany.

In a time of dramatically increasing resistance of microbes to all kinds of antibiotics, natural antimicrobial peptides and synthetic analogs thereof have emerged as compounds with potentially significant therapeutical applications against human pathogens. Only very few of these peptide antibiotics have been tested against protozoan pathogens which are a major cause of morbidity and mortality in large parts of the world. Here, we studied the effect of NK-2, a peptide representing the cationic core region of the lymphocytic effector protein NK-lysin, on the malaria parasite Plasmodium falciparum. Whereas non-infected red blood cells were hardly affected, human erythrocytes infected with the parasite were rapidly permeabilized by NK-2 in the micromolar range. Loss of plasma membrane asymmetry and concomitant exposure of phosphatidylserine upon infection appears to be the molecular basis for the observed target preference of NK-2 as can be demonstrated by annexin V binding. The peptide also affects the viability of the intracellular parasite as evidenced by the drop of DNA content of cultured parasites. Accumulated evidences derived from permeabilization assays using parasites and liposomes as targets and from fluorescence microscopy of infected erythrocytes treated with fluorescently labeled NK-2 indicate that the positively charged peptide electrostatically interacts with the altered and negatively charged plasma membrane of the infected host cell and traverse this membrane as well as the parasitophorous vacuole membrane to reach its final target, the intracellular parasite. The apparent affinity for foreign membranes that resulted in death of a eukaryotic parasite residing in human host cells makes NK-2 a promising template for novel anti-infectives.

[Evaluation of two rapid diagnostic tests, NOW(R) ICT Malaria Pf/Pv and OptiMAL(R), for diagnosis of malaria.] [Article in Spanish]

Mendoza NM, García M, Cortés LJ, Velas C, Erazo R, Pérez P, Ospina OL, Burgos JD.


Introduction. To increase the accessibility of malaria diagnosis, the Instituto Nacional de Salud de Colombia undertook a field trial to evaluate the sensitivity and specificity of two rapid diagnostic tests. Objective. The sensitivity, specificity and concordance was compared for two rapid diagnostic tests for malaria, NOW(R) ICT Malaria Pf/Pv and OptiMAL(R), Materials and methods. A descriptive and concordance study was performed with 214 patients in the southwestern coastal city of Tumaco, Colombia, each of whom presented at least one of the symptoms of the classical malaria triad. Two strategies were applied for patient recruitment—one by passive search and a second through local health brigades. Results. NOW(R) ICT showed a general sensitivity of 98.4% (95%CI: 90.3-99.9), and a general specificity of 98.0% (95%CI: 93.9-99.5). For Plasmodium falciparum, the sensitivity was 98.2% (95%CI: 89.4-99.9) and the specificity 98.1% (95%CI: 94.1-99.5). The sensitivity was lower (80.0%) when parasitemia ranged from 200 to 4,000 parasites/ microl. The sensitivity and specificity of the NOW(R) ICT for P. vivax malaria were 100%. The sensitivity for this test was not affected for the established ranges of parasitemia for P. vivax. The overall figures for OptiMAL(R) were 95.2% (95%CI: 85.8-98.8) sensitivity and 99.3% (95%CI: 95.8-100.0) specificity. For P. falciparum malaria OptiMAL(R) showed 94.7% (95%CI: 84.5-98.6) sensitivity and 99.4% (95%CI: 96.0-100.0) specificity. The sensitivity was lower (60.0%) when samples with 200-4,000 parasites/microl were tested. For P. vivax, OptiMAL(R) presented a 66.7% (95%CI: 24.1-94.0) sensitivity, which diminished to 50% with a parasitemia between 300-2,500 parasites/ microl. Conclusions. Good results for sensitivity and specificity were obtained for malaria diagnosis using NOW(R) ICT and OptiMAL(R), with NOW(R)ICT showing higher sensitivity and specificity values than OptiMAL(R).

Biomedica. 2007 Dec;27(4):559-70.

[Nutritional status of children with malaria in northwest Colombia.] [Article in Spanish]

Uscátegui RM, Correa AM.

Grupo de Investigación en Alimentación y Nutrición Humana, Escuela de Nutrición y Dietética, Universidad de Antioquia, Medellín, Colombia.

Introduction. Malaria and malnutrition coexist within the poorest regions of the world. In the regions of Colombia where malaria is endemic, malnutrition is also a public health problem. Objective. The prevalence of malnutrition in children with malaria was determined and several factors associated with malnutrition were identified. Materials and methods. A total of 93 children with malaria were included. They resided in the municipalities of Turbo and El Bagre (Antioquia, Colombia) and volunteered for an experimental, study to evaluate the outcome of an antimalarial treatment with a vitamin A supplement and an anti-intestinal parasite treatment administered as a single dose. At enrollment, the nutritional status was evaluated by anthropometry, and a survey questionnaire was administered about economic and social conditions of the family. Results. Prevalence of moderate or severe chronic malnutrition was 22.6%-10.8% of children had moderate or severe global malnutrition and 2.3% had acute malnutrition. The prevalence of malnutrition was higher in males and in children from Turbo.
Conclusions. Prevalence of malnutrition was higher than previously reported by the National Health and Demography Survey (2005) and, in Turbo, the prevalence was higher than that reported for the rest of the Urabá region of Antioquia.

8: Bioorg Med Chem. 2008 Mar 16

Interaction between artemisinin and heme. A Density Functional Theory study of structures and interaction energies.

Araújo JQ, Carneiro JW, Araújo MT, Leite FH, Taranto AG.

Programa de Pós-Graduação em Química Orgânica, Universidade Federal Fluminense, Outeiro de São João Batista, s/n, 24020-150, Niterói, RJ, Brazil.

Malaria is an infectious disease caused by the unicellular parasite Plasmodium sp. Currently, the malaria parasite is becoming resistant to the traditional pharmacological alternatives, which are ineffective. Artemisinin is the most recent advance in the chemotherapy of malaria. Since it has been proven that artemisinin may act on intracellular heme, we have undertaken a systematic study of several interactions and arrangements between artemisinin and heme. Density Functional Theory calculations were employed to calculate interaction energies, electronic states, and geometrical arrangements for the complex between the heme group and artemisinin. The results show that the interaction between the heme group and artemisinin at long distances occurs through a complex where the iron atom of the heme group retains its electronic features, leading to a quintet state as the most stable one. However, for interaction at short distances, due to artemisinin reduction by the heme group, the most stable complex has a septet spin state. These results suggest that a thermodynamically favorable interaction between artemisinin and heme may happen.


A novel semi-automatic image processing approach to determine Plasmodium falciparum parasitemia in Giemsa-stained thin blood smears.

Le MT, Bretschneider TR, Kuss C, Preiser PR.

ABSTRACT: BACKGROUND: Malaria parasitemia is commonly used as a measurement of the amount of parasites in the patient's blood and a crucial indicator for the degree of infection. Manual evaluation of Giemsa-stained thin blood smears under the microscope is onerous, time consuming and subject to human error. Although automatic assessments can overcome some of these problems the available methods are currently limited by their inability to evaluate cases that deviate from a chosen "standard" model. RESULTS: In this study reliable parasitemia counts were achieved even for sub-standard smear and image quality. The outcome was assessed through comparisons with manual evaluations of more than 200 sample smears and related to the complexity of cell overlaps. On average an estimation error of less than 1% with respect to the average of manually obtained parasitemia counts was achieved. In particular the results from the proposed approach are generally within one standard deviation of the counts provided by a comparison group of maliariologists yielding a correlation of 0.97. Variations occur mainly for blurred out-of-focus imagery exhibiting larger degrees of cell overlaps in clusters of erythrocytes. The assessment was also carried out in terms of precision and recall and combined in the F-measure providing results generally in the range of 92% to 97% for a variety of smears. In this context the observed trade-off relation between precision and recall guaranteed stable results. Finally, relating the F-measure with the degree of cell overlaps, showed that up to 50% total cell overlap can be tolerated if the smear image is well-focused and the smear itself adequately stained. CONCLUSIONS: The automatic analysis has proven to be comparable with manual evaluations in terms of accuracy. Moreover, the test results have shown that the proposed comparison-based approach, by
exploiting the interrelation between different images and color channels, has successfully overcome most of the inherent limitations possibly occurring during the sample preparation and image acquisition phase. Eventually, this can be seen as an opportunity for developing low-cost solutions for mass screening.


Microsatellite data suggest significant population structure and differentiation within the malaria vector Anopheles darlingi in Central and South America.

Mirabello L, Vineis JH, Yanoviak SP, Scarpassa VM, Povoa MM, Padilla N, Achee NL, Conn JE.

ABSTRACT: BACKGROUND: Anopheles darlingi is the most important malaria vector in the Neotropics. An understanding of A. darlingi's population structure and contemporary gene flow patterns is necessary if vector populations are to be successfully controlled. We assessed population genetic structure and levels of differentiation based on 1,378 samples from 31 localities throughout the Peruvian and Brazilian Amazon and Central America using 5-8 microsatellite loci. RESULTS: We found high levels of polymorphism for all of the Amazonian populations (mean RS = 7.37, mean HO = 0.747), and low levels for the Belize and Guatemalan populations (mean RS = 4.3, mean HO = 0.456). The Bayesian clustering analysis revealed five population clusters: northeastern Amazonian Brazil, southeastern and central Amazonian Brazil, western and central Amazonian Brazil, Peruvian Amazon, and the Central American populations. Within Central America there was low non-significant differentiation, except for between the populations separated by the Maya Mountains. Within Amazonia there was a moderate level of significant differentiation attributed to isolation by distance. Within Peru there was no significant population structure and low differentiation, and some evidence of a population expansion. The pairwise estimates of genetic differentiation between Central America and Amazonian populations were all very high and highly significant (FST = 0.1859 - 0.3901, P < 0.05). Both the DA and FST distance-based trees illustrated the main division to be between Central America and Amazonia. CONCLUSIONS: We detected a large amount of population structure in Amazonia, with three population clusters within Brazil and one including the Peru populations. The considerable differences in Ne among the populations may have contributed to the observed genetic differentiation. All of the data suggest that the primary division within A. darlingi corresponds to two white gene genotypes between Amazonia (genotype 1) and Central America, parts of Colombia and Venezuela (genotype 2), and are in agreement with mitochondrial COI gene sequences interpreted as incipient species. Overall, it appears that two main factors have contributed to the genetic differentiation between the population clusters: physical distance between the populations and the differences in effective population sizes among the subpopulations.


The molecular evolution of four anti-malarial immune genes in the Anopheles gambiae species complex.


ABSTRACT: BACKGROUND: If the insect innate immune system is to be used as a potential blocking step in transmission of malaria, then it will require targeting one or a few genes with highest relevance and ease of manipulation. The problem is to identify and manipulate those of most importance to malaria infection without the risk of decreasing the mosquito's ability to stave off infections by microbes in general. Molecular evolution methodologies and concepts can help identify such genes. Within the setting of a comparative molecular population genetic and phylogenetic framework, involving six species of the
Anopheles gambiae complex, we investigated whether a set of four pre-selected immunity genes (gambicin, NOS, Rel2 and FBN9) might have evolved under selection pressure imposed by the malaria parasite. RESULTS: We document varying levels of polymorphism within and divergence between the species, in all four genes. Introgression and the sharing of ancestral polymorphisms, two processes that have been documented in the past, were verified in this study in all four studied genes. These processes appear to affect each gene in different ways and to different degrees. However, there is no evidence of positive selection acting on these genes. CONCLUSIONS: Considering the results presented here in concert with previous studies, genes that interact directly with the Plasmodium parasite, and play little or no role in defense against other microbes, are probably the most likely candidates for a specific adaptive response against P. falciparum. Furthermore, since it is hard to establish direct evidence linking the adaptation of any candidate gene to P. falciparum infection, a comparative framework allowing at least an indirect link should be provided. Such a framework could be achieved, if a similar approach like the one involved here, was applied to all other anopheline complexes that transmit P. falciparum malaria.


Rectal artemisinins for malaria: a review of efficacy and safety from individual patient data in clinical studies.

Gomes M, Ribeiro I, Warsame M, Karunajeewa H, Petzold M.

ABSTRACT: BACKGROUND: Rectal administration of artemisinin derivatives has potential for early treatment for severe malaria in remote settings where injectable antimalarial therapy may not be feasible. Preparations available include artesunate, artemisinin, arteether and dihydroartemisinin. However each may have different pharmacokinetic properties and more information is needed to determine optimal dose and comparative efficacy with each another and with conventional parenteral treatments for severe malaria. METHODS: Individual patient data from 1167 patients in 15 clinical trials of rectal artemisinin derivative therapy (artesunate, artemisinin and arteether) were pooled in order to compare the rapidity of clearance of Plasmodium falciparum parasitaemia and the incidence of reported adverse events with each treatment. Data from patients who received comparator treatment (parenteral artemisinin derivative or quinine) were also included. Primary endpoints included percentage reductions in parasitaemia at 12 and 24 hours. A parasite reduction of >90% at 24 hours was defined as parasitological success. RESULTS: Artemisinin and artesunate treatment cleared parasites more rapidly than parenteral quinine during the first 24 hours of treatment. A single higher dose of rectal artesunate treatment was five times more likely to achieve >90% parasite reductions at 24 hours than were multiple lower doses of rectal artesunate, or a single lower dose administration of rectal arteether. CONCLUSIONS: Artemisinin and artesunate suppositories rapidly eliminate parasites and appear to be safe. There are less data on arteether and dihydroartemisinin suppositories. The more rapid parasite clearance of single high-dose regimens suggests that achieving immediate high drug concentrations may be the optimal strategy.


A malaria parasite formin regulates actin polymerization and localizes to the parasite-erythrocyte moving junction during invasion.


Infection and Immunity Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3050, Australia.
Malaria parasites invade host cells using actin-based motility, a process requiring parasite actin filament nucleation and polymerization. Malaria and other apicomplexan parasites lack Arp2/3 complex, an actin nucleator widely conserved across eukaryotes, but do express formins, another type of actin nucleator. Here, we demonstrate that one of two malaria parasite formins, Plasmodium falciparum formin 1 (PfFormin 1), and its ortholog in the related parasite Toxoplasma gondii, follows the moving tight junction between the invading parasite and the host cell, which is the predicted site of the actomyosin motor that powers motility. Furthermore, in vitro, the PfFormin1 actin-binding formin homology 2 domain is a potent nucleator, stimulating actin polymerization and, like other formins, localizing to the barbed end during filament elongation. These findings support a conserved molecular mechanism underlying apicomplexan parasite motility and, given the essential role that actin plays in cell invasion, highlight formins as important determinants of malaria parasite pathogenicity.

14: Cell Host Microbe. 2008 Feb 14;3(2):63-5.

New pieces for the malaria liver stage puzzle: where will they fit?

Mota MM, Rodriguez A. Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, 1649-028 Lisboa, Portugal.

Malaria starts with the infection of the liver by Plasmodium parasites. Although extensive analysis of Plasmodium has revealed the expression patterns of this parasite in every stage of its life cycle, the liver stage remained unexplored. Recently, Tarun et al. have published the first complete transcriptome and proteome analysis of this intriguing parasite stage, providing a list of potential candidate target genes for antimalarial vaccines and drugs.


Pharmacokinetics of oral artesunate in thai patients with uncomplicated falciparum malaria.

Karbwang J, Na-Bangchang K, Congpoung K, Thanavibul A, Harinasuta T.

Clinical Pharmacology Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

The pharmacokinetics of artesunate and its major plasma metabolite, dihydroartemisinin, were investigated in 11 Thai male patients with acute uncomplicated falciparum malaria during the acute and recovery phases. Patients were given an oral dose of 200mg artesunate (Guilin Pharmaceutical) on the first day, followed by 100mg 12 hours later, then 100mg daily for another 4 days (total dose of 700mg). All the patients showed a rapid initial response with median (range) parasite and fever clearance times of 30 (18 to 60) and 24 (4 to 94) hours, respectively; no patients showed reappearance of parasites during the 28-day follow-up period. No significant clinical adverse effects were detected in any patient. Acute phase malaria infection significantly influenced the pharmacokinetics of artesunate and its active metabolite, dihydroartemisinin. Maximum plasma drug concentration (C(max)), absorption half-life (t((1/2)a)), area under the plasma concentration-time curve from zero to the last observed time (AUC) and terminal elimination half-life (t((1/2)z)) of artesunate were decreased, while apparent total body clearance (CL/f) was increased during the acute phase, compared with the recovery phase. In addition, a decrease in the C(max) and an increase in the AUC(DHA/ARS ) ratio were found. Optimisation of therapy with oral artesunate should therefore be based on the kinetics of the drug and dihydroartemisinin in malaria patients with acute phase infection.
Contribution of influenza immunity and virosomal-formulated synthetic peptide to cellular immune responses in a phase I subunit malaria vaccine trial.

Swiss Tropical Institute, Molecular Immunology, CH 4002 Basel, Switzerland.

We have demonstrated recently in a phase Ia clinical trial that synthetic malaria peptides delivered by immuno-potentiating reconstituted influenza virosomes (IRIV) induced long-lived peptide-specific antibody responses in all volunteers. In the current ancillary study to this clinical trial we have investigated the cellular immune responses specific for IRIV and the surface bound synthetic malaria peptides tested. After vaccination, in 50% (8/16) of the volunteers at least one positive lymphoproliferative response specific for the 49mer peptide derived from the Plasmodium falciparum apical membrane antigen-1 (AMA-1) was observed with stimulation indices ranging from 2 to 4.5. All volunteers showed pre-existing IRIV specific cellular immunity assessed by ex vivo IFN-gamma ELISpot analysis and lymphoproliferation. The pre-existing influenza specific T cell responses did not interfere negatively with the induction of malaria peptide-specific humoral and cellular immune responses. Our results support the view that IRIV constitute a safe antigen delivery system for induction of peptide-specific immune responses in human populations.


Kifude CM, Rajasekariah HG, Sullivan DJ Jr, Stewart VA, Angov E, Martin SK, Diggs CL, Waitumbi JN.
Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya; Cellabs Pty Ltd, Brookvale, NSW 2001, Australia; Department of Molecular Microbiology and Immunology, Johns Hopkins University, Bloomberg School of Public Health, 615 N. Wolfe St., Baltimore, MD, USA; Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, USA; Malaria Vaccine Development Program, United States Agency for International Development, Washington, DC, USA.

Microscopy, the gold standard for detection and quantification of malaria parasitemia, is in many aspects deficient for this purpose. The method is poorly reproducible, and can be inaccurate because 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 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 ten repeat experiments, the linear range was from an optical density 450-650 nm (OD) of 0.05+/-0.002 to 2.28+/-0.042, corresponding to 3.91-250 ng/mL of rPfHRP2. The coefficient of variation (CV) at each target concentration was 1.93-8.07%. Using cultured parasites, we confirmed the linear range of optical densities, and the association between the PfHRP2 ELISA results and microscopic parasite density. For whole blood spiked with cultured washed ring-stage infected red cells (iRBC), the linear range was 11.7-750 iRBC/microL, with CVs of 0.29-7.56%. The same spiked samples evaluated by microscopists had a similar sensitivity, but CVs were unacceptably high (20.7-161.6%). Stock rPfHRP2 was stable through 4 freeze thaw cycles (P < 0.05, paired T-test). When comparing different patient sample types at different concentrations within the linear range of the assay, the recovery of PfHRP2 from blood and serum was within +/-20%, whereas the recovery for plasma
ranged between +35 to -41%. We conclude that PfHRP2 ELISA in whole blood and serum is a suitable adjunct to microscopy, which ultimately could benefit malaria intervention trials.

18: Eur J Clin Microbiol Infect Dis. 2008 Mar 29

High frequency of Plasmodium falciparum CICNI/SGEAA and CVIET haplotypes without association with resistance to sulfadoxine/pyrimethamine and chloroquine combination in the Daraweesh area, in Sudan.

A-Elbasit IE, Khalil IF, Elbashir MI, Masuadi EM, Bygbjerg IC, Alifrangis M, Giha HA. Malaria Research Centre (MalRC), Department of Biochemistry, Faculty of Medicine, University of Khartoum, P.O. Box 102, Khartoum, Sudan, ishraga20@yahoo.co.uk

Estimation of the prevalence of the molecular markers of sulfadoxine/pyrimethamine (SP) and chloroquine (CQ) resistance and validation of the association of mutations with resistance in different settings is needed for local policy guidance and for contributing to a global map for anti-malarial drug resistance. In this study, malaria patients treated with SP alone (60) and SP with CQ (194) had a total treatment failure (TF) of 35.4%, with no difference between the two arms. The polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA) method was used to identify polymorphisms in 15 loci in the dhfr, dhps and pfcrf genes in a subset of 168 infections. The results revealed a similar frequency of all single nucleotide polymorphisms (SNPs) in the two arms, except dhps 581G, which was over-represented in infections that failed to respond to SP alone (TF). In all infections, a high frequency of dhfr CICNI haplotype (51I and 108N) was found, but without discrimination between the adequate clinical and parasitological response (ACPR, 75.6%) and TF (82.9%). Similarly, the dhps SGEAA haplotype (437G and 540E) (ACPR, 60.5%; TF, 65.9%) and the combined CICNI/SGEAA haplotype (ACPR, 50%; TF 55%) were not associated with TF. In contrast to other studies in Africa, the triple 51I/59R/108N mutation was rare (0.6%). In addition, the pfcrf CVIET haplotype (93%) was found to be associated with the CICNI/SGEAA haplotype. Finally, these data represent a baseline for SP resistance molecular markers needed before the deployment of SP/artesunate combination therapy in the Sudan.

19: Exp Parasitol. 2008 Feb 9

Plasmodium falciparum: Genetic polymorphism in apical membrane antigen-1 gene from Indian isolates.

Rajesh V, Singamsetti VK, Vidya S, Gowrishankar M, Elamaran M, Tripathi J, Radhika NB, Kochar D, Ranjan A, Roy SK, Das A.

Biological Sciences Group, Birla Institute of Technology and Science, Centre for Biotechnology, Pilani 333031, Rajasthan, India.

A number of stage-specific antigens have been characterized for vaccine development against Plasmodium falciparum malaria. This study presents a comprehensive analysis of the sequence polymorphism in Plasmodium falciparum apical membrane antigen-1 (PfAMA-1) in population samples from the eastern and western parts of India. This is the first study of its kind for the nearly full length PfAMA-1 gene from these regions in India. Our observations confirmed that sequence diversity of PfAMA-1 confines only to point mutations and shows 4-8% variation as compared to the prototypes. As opposed to the previous studies on PfAMA-1, our study revealed a greater degree of polymorphism in the Domain II region of PfAMA-1 protein, though signature for diversifying selection is seen throughout the gene. Our present investigation also indicates a very high degree of variation in the reported T- and B-cell epitopes of PfAMA-1. Few noteworthy and unique observations made in this study are the substitution of Cysteine
residues responsible for the disulfide bond structure of the protein and the presence of premature termination after 595 amino acids in 3 of the 13 isolates under consideration. These crucial findings add new perspectives to the future of AMA-1 research and could have major implications in establishing AMA-1 as a vaccine candidate.


Malaria vaccines: the case for a whole-organism approach.

Pinzon-Charry A, Good MF. PO Royal Brisbane Hospital, Queensland Institute of Medical Research, Molecular Immunology Laboratory, Brisbane 4029, Australia.

BACKGROUND: Malaria is a significant health problem causing morbidity and mortality worldwide. Vaccine development has been an imperative for decades. However, the intricacy of the parasite's lifecycle coupled with the lack of evidence for robust infection-induced immunity has made vaccine development exceptionally difficult. OBJECTIVE: To review some of the key advances in the field and discuss potential ways forward for a whole-organism vaccine. METHODS: The authors searched PubMed using the words 'malaria and vaccine'. We searched for manuscripts detailing antigen characterisation and vaccine strategies with emphasis on subunit versus whole-parasite approaches. Abstracts were selected and relevant articles are discussed. The searches were not restricted by language or date. CONCLUSIONS: The early cloning of malaria antigens has fuelled rapid development of subunit vaccines. However, the disappointing results of clinical trials have resulted in reappraisal of current strategies. Whole-parasite approaches have re-emerged as an alternative strategy. Immunization using radiation or genetically attenuated sporozoites has been shown to result in sterile immunity and immunization with blood-stage parasites curtailed by antimalarial has demonstrated delayed parasitemia in rodent models as well as in human malaria.


Plasmodium falciparum malaria vaccines in development.

Vekemans J, Ballou WR. GlaxoSmithKline Biologicals, Emerging Diseases, Global Clinical Research and Development Vaccines, Rixensart, Belgium.

johan.vekemans@gskbio.com

The development and implementation of a malaria vaccine would constitute a major breakthrough for global health. Recently, numerous new candidates have entered clinical testing, following strategies that are as diverse as the malaria cycle is complex. While promising results have been obtained, some candidate vaccines have not fulfilled expectations. The challenges are not merely scientific; further progresses will require the development of competent investigator networks, partnerships between academics, industry and funding agencies, and continuous political commitment. In this review, we present the developmental status of all malaria vaccine candidates that are currently in human clinical testing against Plasmodium falciparum, as well as selected malaria vaccine candidates at preclinical development stage, and discuss the main challenges facing the field of malaria vaccine development.


Human fortilin is a molecular target of dihydroartemisinin.


Division of Cardiology, Department of Internal Medicine, University of Texas Medical Branch, 301, University Boulevard, John Sealy Annex Suite 5.160G,
Dehydroartemisinin (DHA) is an effective anti-malaria agent. Fortilin is an anti-apoptotic molecule overexpressed in many human cancers. Here, we show that DHA binds human fortilin, increases the ubiquitination of fortilin, shortens fortilin's half-life in a proteasome-dependent fashion, and reduces cellular levels of fortilin in varieties of cells. DHA induced DNA fragmentation in U2OS cells in a fortilin-dependent manner. The fortilin-knocked-down cells were less susceptible-and fortilin-overexpressing cells more susceptible-to DHA than were wild-type cells, suggesting that apoptotic effects of DHA are-at least partly-conferrered through fortilin. Together, these data suggest that fortilin is a molecular target of DHA. DHA and its derivative may prove to be viable anti-cancer agents in fortilin-overexpressing cancers.

23: Genes Dev. 2008 Mar 26

The conserved plant sterility gene HAP2 functions after attachment of fusogenic membranes in Chlamydomonas and Plasmodium gametes.

Liu Y, Tewari R, Ning J, Blagborough AM, Garbom S, Pei J, Grishin NV, Steele RE, Sinden RE, Snell WJ, Billker O.

Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA;

The cellular and molecular mechanisms that underlie species-specific membrane fusion between male and female gametes remain largely unknown. Here, by use of gene discovery methods in the green alga Chlamydomonas, gene disruption in the rodent malaria parasite Plasmodium berghei, and distinctive features of fertilization in both organisms, we report discovery of a mechanism that accounts for a conserved protein required for gamete fusion. A screen for fusion mutants in Chlamydomonas identified a homolog of HAP2, an Arabidopsis sterility gene. Moreover, HAP2 disruption in Plasmodium blocked fertilization and thereby mosquito transmission of malaria. HAP2 localizes at the fusion site of Chlamydomonas minus gametes, yet Chlamydomonas minus and Plasmodium hap2 male gametes retain the ability, using other, species-limited proteins, to form tight prefusion membrane attachments with their respective gamete partners. Membrane dye experiments show that HAP2 is essential for membrane merger. Thus, in two distantly related eukaryotes, species-limited proteins govern access to a conserved protein essential for membrane fusion.

24: Health Policy Plan. 2008 Mar 4

Malaria overdiagnosis: is patient pressure the problem?

Chandler CI, Mwangi R, Mbakilwa H, Olomi R, Whitty CJ, Reyburn H.

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK.

OBJECTIVE In Africa antimalarials are often prescribed when malaria is unlikely, a problem that is becoming critical as more expensive antimalarials replace established drugs. However, little is known about what drives the overuse of antimalarials. We conducted this study to explore to what extent current prescribing behaviour in hospitals is driven by patient demand. METHODS Consultations were observed followed by exit interviews with patients or caretakers. Five district hospitals where microscopy was routinely available were selected in areas of low (n = 3) and high (n = 2) malaria transmission in north-eastern Tanzania. All outpatient consultations during the study period were observed (n = 669). Those sent for a malaria blood slide or treated with antimalarials presumptively were interviewed (n = 326). At the end of the study,
clinicians were interviewed for their opinions on the use of antimalarials. Findings: Patients were not observed to demand antimalarials from clinicians, but occasionally asked for a malaria slide. Patient satisfaction on exit was similar between those prescribed antimalarials and those not prescribed antimalarials, but more patients or carers expressed satisfaction when the patient had been tested than when not. Clinicians rarely reported perceiving patient demand for antimalarials and asserted that such demand for medication would not affect their prescribing behaviour. CONCLUSIONS: Patient demand was not found to be driving the over-prescription of antimalarials found in the hospitals in our setting. To the contrary, the involvement of patients may provide an opportunity to improve prescribing practice if their expectations for testing and treatment in line with test results can be effectively communicated to clinicians.

25: Hum Genet. 2008 Mar 4

Evolutionary analysis of genes of two pathways involved in placental malaria infection.

Sikora M, Ferrer-Admetlla A, Mayor A, Bertranpetit J, Casals F.

Evolutionary Biology Unit, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona Biomedical Research Park, Carrer Dr Aiguader 88, 08003, Barcelona, Catalonia, Spain, martin.sikora@upf.edu

Placental malaria is a special form of malaria that causes up to 200,000 maternal and infant deaths every year. Previous studies show that two receptor molecules, hyaluronic acid and chondroitin sulphate A, are mediating the adhesion of parasite-infected erythrocytes in the placenta of patients, which is believed to be a key step in the pathogenesis of the disease. In this study, we aimed at identifying sites of malaria-induced adaptation by scanning for signatures of natural selection in 24 genes in the complete biosynthesis pathway of these two receptor molecules. We analyzed a total of 24 Mb of publicly available polymorphism data from the International HapMap project for three human populations with European, Asian and African ancestry, with the African population from a region of presently and historically high malaria prevalence. Using the methods based on allele frequency distributions, genetic differentiation between populations, and on long-range haplotype structure, we found only limited evidence for malaria-induced genetic adaptation in this set of genes in the African population; however, we identified one candidate gene with clear evidence of selection in the Asian population. Although historical exposure to malaria in this population cannot be ruled out, we speculate that it might be caused by other pathogens, as there is growing evidence that these molecules are important receptors in a variety of host-pathogen interactions. We propose to use the present methods in a systematic way to help identify candidate regions under positive selection as a consequence of malaria.

26: Infect Immun. 2008 Mar 3

Breadth and magnitude of antibody responses to multiple Plasmodium falciparum merozoite antigens are associated with protection from clinical malaria.


KEMRI Centre for Geographic Medicine Research, Coast, P.O. Box 230-80108, Kilifi, Kenya; London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK; Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP; Dipartimento di Scienze di Sanita' Pubblica, Sezione di Parassitologia, University of Rome "La Sapienza", 00185 Rome, Italy; BPRC, Department of Parasitology, P.O. Box 3306, 2280, GH Rijswijk, The Netherlands;
Individuals living in malaria-endemic areas are repeatedly exposed to many different malaria parasite antigens. Studies on naturally-acquired antibody-mediated immunity to clinical malaria have largely focused on the presence of responses to individual antigens and their associations with decreased morbidity. We hypothesized that the breadth (number of important targets to which antibodies were made) and magnitude (antibody level measured in a random serum sample) of the antibody response were important predictors of protection from clinical malaria. We analyzed naturally-acquired antibodies to five leading P. falciparum merozoite stage vaccine candidate antigens, and schizont extract, in Kenyan children monitored for uncomplicated malaria for 6 months (n=119). Serum antibody levels to apical membrane antigen 1(AMA1), and merozoite surface protein antigens (MSP-1 block 2, MSP-2, MSP-3) were inversely related to the probability of developing malaria, but levels to MSP-119 and erythrocyte binding antigen (EBA-175) were not. The risk of malaria was also inversely associated with increasing breadth of antibody specificities, with none of the children who simultaneously had high antibody levels to five or more antigens experiencing a clinical episode, (17/119, 15%) P=0.0006. Particular combinations of antibodies (AMA1, MSP-2, MSP-3) were more strongly predictive of protection than others. The results were validated in a larger, separate case-control study whose end-point was malaria severe enough to warrant hospital admission (n=387). These findings suggest that under natural exposure, immunity to malaria may result from high titer antibodies to multiple antigenic targets and support the idea of testing combination blood stage vaccines optimized to induce similar antibody profiles.


Mlambo G, Maciel J, Kumar N.

Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, Department of Molecular Microbiology and Immunology, 615 N. Wolfe Street, Baltimore, MD 21205, USA.

Currently, there is no animal model for Plasmodium falciparum challenge to evaluate malaria transmission blocking vaccines (TBV) based on well established Pfs25 target antigen. The biological activity of transmission blocking antibodies is assessed using an assay known as the membrane feeding assay (MFA). It is an in vitro method that involves mixing antibodies with cultured P. falciparum gametocytes and feeding them to mosquitoes through an artificial membrane followed by assessment of infection in the mosquitoes. We genetically modified P. berghei to express Pfs25 and demonstrate that the transgenic parasites (TrPfs25Pb) are susceptible to anti-Pfs25 antibodies during mosquito stage development. The asexual growth kinetics and mosquito infectivity of TrPfs25Pb were comparable to that of wild-type parasites and TrPfs25Pb displayed Pfs25 on the surface of ookinetes. Immune sera from non-human primates immunized with Pfs25 based vaccine when passively transferred to mice blocked transmission of TrPfs25Pb to An. stephensi. Furthermore, mice immunized with Pfs25 DNA vaccine and challenged with TrPfs25Pb displayed reduced malaria transmission as compared to mice immunized with wild-type plasmid. These studies describe development of an animal malaria model alternate to in vitro MFA and that it can facilitate P. falciparum transmission blocking vaccine evaluation based on the target antigen, Pfs25. We believe that using an animal model to test transmission blocking
vaccines would be superior to MFA since there could be additional immune factors that may synergize transmission blocking activity of antibodies in vivo.


Single-day, three-dose treatment with fixed dose combination artesunate/sulfamethoxypyrazine/pyrimethamine to cure Plasmodium falciparum malaria.

Penali LK, Jansen FH. Unité de Paludologie, Institut Pasteur, Abidjan, Ivory Coast.

OBJECTIVES: Malaria kills approximately 1.5 to 2.7 million people each year. Despite the introduction of artemisinin-based combination therapies (ACTs), the treatment of malaria is hampered by problems such as inadequate efficacy, recrudescence, early re-infection, low patient compliance, and high cost price of drugs. This study tested the hypothesis that the co-formulated fixed dose combination (FDC) artesunate/sulfamethoxypyrazine/pyrimethamine (As/SMP) administered as a 24-hour therapy with a dose interval of 12hours is as efficacious and safe as the administration of the same drug over 3 days given with a dose interval of 24hours, for the treatment of uncomplicated Plasmodium falciparum malaria in Ivory Coast. METHOD: Two hundred and twenty-one patients presenting with uncomplicated P. falciparum malaria were randomly assigned to either one of the two dosing schemes. Treatment efficacy was assessed using the current 28-day World Health Organization protocol, success being determined by absence of recrudescence and parasitemia on day 28. RESULTS: Both treatment regimens were highly efficacious, with a success rate of 100% (111/111) for the 3-day therapy and 99% (109/110) for the 24-hour therapy. Only one patient in the 24-hour therapy group showed late treatment failure. No serious adverse events or significant laboratory abnormalities were seen. CONCLUSION: The 24-hour therapy is as well tolerated and efficacious as the same medicament administered over 3 days. This low cost and simplified three-pill treatment is certain to improve compliance.


Association of HIV and Malaria With Mother-to-Child Transmission, Birth Outcomes, and Child Mortality.


From the *Department of Population and Family Health Sciences, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD; †Department of Molecular Microbiology and Immunology, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD; ‡Rakai Project, Uganda Virus Research Institute, Entebbe, Uganda; §Department of Pathology, Johns Hopkins School of Medicine, Baltimore, MD; †Director, Institute of Public Health, Makerere University, Kampala, Uganda; ‡School of Public Health, Makerere University, Kampala, Uganda; #School of Medicine, Makerere University, Kampala, Uganda; and the **Heilbrun Center for Population and Family Health, Columbia University, Joseph L. Mailman School of Public Health, New York, NY.

OBJECTIVE:: To assess the impact of HIV and malaria coinfection on mother-to-child HIV transmission (MTCT) and adverse birth outcomes. METHODS:: One hundred nine HIV-positive mother-infant pairs with a malaria diagnosis were identified in a community cohort and followed up postpartum. Maternal malaria was diagnosed by a rapid immunochromatographic test (ICT) on sera and histopathologic examination of placenta. Infant HIV was diagnosed within 6 weeks of birth using polymerase chain reaction (PCR) to capture in-utero and intrapartum HIV transmission. Log binomial models were used to assess the relative risk of MTCT,
low birth weight, and preterm birth associated with malaria. RESULTS:
Approximately 17.4% of infants were HIV positive at or around birth, and the
prevalence of serologic and placental malaria were 31% and 32%, respectively.
HIV-positive mothers with serological ICT malaria were significantly more likely
to have low-birth-weight infants, and low-birth-weight infants had significantly
higher risk of MTCT compared with infants of normal birth weight. Although
placental and serologic ICT malaria were significantly associated with MTCT,
after adjusting for maternal HIV viral load, the risk of MTCT was significantly
increased only for mothers coinfected with placental malaria (relative risk [RR]
= 7.9, P = 0.025). CONCLUSIONS: Placental malaria increases the risk of MTCT
after adjustment for viral load. Programs should focus on enhanced malaria
prevention during pregnancy to decrease the risk of adverse birth outcomes and
MTCT.


Optimization of an LC-MS Method for the Determination of Artesunate and
Dihydroartemisinin Plasma Levels using Liquid-Liquid Extraction.

Van Quekelberghe SA, Soomro SA, Cordonnier JA, Jansen FH.
Chemiphar, L. Bauwensstraat 4, B-8200 Brugge, Belgium.

Artesunate is a derivate of artemisinin, an antimalarial drug used for the
treatment of malaria caused by Plasmodium falciparum and related parasites.
Artesunate is hydrolyzed rapidly to dihydroartemisinin in vivo. It has been found
that artemisinin and its derivatives may have neurotoxic effects. A method was
developed to analyze human plasma samples for the contents of artesunate and
dihydroartemisinin. The plasma samples are extracted with ethyl acetate,
concentrated, and redissolved in water/acetonitrile. Analyses was performed with
liquid chromatography-mass spectrometry using a binary gradient program with
aqueous formic acid and acetonitrile formic acid on a X Terra MS C18-column. The
mass spectrometer was operated in the positive atmospheric pressure chemical
ionization mode with single ion recording. The lower limits of detection were 10
and 25 ng/mL plasma for DHA and artesunate, respectively. The method was
validated according to the guidelines for validation of bioanalytical methods.

31: J Ethnopharmacol. 2008 Mar 10

Anti-protozoan activities of Harungana madagascariensis stem bark extract on
trichomonads and malaria.

Iwalewa EO, Omisore NO, Adewunmi CO, Gbolade AA, Ademowo OG, Nneji C, Agboola OI,
Daniyan OM.
Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University,
Ile-Ife, Nigeria; Department of Paraclinical Sciences (Phytomedicine Programme),
Faculty of Veterinary Sciences, University of Pretoria, PB 0X4, Onderstepoort
0110, Pretoria, South Africa.

AIM OF THE STUDY: The ethanolic stem bark extract of Harungana madagascariensis
(Hypericaceae), (Choisy) Poir were evaluated for their activities on Trichomonas
gallinae (Rivolta) Stabler isolated from the pigeon (Columba livia). It was also
tested for their anti-malarial activity on N67 Plasmodium yoelii nigeriensis (in
vivo) in mice and on Plasmodium falciparum isolates in vitro. MATERIALS AND
METHODS: The anti-trichomonal screening was performed in vitro using Trichomonas
gallinae culture. The minimum lethal concentration (MLC) is the lowest
concentration of the test extract in which no motile organisms were observed. The
anti-malarial effects were determined in-vivo for suppressive, curative and
prophylactic activities in mice receiving a standard inoculum size of 1x10(7)
(0.2ml) infected erythrocytes of Plasmodium yoelii nigeriensis intraperitoneally,
and the in vitro was performed against 3 isolates of Plasmodium falciparum in a candle jar procedures. RESULTS: The IC(50) of the extract and metronidazole (MDZ) (Flagyl) on Trichomonas gallinae at 48h are 187 and 1.56µg/ml. The IC(50) of the extract, chloroquine (CQ) and artemether (ART) on Plasmodium falciparum are between 0.052 and 0.517µg/ml for the extract and 0.021 and 0.0412µg/ml for ART and CQ, respectively. The actions of the extract in in vivo study on Plasmodium yoelii nigeriensis showed that in both suppressive and prophylactic tests the percentages chemo-suppressive were between 28.6-44.8% and 30.2-78.2% respectively, while only 80mg/kg of the extract reduced the parasitaemia level compared to the control and the standard drugs in curative test. CONCLUSIONS: Harungana madagascariensis stem bark extract therefore exhibited significant anti-protozoan effects against Trichomonas and Plasmodium both in vivo and in vitro.

32: J Trop Pediatr. 2008 Mar 29

Possible Risk Factors for Congenital Malaria at a Tertiary Care Hospital in Sagamu, Ogun State, South-West Nigeria.

Sotimehin SA, Runsewe-Abiodun TI, Oladapo OT, Njokanma OF, Olanrewaju DM.

Department of Pediatrics, Obafemi Awolowo College of Health Sciences/Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Ogun State, Nigeria.

Congenital malaria, defined as the presence of malaria parasites in the erythrocytes of newborns aged <7 days, was considered rare in endemic areas until recent studies started reporting high prevalence rates. Various theories have been postulated to explain this phenomenon, but they are not proven conclusively from research. Against this background, a prospective study was designed with the following objectives. To determine the prevalence of congenital malaria parasitaemia and identify possible risk factors amongst newborns delivered in O.O.U.T.H Sagamu, Ogun State. Over a 6-month period, 192 live newborns and their mothers were consecutively recruited into the study. Within 3 days of life, neonatal peripheral blood samples were collected for malaria screening by blood film microscopy and detection of plasmodium lactate dehydrogenase (pLDH) with the OptiMAL(R) Rapid Malaria Test kit. Maternal peripheral blood samples were taken simultaneously, to check for malaria infestation by blood film microscopy, and questionnaires were administered on the mothers to identify possible factors associated with the development of neonatal parasitaemia. Neonatal clinical and laboratory data were recorded in a proforma designed for the study. Data analysis was done with Epi-info version 6 software and level of significance set at <5%. Twenty-one of 192 newborns delivered in O.O.U.T.H within the study period were diagnosed as having congenital malaria by blood film microscopy, giving a prevalence rate of 10.9%. The main identified innate neonatal risk factor for congenital malaria parasitaemia was prematurity. First-order pregnancy, history of fever within 3 months of delivery and peripheral parasitaemia at delivery (p < 0.001) were the variables that were significantly higher in the mothers of the parasitaemic newborns. We conclude that congenital malaria parasitaemia in tropical endemic areas is not rare. Pre-term neonates, infants of primigravidae, women with history of fever within 3 months of delivery and women with post-partum peripheral parasitaemia may benefit from routine screening for malaria.
Lessons from the past: managing insecticide resistance in malaria control and eradication programmes.

Kelly-Hope L, Ranson H, Hemingway J. Vector Group, Liverpool School of Tropical Medicine, Liverpool, UK.

The distribution of insecticide-treated bednets to help combat the burden of malaria in sub-Saharan Africa has accelerated in the past 5 years. Additionally, many countries are also considering, or have already begun, indoor residual spraying campaigns. These are positive developments, since vector control has repeatedly proven to be an effective means of reducing malaria transmission. However, the sustainability of these insecticide-based interventions relies on the continuing susceptibility of the anopheles vectors to the limited number of available insecticides. Continual monitoring for early signs of insecticide resistance and the adoption of carefully considered resistance management strategies are therefore required. Regrettably, this essential monitoring component is frequently given a low priority in the push to meet ambitious coverage targets. We outline the key requirements for establishing an insecticide resistance surveillance system and urge all those involved in malaria vector control, either directly or as facilitators, to ensure that these measures are incorporated into control programmes. Failure to act now will inevitably lead to a future breakdown in disease control and jeopardise hopes of eradicating this major public-health problem.

Characterization of VAR2CSA-deficient Plasmodium falciparum-infected erythrocytes selected for adhesion to the BeWo placental cell line.


ABSTRACT: BACKGROUND: Malaria in pregnancy is characterized by accumulation of infected erythrocytes (IE) in the placenta. The key ligand identified as mediating this process is a Plasmodium falciparum erythrocyte membrane protein 1 family member, termed VAR2CSA. VAR2CSA appears to be the main ligand responsible for adhesion to chondroitin sulphate A (CSA). Whether other PfEMP1 molecules can also mediate placental adhesion, independent of CSA binding, is unclear. METHODS: The parasite line CS2 carrying a disrupted var2csa gene (CS2KO) was selected for adhesion to the BeWo choriocarcinoma cell line, which has been proposed as a model for placental malaria. The selected and control IE were tested for adhesion to placental sections and flow cytometry was used to measure recognition of IE by three serum sets from malaria-exposed men and women. RESULTS: Wild-type CS2 adhere to BeWo and placental tissue via CSA. CS2KO IE were successfully selected for adhesion to BeWo, and adhered by a CSA-independent mechanism. They bound to immobilized ICAM-1 and CD36. BeWo-selected CS2KO bound at moderate levels to placental sections, but most binding was to placental villi rather than to the syncytiotrophoblast to which IE adherence occurs in vivo. This binding was inhibited by a blocking antibody to CD36 but not to ICAM-1. As expected, sera from malaria-exposed adults recognized CS2 IE in a gender and parity dependent manner. In one serum set, there was a similar but less pronounced pattern of antibody binding to selected CS2KO IE, but this was not seen in two others. One var gene, It4var19, was particularly abundant in the selected line and was detected as full length transcripts in BeWo-selected IE, but not unselected CS2KO. CONCLUSIONS: This study suggests that IE with characteristics similar to the CS2KO have a limited role in the pathogenesis of placental malaria. VAR2CSA appear to be the major ligand for placental adhesion, and could be the basis for a vaccine against pregnancy malaria.
Process and effects of a community intervention on malaria in rural Burkina Faso: randomized controlled trial.


ABSTRACT: BACKGROUND: In the rural areas of sub-Saharan Africa, the majority of young children affected by malaria have no access to formal health services. Home treatment through mothers of febrile children supported by mother groups and local health workers has the potential to reduce malaria morbidity and mortality. METHODS: A cluster-randomized controlled effectiveness trial was implemented from 2002-2004 in a malaria endemic area of rural Burkina Faso. Six and seven villages were randomly assigned to the intervention and control arms respectively. Febrile children from intervention villages were treated with chloroquine (CQ) by their mothers, supported by local women group leaders. CQ was regularly supplied through a revolving fund from local health centres. The trial was evaluated through two cross-sectional surveys at baseline and after two years of intervention. The primary endpoint of the study was the proportion of moderate to severe anaemia in children aged 6-59 months. For assessment of the development of drug efficacy over time, an in vivo CQ efficacy study was nested into the trial. The study is registered under www.controlled.trials.com (ISRCTN 34104704). RESULTS: The intervention was shown to be feasible under program conditions and a total of 1,076 children and 999 children were evaluated at baseline and follow-up time points respectively. Self-reported CQ treatment of fever episodes at home as well as referrals to health centres increased over the study period. At follow-up, CQ was detected in the blood of high proportions of intervention and control children. Compared to baseline findings, the prevalence of anaemia (29% vs 16%, p<0.0001) and malaria parameters such as prevalence of P. falciparum parasitaemia, fever and palpable spleens was lower at follow-up but there were no differences between the intervention and control group. CQ efficacy decreased over the study period but this was not associated with the intervention. DISCUSSION: The decreasing prevalence of malaria morbidity including anaemia over the study period can be explained by an overall increase of malaria prevention and treatment activities in the study area. The lack of effectiveness of the intervention was likely caused by contamination, pre-existing differences in the coverage of malaria treatment in both study groups and an unexpectedly rapid increase of resistance against CQ, the first-line treatment drug at the time of the study.

Real-time PCR/MCA assay using fluorescence resonance energy transfer for the genotyping of resistance related DHPS-540 mutations in Plasmodium falciparum.

Mens PF, van Overmeir C, Bonnet M, Dujardin JC, d'Alessandro U.

BACKGROUND: Sulphadoxine-pyrimethamine has been abandoned as first- or second-line treatment by most African malaria endemic countries in favour of artemisinin-based combination treatments, but the drug is still used as intermittent preventive treatment during pregnancy. However, resistance to sulphadoxine-pyrimethamine has been increasing in the past few years and, although the link between molecular markers and treatment failure has not been firmly established, at least for pregnant women, it is important to monitor such markers. METHODS: This paper reports a novel sensitive, semi-quantitative and specific real-time PCR and melting curve analysis (MCA) assay using fluorescence resonance energy transfer (FRET) for the detection of DHPS-540, an important marker related to resistance to sulphadoxine-pyrimethamine.

Environmental Health at USAID – Malaria Bulletin, April 2008
predictor for SP resistance. FRET/MCA was evaluated using 78 clinical samples from malaria patients and compared to PCR-RFLP. RESULTS: Sixty-two samples were in perfect agreement between both assays. One sample showed a small wild type signal with FRET/MCA that indicates a polyclonal infection. Four samples were not able to generate enough material in both assays to distinguish mutant from wild-type infection, six samples gave no signal in PCR-RFLP and five samples gave no amplification in FRET/MCA. CONCLUSION: FRET/MCA is an effective tool for the identification of SNPs in drug studies and epidemiological surveys on resistance markers in general and DHPS-540 mutation in particular.

Malar J. 2008 Mar 10;7(1):47

No miRNA were found in Plasmodium and the ones identified in erythrocytes could not be correlated with infection.

Xue X, Zhang Q, Huang Y, Feng L, Pan W.

ABSTRACT: BACKGROUND: The transcriptional regulation of Plasmodium during its complex life cycle requires sequential activation and/or repression of different genetic programmes. MicroRNAs (miRNAs) are a highly conserved class of non-coding RNAs that are important in regulating diverse cellular functions by sequence-specific inhibition of gene expression. What is know about double-stranded RNA-mediated gene silencing (RNAi) and posttranscriptional gene silencing (PTGS) in Plasmodium parasites entice us to speculate whether miRNAs can also function in Plasmodium-infected RBCs. RESULTS: Of 132 small RNA sequences, no Plasmodium-specific miRNAs have been found. However, a human miRNA, miR-451, was highly expressed, comprising approximately one third of the total identified miRNAs. Further analysis of miR-451 expression and malaria infection showed no association between the accumulation of miR-451 in Plasmodium falciparum-iRBCs, the life cycle stage of P. falciparum in the erythrocyte, or of P. berghei in mice. Moreover, treatment with an antisense oligonucleotide to miR-451 had no significant effect on the growth of the erythrocytic-stage P. falciparum. METHODS: Short RNAs from a mixed-stage of P. falciparum-iRBC were separated in a denaturing polyacrylamide gel and cloned into T vectors to create a cDNA library. Individual clones were then sequenced and further analysed by bioinformatics prediction to discover probable miRNAs in P. falciparum-iRBC. The association between miR-451 expression and the parasite were analysed by Northern blotting and antisense oligonucleotide (ASO) of miR-451. CONCLUSION: These results contribute to eliminate the probability of miRNAs in P. falciparum. The absence of miRNA in P. falciparum could be correlated with absence of argonaute/dicer genes. In addition, the miR-451 accumulation in Plasmodium-infected RBCs is independent of parasite infection. Its accumulation might be only the residual of erythroid differentiation or a component to maintain the normal function of mature RBCs.

Malar J. 2008 Feb 29;7:42.

Immunologic activation of human syncytiotrophoblast by Plasmodium falciparum.

Lucchi NW, Peterson DS, Moore JM. Department of Infectious Diseases and Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602, USA. frd9@cdc.gov

BACKGROUND: Malaria during pregnancy is characterized by the sequestration of malaria-infected red blood cells (iRBC) in the intervillous spaces of the placenta, often accompanied by the infiltration of maternal mononuclear cells, causing substantial maternal and foetal/infant morbidity. The iRBC bind to receptors expressed by the syncytiotrophoblast (ST). How ST responds to this interaction remains poorly understood. Because it is known that ST is immunoactive and can respond to infectious agents, the consequences of this ST-iRBC interaction should be investigated. METHODS: An in vitro system was used
to assess the biochemical and immunological changes induced in ST by ST-adherent iRBCs. Changes in ST mitogen-activated protein kinase (MAPK) activation were assessed by immunoblotting and mRNA expression levels of selected cytokine and chemokines in primary ST bound by iRBC were determined using real-time, reverse transcription PCR. In addition, secreted cytokine and chemokine proteins were assayed by standard ELISA, and chemotaxis of PBMC was assessed using a two-chamber assay system. RESULTS: Following iRBC/ST interaction, ST C-Jun N-terminal kinase 1 (JNK1) was activated and modest increases in the mRNA expression of TGF-beta and IL-8/CXCL8 were observed. In addition, this interaction increased secretion of MIF and MIP-1alpha/CCL3 by ST and induced migration of PBMC towards iRBC-stimulated ST. CONCLUSION: Results from this study provide the first evidence that ST participates in shaping the local immunological milieu and in the recruitment of maternal immune cells to the maternal blood space during placental malaria infection.


Cytokine-associated neutrophil extracellular traps and antinuclear antibodies in Plasmodium falciparum infected children under six years of age.


Department of Biological Science, Florida State University, Tallahassee, Florida, USA. Bakerv@chipola.edu

BACKGROUND: In Plasmodium falciparum-infected children, the relationships between blood cell histopathology, blood plasma components, development of immunocompetence and disease severity remain poorly understood. Blood from Nigerian children with uncomplicated malaria was analysed to gain insight into these relationships. This investigation presents evidence for circulating neutrophil extracellular traps (NETs) and antinuclear IgG antibodies (ANA). The presence of NETs and ANA to double-stranded DNA along with the cytokine profiles found suggests autoimmune mechanisms that could produce pathogenesis in children, but immunoprotection in adults. METHODS: Peripheral blood smear slides and blood samples obtained from 21 Nigerian children under six years of age, presenting with uncomplicated malaria before and seven days after initiation of sulphadoxine-pyrimethamine (SP) treatment were analysed. The slides were stained with Giemsa and with DAPI. Levels of the pro-inflammatory cytokines IFN-gamma, IL-2, TNF, CRP, and IL-6, select anti-inflammatory cytokines TGF-beta and IL-10, and ANA were determined by immunoassay. RESULTS: The children exhibited circulating NETs with adherent parasites and erythrocytes, elevated ANA levels, a Th2 dominated cytokine profile, and left-shifted leukocyte differential counts. Nonspecific ANA levels were significant in 86% of the children pretreatment and in 100% of the children seven days after SP treatment, but in only 33% of age-matched control samples collected during the season of low parasite transmission. Levels of ANA specific for dsDNA were significant in 81% of the children both pre-treatment and post treatment. CONCLUSION: The results of this investigation suggest that NET formation and ANA to dsDNA may induce pathology in falciparum-infected children, but activate a protective mechanism against falciparum malaria in adults. The significance of in vivo circulating chromatin in NETs and dsDNA ANA as a causative factor in the hyporesponsiveness of CpG oligonucleotide-based malaria vaccines is discussed.

Ogungbamigbe T, Ojurongbe O, Ogunro P, Okanlawon B, Kolawole S.

Malaria Research Clinic & Laboratory, College of Health Sciences, Ladoke Akintola University Teaching Hospital, Osogbo, Nigeria.

Chloroquine (CQ) resistance in Plasmodium falciparum contributes to increasing malaria-attributable morbidity and mortality in Sub-Saharan Africa. Despite a change in drug policy, continued prescription of CQ did not abate. Therefore the therapeutic efficacy of CQ in uncomplicated falciparum malaria patients was assessed in a standard 28-day protocol in 116 children aged between six and 120 months in Osogbo, Southwest Nigeria. Parasitological and clinical assessments of response to treatment showed that 72 (62.1%) of the patients were cured and 44 (37.9%) failed the CQ treatment. High initial parasite density and young age were independent predictors for early treatment failure. Out of the 44 patients that failed CQ, 24 received amodiaquine + sulphadoxine/pyrimethamine (AQ+SP) and 20 received chlorpheniramine + chloroquine (CH+CQ) combinations. Mean fever clearance time in those treated with AQ+SP was not significantly different from those treated with CH+CQ (p = 0.05). There was no significant difference in the mean parasite density of the two groups. The cure rate for AQ+SP group was 92% while those of CH+CQ was 85%. There was a significant difference in parasite clearance time (p = 0.01) between the two groups. The 38% treatment failure for CQ reported in this study is higher than the 10% recommended by World Health Organization in order to effect change in antimalarial treatment policy. Hence we conclude that CQ can no more be solely relied upon for the treatment of falciparum malaria in Osogbo, Nigeria. AQ+SP and CH+CQ are effective in the treatment of acute uncomplicated malaria and may be considered as useful alternative drugs in the absence of artemisinin-based combination therapies.

Patterns of co-association of C-reactive protein and nitric oxide in malaria in endemic areas of Iran.

Nahrevanian H, Gholizadeh J, Farahmand M, Assmar M. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran.

In addition to numerous immune factors, C-reactive protein (CRP) and nitric oxide (NO) are believed to be molecules of malaria immunopathology. The objective of this study was to detect CRP and NO inductions by agglutination latex test and Griess microassay respectively in both control and malaria groups from endemic areas of Iran, including Southeastern (SE) (Sistan & Balouchestan, Hormozgan, Kerman) and Northwestern (NW) provinces (Ardabil). The results indicated that CRP and NO are produced in all malaria endemic areas of Iran. In addition, more CRP and NO positive cases were observed amongst malaria patients in comparison with those in control group. A variable co-association of CRP/NO production were detected between control and malaria groups, which depended upon the malaria endemic areas and the type of plasmodia infection. The percentage of CRP/NO positive cases was observed to be lower in NW compare to SE region, which may be due to the different type of plasmodium in the NW (Plasmodium vivax) with SE area (P. vivax, Plasmodium falciparum, mixed infection). The fluctuations in CRP/NO induction may be consistent with genetic background of patients. Although, CRP/NO may play important role in malaria, their actual function and interaction in clinical forms of disease remains unclear.
Evaluation of a malaria antibody enzyme immunoassay for use in blood screening.

Oh JS, Kim JS, Lee CH, Nam DH, Kim SH, Park DW, Lee CK, Lim CS, Park GH. Laboratory of Cellular Oncology.

Transfusion-transmitted malaria is rare, but it may produce severe problem in the safety of blood transfusion due to the lack of reliable procedure to evaluate donors potentially exposed to malaria. Here, we evaluated a new enzyme-linked immunosorbent assay malaria antibody test (ELISA malaria antibody test, DiaMed, Switzerland) to detect antibodies to Plasmodium vivax (the indigenous malaria) in the blood samples in the Republic of Korea (ROK). Blood samples of four groups were obtained and analyzed; 100 samples from P.vivax infected patients, 35 from recovery patients, 366 from normal healthy individuals, and 325 from domestic travelers of non-endemic areas residents to risky areas of ROK. P.vivax antibody levels by ELISA were then compared to the results from microscopic examination and polymerase chain reaction (PCR) test. As a result, the ELISA malaria antibody test had a clinical sensitivity of 53.0% and a clinical specificity of 94.0% for P.vivax. Twenty out of 325 domestic travelers (6.2%) were reactive and 28 cases (8.6%) were doubtful. Of the reactive and doubtful cases, only two were confirmed as acute malaria by both microscopy and PCR test. Thus we found that the ELISA malaria antibody test was insufficiently sensitive for blood screening of P.vivax in ROK.


Reverse Genetics Analysis of Antiparasitic Responses in the Malaria Vector, Anopheles gambiae.

Blandin SA, Levashina EA. IBMC, Strasbourg, France.

Anopheles mosquitoes are the major vectors of human malaria parasites. Mosquito-parasite interactions are critical for disease transmission and therefore represent a potential target for malaria control strategies. Mosquitoes mount potent antiparasitic responses, and identification of mosquito factors that limit parasite development is one of the major objectives in the field. To address this question, we have developed a convenient reverse genetics approach by injection of double-stranded RNA (dsRNA) in adult mosquitoes, to evaluate the function of candidate genes in mosquito antiparasitic responses.

37: Microbes Infect. 2008 Mar;10(3):269-75.

A comparison of Anopheles gambiae and Plasmodium falciparum genetic structure over space and time.

Prugnolle F, Durand P, Jacob K, Razakandrainibe F, Arnathau C, Villarreal D, Rousset F, de Meeûs T, Renaud F.

Génétique et Evolution des Maladies Infectieuses, UMR 2274 IRD-CNRS-UMI, Centre IRD, 911 avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France.

Population genetic structure and subdivision are key factors affecting the evolution of organisms. In this study, we analysed and compared the population genetic structure of the malaria parasite Plasmodium falciparum and its mosquito vector Anopheles gambiae over space and time in the Nianza Province, near Victoria Lake in Kenya. The parasites were collected from mosquitoes caught in six villages separated by up to 68km in 2002 and 2003. A total of 545 oocysts were dissected from 122 infected mosquitoes and genotyped at seven microsatellite markers. Five hundred and forty-seven mosquitoes, both infected and uninfected,
were genotyped at eight microsatellites. For the parasite and the vector, the analysis revealed no (or very little) genetic differentiation among villages. This may be explained by high local population sizes for the parasite and the mosquito. The small level of genetic differentiation observed between populations may explain the speed at which antimalarial drug resistance and insecticide resistance spread into the African continent.


*Malaria combination therapies: advantages and shortcomings.*

Martinelli A, Moreira R, Ravo PV.

Instituto de Higiene e Medicina Tropical, Rua da Junqueira 96. 1349-008 Lisbon, Portugal. amartinelli@ihmt.unl.pt and pcravo@ihmt.unl.pt

Drug combination therapies have been devised to delay the development and spread of resistant malaria parasites. However, poor design often leads to ineffective combinations. Here, the properties of various drug combinations are reviewed in relationship to drug resistance and their pharmacokinetic compatibility.


*Binding affinity of Plasmodium falciparum-infected erythrocytes from infected placetas and laboratory selected strains to chondroitin 4-sulfate.*

Achur RN, Muthusamy A, Madhunapantula SV, Gowda DC.

Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, PA 17033, United States.

The adherence of Plasmodium falciparum-infected red blood cells (IRBCs) in human placenta is mediated by chondroitin 4-sulfate (C4S). The C4S-adherent parasites selected from laboratory strains have been widely used for determining the C4S structural elements involved in IRBC binding and for the identification of parasite adhesive protein(s). However, as far as we know, the relative binding strength of the placental versus laboratory-selected parasites has not been reported. In this study, we show that IRBCs from the infected placetas bind to C4S about 3-fold higher than those selected for C4S adherence from laboratory strains. Although adherent parasites selected from several laboratory strains have comparable binding strengths, the one obtained from 3D7 parasites designated as 3D7N61 used for malaria genome sequencing, exhibits markedly lower binding strength. Furthermore, 3D7N61-CSA parasites lose most of the binding capacity by tenth generation in continuous culture.

*Mol Biochem Parasitol.* 2008 Feb 14

*Truncation of Plasmodium berghei merozoite surface protein 8 does not affect in vivo blood-stage development.*

de Koning-Ward TF, Drew DR, Chesson JM, Beeson JG, Crabb BS.

The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, VIC 3050, Australia.

Merozoite surface protein 8 (MSP8) has shown promise as a vaccine candidate in the Plasmodium yoelii rodent malaria model and has a proposed role in merozoite invasion of erythrocytes. However, the temporal expression and localisation of MSP8 are unusual for a merozoite antigen. Moreover, in Plasmodium falciparum the MSP8 gene could be disrupted with no apparent effect on invitro growth. To address the invivo function of full-length MSP8, we truncated MSP8 in the rodent
parasite Plasmodium berghei. PbDeltaMSP8 disruptant parasites displayed a normal blood-stage growth rate but no increase in reticulocyte preference, a phenomenon observed in P. yoelii MSP8 vaccinated mice. Expression levels of erythrocyte surface antigens were similar in P. berghei wild-type and PbDeltaMSP8-infected erythrocytes, suggesting that a parasitophorous vacuole function for MSP8 does not involve global trafficking of such antigens. These data demonstrate that a full-length membrane-associated form of PbMSP8 is not essential for blood-stage growth.


Reduced immune complex binding capacity and increased complement susceptibility of red cells from children with severe malaria-associated anemia.

Owuor BO, Odhiambo CO, Otieno WO, Adhiambo C, Makawiti DW, Stoute JA.
The US Army Medical Research Unit, Kenya, and the Kenya Medical Research Institute, Nairobi, Kenya.

Plasmodium falciparum malaria causes 1-2 million deaths per year. Most deaths occur as a result of complications such as severe anemia and cerebral malaria (CM) (coma). Red cells of children with severe malaria-associated anemia (SMA) have acquired deficiencies in the complement regulatory proteins complement receptor 1 (CR1, CD35) and decay accelerating factor (DAF, CD55). We investigated whether these deficiencies affect the ability of erythrocytes to bind immune complexes (ICs) and regulate complement activation. We recruited 75 children with SMA (Hb </= 6 g/dL) from the holoendemic malaria region of the Lake Victoria basin, western Kenya, and 74 age- and gender-matched uncomplicated malaria controls. In addition, we recruited 32 children with CM and 52 age- and gender-matched controls. Deficiencies in red cell CR1 and CD55 in children with SMA were accompanied by a marked decline in IC binding capacity and increased C3b deposition in vivo and ex vivo. Importantly, these changes were specific because they were not seen in red cells of children with CM or their controls. These data suggest that the declines in red cell CR1 and CD55 seen in children with SMA are of physiologic significance and may predispose erythrocytes to complement-mediated damage and phagocytosis in vivo.


MSP1(19) miniproteins can serve as targets for invasion inhibitory antibodies in Plasmodium falciparum provided they contain the correct domains for cell surface trafficking.

Gilson PR, O'Donnell RA, Neb1 T, Sanders PR, Wickham ME, McElwain TF, de Koning-Ward TF, Crabb BS.
The Walter and Eliza Hall Institute of Medical Research, Melbourne, Vic. 3050, Australia.

Antibodies from malaria-exposed individuals can agglutinate merozoites released from Plasmodium schizonts, thereby preventing them from invading new erythrocytes. Merozoite coat proteins attached to the plasma membrane are major targets for host antibodies and are therefore considered important malaria vaccine candidates. Prominent among these is the abundant glycosylphosphatidylinositol (GPI)-anchored merozoite surface protein 1 (MSP1) and particularly its C-terminal fragment (MSP1(19)) comprised of two epidermal growth factor (EGF)-like modules. In this paper, we revisit the role of agglutination and immunity using transgenic fluorescent marker proteins. We describe expression of heterologous MSP1(19)'miniproteins' on the surface of Plasmodium falciparum merozoites. To correctly express these proteins, we determined that GPI-anchoring and the presence of a signal sequence do not allow...
default export of proteins from the endoplasmic reticulum to merozoite surface and that extra sequence elements are required. The EGFs are insufficient for correct trafficking unless they are fused to additional residues that normally reside upstream of this fragment. Antibodies specifically targeting the surface-expressed miniprotein can inhibit erythrocyte invasion in vitro despite the presence of endogenous MSP1. Using a line expressing a green fluorescent protein-MSP1 fusion protein, we demonstrate that one mode of inhibition by antibodies targeting the MSP1(19) domain is the rapid agglutinating of merozoites prior to erythrocyte attachment.


Synthetic GPI array to study antitoxic malaria response.


Laboratory for Organic Chemistry, Swiss Federal Institute of Technology (ETH) Zurich, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland.

Parasite glycosylphosphatidylinositol (GPI) is an important toxin in malaria disease, and people living in malaria-endemic regions often produce high levels of anti-GPI antibodies. The natural anti-GPI antibody response needs to be understood to aid the design of an efficient carbohydrate-based antitoxin vaccine. We present a versatile approach based on a synthetic GPI glycan array to correlate anti-GPI antibody levels and protection from severe malaria.

43: Parasitology. 2008 Mar 27;:1-12

HIV-1/parasite co-infection and the emergence of new parasite strains.

Lloyd-Smith JO, Poss M, Grenfell BT. Center for Infectious Disease Dynamics, Pennsylvania State University, 208 Mueller Lab, University Park PA, 16802, USA.

SUMMARY: HIV-1 and parasitic infections co-circulate in many populations, and in a few well-studied examples HIV-1 co-infection is known to amplify parasite transmission. There are indications that HIV-1 interacts significantly with many other parasitic infections within individual hosts, but the population-level impacts of co-infection are not well-characterized. Here we consider how alteration of host immune status due to HIV-1 infection may influence the emergence of novel parasite strains. We review clinical and epidemiological evidence from five parasitic diseases (malaria, leishmaniasis, schistosomiasis, trypanosomiasis and strongyloidiasis) with emphasis on how HIV-1 co-infection alters individual susceptibility and infectiousness for the parasites. We then introduce a simple modelling framework that allows us to project how these individual-level properties might influence population-level dynamics. We find that HIV-1 can facilitate invasion by parasite strains in many circumstances and we identify threshold values of HIV-1 prevalence that allow otherwise unsustainable parasite strains to invade successfully. Definitive evidence to test these predicted effects is largely lacking, and we conclude by discussing challenges in interpreting available data and priorities for future studies.

44: Phytomedicine. 2008 Mar 10

In vitro antiplasmodial activity of extract and constituents from Esenbeckia febrifuga, a plant traditionally used to treat malaria in the Brazilian Amazon.

Dolabela MF, Oliveira SG, Nascimento JM, Peres JM, Wagner H, Póvoa MM, de Oliveira AB.

Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brazil.
Esenbeckia febrifuga (Rutaceae) is a plant traditionally used to treat malaria in the Brazilian Amazon region. Ethanol extract of stems displayed a good antiplasmodial activity against Plasmodium falciparum strains W-2 (IC(50) 15.5±0.71µg/ml) and 3D7 (IC(50) 21.0±1.4µg/ml). Two coumarins (bergaptene 1 and isopimpinellin 2), five alkaloids (flindersiamine 3, kokusaginine 4, skimmiamine 5, gamma-fagarine 6 and 1-hydroxy-3-methoxy-N-methylacridone, 7), besides a limonoid (rutaevine 8), have been isolated for the first time from this species. Antiplasmodial activity of compounds 3, 5-8 has been evaluated in vitro against P. falciparum strains (W-2 and 3D7) and the furoquinolines 5 and 6 were the most potent displaying IC(50) values <50µg/ml; flindersiamine (3) showed a weak activity while alkaloid 7 and rutaevine (8) were inactive (IC(50)>100µg/ml).


*Increased microerythrocyte count in homozygous alpha(+)-thalassaemia contributes to protection against severe malarial anaemia.*

Fowkes FJ, Allen SJ, Allen A, Alpers MP, Weatherall DJ, Day KP.

Peter Medawar Building for Pathogen Research and Department of Zoology, University of Oxford, Oxford, United Kingdom.

**BACKGROUND:** The heritable haemoglobinopathy alpha(+)-thalassaemia is caused by the reduced synthesis of alpha-globin chains that form part of normal adult haemoglobin (Hb). Individuals homozygous for alpha(+)-thalassaemia have microcytosis and an increased erythrocyte count. Alpha(+)-thalassaemia homozygosity confers considerable protection against severe malaria, including severe malarial anaemia (SMA) (Hb concentration < 50 g/l), but does not influence parasite count. We tested the hypothesis that the erythrocyte indices associated with alpha(+)-thalassaemia homozygosity provide a haematological benefit during acute malaria. **METHODS AND FINDINGS:** Data from children living on the north coast of Papua New Guinea who had participated in a case-control study of the protection afforded by alpha(+)-thalassaemia against severe malaria were reanalysed to assess the genotype-specific reduction in erythrocyte count and Hb levels associated with acute malarial disease. We observed a reduction in median erythrocyte count of approximately 1.5 x 10(12)/l in all children with acute falciparum malaria relative to values in community children (p < 0.001). We developed a simple mathematical model of the linear relationship between Hb concentration and erythrocyte count. This model predicted that children homozygous for alpha(+)-thalassaemia lose less Hb than children of normal genotype for a reduction in erythrocyte count of >1.1 x 10(12)/l as a result of the reduced mean cell Hb in homozygous alpha(+)-thalassaemia. In addition, children homozygous for alpha(+)-thalassaemia require a 10% greater reduction in erythrocyte count than children of normal genotype (p = 0.02) for Hb concentration to fall to 50 g/l, the cutoff for SMA. We estimated that the haematological profile in children homozygous for alpha(+)-thalassaemia reduces the risk of SMA during acute malaria compared to children of normal genotype (relative risk 0.52; 95% confidence interval [CI] 0.24-1.12, p = 0.09). **CONCLUSIONS:** The increased erythrocyte count and microcytosis in children homozygous for alpha(+)-thalassaemia may contribute substantially to their protection against SMA. A lower concentration of Hb per erythrocyte and a larger population of erythrocytes may be a biologically advantageous strategy against the significant reduction in erythrocyte count that occurs during acute infection with the malaria parasite Plasmodium falciparum. This haematological profile may reduce the risk of anaemia by other Plasmodium species, as well as other causes of anaemia. Other host polymorphisms that induce an increased erythrocyte count and microcytosis may confer a similar advantage.
Genome-wide detection of serpentine receptor-like proteins in malaria parasites.

Madeira L, Galante PA, Budu A, Azevedo MF, Malnic B, Garcia CR.

Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.

Serpentine receptors comprise a large family of membrane receptors distributed over diverse organisms, such as bacteria, fungi, plants and all metazoans. However, the presence of serpentine receptors in protozoan parasites is largely unknown so far. In the present study we performed a genome-wide search for proteins containing seven transmembrane domains (7-TM) in the human malaria parasite Plasmodium falciparum and identified four serpentine receptor-like proteins. These proteins, denoted PfSR1, PfSR10, PfSR12 and PfSR25, show membrane topologies that resemble those exhibited by members belonging to different families of serpentine receptors. Expression of the pfsrs genes was detected by Real Time PCR in P. falciparum intraerythrocytic stages, indicating that they potentially code for functional proteins. We also found corresponding homologues for the PfsRs in five other Plasmodium species, two primate and three rodent parasites. PfSR10 and 25 are the most conserved receptors among the different species, while PfSR1 and 12 are more divergent. Interestingly, we found that PfSR10 and PfSR12 possess similarity to orphan serpentine receptors of other organisms. The identification of potential parasite membrane receptors raises a new perspective for essential aspects of malaria parasite host cell infection.

Malaria liver stage susceptibility locus identified on mouse chromosome 17 by congenic mapping.

Gonçalves LA, Almeida P, Mota MM, Penha-Gonçalves C.

Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Host genetic variants are known to confer resistance to Plasmodium blood stage infection and to control malaria severity both in humans and mice. This work describes the genetic mapping of a locus for resistance to liver stage parasite in the mouse. First, we show that decreased susceptibility to the liver stage of Plasmodium berghei in the BALB/c mouse strain is attributable to intra-hepatic factors and impacts on the initial phase of blood stage infection. We used QTL mapping techniques to identify a locus controlling this susceptibility phenotype (LOD score 4.2) on mouse chromosome 17 (belr1 locus). Furthermore, analysis of congenic mouse strains delimited the belr1 locus boundaries distally to the H2 region. Quantification of parasites in the liver of infected congenic mice strongly suggested that the belr1 locus represents a genetic factor controlling the expansion of P. berghei in the hepatic tissue. The mapping of belr1 locus raises the hypothesis that host gene variation is able to control the progression of Plasmodium liver stage infection and opens the possibility that the human genomic region orthologue to belr1 may contain genes that confer resistance to the human malaria liver stage.

Plasmodium falciparum transcriptome analysis reveals pregnancy malaria associated gene expression.

BACKGROUND: Pregnancy-associated malaria (PAM) causing maternal anemia and low birth weight is among the multiple manifestations of Plasmodium falciparum malaria. Infected erythrocytes (iRs) can acquire various adhesive properties that mediate the clinical severity of malaria. Recent advances on the molecular basis of virulence and immune evasion have helped identify var2csa as a PAM-specific var gene. METHODOLOGY/PRINCIPAL FINDINGS: The present study presents a genome-wide microarray transcript analysis of 18 P. falciparum parasite isolates freshly collected from the placenta. The proportion of PAM over-expressed genes located in subtelomeric regions as well as that of PAM over-expressed genes predicted to be exported were higher than expected compared to the whole genome. The identification of novel parasite molecules with specificity to PAM and which are likely involved in host-pathogen interactions and placental tropism is described. One of these proteins, PFI1785w, was further characterized as the product of a two-exon PHIST gene, and was more often recognized by serum samples from P. falciparum-exposed women than from men. CONCLUSIONS/SIGNIFICANCE: These findings suggest that other parasite proteins, such as PFI1785w, may contribute beside VAR2CSA to the pathogenesis of PAM. These data may be very valuable for future vaccine development.

**PLoS ONE. 2008 Mar 12;3(3):e1767.**

Prospects for malaria eradication in sub-Saharan Africa.

Aguas R, White LJ, Snow RW, Gomes MG.

Instituto Gulbenkian de Ciência, Oeiras, Portugal.

BACKGROUND: A characteristic of Plasmodium falciparum infections is the gradual acquisition of clinical immunity resulting from repeated exposures to the parasite. While the molecular basis of protection against clinical malaria remains unresolved, its effects on epidemiological patterns are well recognized. Accumulating epidemiological data constitute a valuable resource that must be intensively explored and interpreted as to effectively inform control planning. METHODOLOGY/PRINCIPAL FINDING: Here we apply a mathematical model to clinical data from eight endemic regions in sub-Saharan Africa. The model provides a quantitative framework within which differences in age distribution of clinical disease are assessed in terms of the parameters underlying transmission. The shorter infectious periods estimated for clinical infections induce a regime of bistability of endemic and malaria-free states in regions of mesoendemic transmission. The two epidemiological states are separated by a threshold that provides a convenient measure for intervention design. Scenarios of eradication and resurgence are simulated. CONCLUSIONS/SIGNIFICANCE: In regions that support mesoendemic transmission, intervention success depends critically on reducing prevalence below a threshold which separates endemic and malaria-free regimes.

**PLoS ONE. 2008 Mar 5;3(3):e1779.**

Open-Label Comparative Clinical Study of Chlorproguanil-Dapsone Fixed Dose Combination (Lapdap trade mark) Alone or with Three Different Doses of Artesunate for Uncomplicated Plasmodium falciparum Malaria.


Department of Pharmacology & Therapeutics, University of Liverpool, Liverpool, United Kingdom.
The objective of this study was to determine the appropriate dose of artesunate for use in a fixed dose combination therapy with chlorproguanil-dapsone (CPG-DDS) for the treatment of uncomplicated falciparum malaria. METHODS: Open-label clinical trial comparing CPG-DDS alone or with artesunate 4, 2, or 1 mg/kg at medical centers in Blantyre, Malawi and Farafenni, The Gambia. The trial was conducted between June 2002 and February 2005, including 116 adults (median age 27 years) and 107 children (median age 38 months) with acute uncomplicated Plasmodium falciparum malaria. Subjects were randomized into 4 groups to receive CPG-DDS alone or plus 4, 2 or 1 mg/kg of artesunate once daily for 3 days. Assessments took place on Days 0-3 in hospital and follow-up on Days 7 and 14 as out-patients. Efficacy was evaluated in the Day 3 per-protocol (PP) population using mean time to reduce baseline parasitemia by 90% (PC90). A number of secondary outcomes were also included. Appropriate artesunate dose was determined using a pre-defined decision matrix based on primary and secondary outcomes. Treatment emergent adverse events were recorded from clinical assessments and blood parameters. Safety was evaluated in the intent to treat (ITT) population. RESULTS: In the Day 3 PP population for the adult group (N = 85), mean time to PC90 was 19.1 h in the CPG-DDS group, significantly longer than for the +artesunate 1 mg/kg (12.5 h; treatment difference -6.6 h [95%CI -11.8, -1.5]), 2 mg/kg (10.7 h; -8.4 h [95%CI -13.6, -3.2]) and 4 mg/kg (10.3 h; -8.7 h [95%CI -14.1, -3.2]) groups. For children in the Day 3 PP population (N = 92), mean time to PC90 was 21.1 h in the CPG-DDS group, similar to the +artesunate 1 mg/kg group (17.7 h; -3.3 h [95%CI -8.6, 2.0]), though the +artesunate 2 mg/kg and 4 mg/kg groups had significantly shorter mean times to PC90 versus CPG-DDS; 14.4 h (treatment difference -6.4 h [95%CI -11.7, -1.0]) and 12.8 h (-7.4 h [95%CI -12.9, -1.8]), respectively. An analysis of mean time to PC90 for the Day 14 PP and ITT populations was consistent with the primary analysis. Treatment emergent, drug-related adverse events were experienced in 35.3% (41/116) of adults and 70.1% (75/107) of children; mostly hematological and gastroenterological. The nature and incidence of adverse events was similar between the groups. No dose-related changes in laboratory parameters were observed. Nine serious adverse events due to any cause occurred in five subjects including two cases of hemolysis believed to be associated with drug treatment (one adult, one child). One adult died of anaphylactic shock, not associated with investigational therapy. CONCLUSIONS: CPG-DDS plus artesunate demonstrated advantages over CPG-DDS alone for the primary efficacy endpoint (mean time to PC90) except in children for the 1 mg/kg artesunate dose. Based on a pre-defined decision matrix, the primary endpoint in the child group supported an artesunate dose of 4 mg/kg. Secondary endpoints also supported a 4 mg/kg artesunate dose to take forward into the remainder of the development program. TRIAL REGISTRATION: ClinicalTrials.gov NCT00519467.


Identification and Characterization of a Novel Plasmodium falciparum Merozoite Apical Protein Involved in Erythrocyte Binding and Invasion.

Wickramarachchi T, Devi YS, Mohmmed A, Chauhan VS.

Malaria Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, India.

Proteins that coat Plasmodium falciparum merozoite surface and those secreted from its apical secretory organelles are considered promising candidates for the vaccine against malaria. In the present study, we have identified an asparagine rich parasite protein (PfAARP; Gene ID PFDD1105w), that harbors a predicted signal sequence, a C-terminal transmembrane region and whose transcription and translation patterns are similar to some well characterized merozoite surface/apical proteins. PfAARP was localized to the apical end of the merozoites by GFP-targeting approach using an inducible, schizont-stage expression system,
by immunofluorescence assays using anti-PfAARP antibodies. Immuno-electron
microscopic studies showed that PfAARP is localized in the apical ends of the
rhoptries in the merozoites. RBC binding assays with PfAARP expressed on COS
cells surface showed that it binds to RBCs through its N-terminal region with a
receptor on the RBC surface that is sensitive to trypsin and neuraminidase
treatments. Sequencing of PfAARP from different P. falciparum strains as well as
field isolates showed that the N-terminal region is highly conserved. Recombinant
protein corresponding to the N-terminal region of PfAARP (PfAARP-N) was produced
in its functional form in E. coli. PfAARP-N showed reactivity with immune sera
from individuals residing in P. falciparum endemic area. The anti-PfAARP-N rabbit
antibodies significantly inhibited parasite invasion in vitro. Our data on
localization, functional assays and invasion inhibition, suggest a role of PfAARP
in erythrocyte binding and invasion by the merozoite.


Evolution of malaria parasite plastid targeting sequences.

Tonkin CJ, Foth BJ, Ralph SA, Struck N, Cowman AF, McFadden GI.
School of Botany, University of Melbourne, Melbourne, Victoria 3010, Australia.

The transfer of genes from an endosymbiont to its host typically requires
acquisition of targeting signals by the gene product to ensure its return to the
endosymbiont for function. Many hundreds of plastid-derived genes must have
acquired transit peptides for successful relocation to the nucleus. Here, we
explore potential evolutionary origins of plastid transit peptides in the malaria
parasite Plasmodium falciparum. We show that exons of the P. falciparum genome
could serve as transit peptides after exon shuffling. We further demonstrate that
numerous randomized peptides and even whimsical sequences based on English words
can also function as transit peptides in vivo. Thus, facile acquisition of
transit peptides from existing sequence likely expedited endosymbiont integration
through intracellular gene transfer.


Chemokine receptor CXCR3 and its ligands CXCL9 and CXCL10 are required for the
development of murine cerebral malaria.

Campanella GS, Tager AM, El Khoury JK, Thomas SY, Abrazinski TA, Manice LA,
Colvin RA, Luster AD.
Center for Immunology and Inflammatory Diseases, Division of Rheumatology,
Allergy, and Immunology, Massachusetts General Hospital, Harvard Medical School,
Charlestown, MA 02129, USA.

Cerebral malaria is a significant cause of global mortality, causing an estimated
two million deaths per year, mainly in children. The pathogenesis of this disease
remains incompletely understood. Chemokines have been implicated in the
development of cerebral malaria, and the IFN-inducible CXCR3 chemokine ligand
IP-10 (CXCL10) was recently found to be the only serum biomarker that predicted
cerebral malaria mortality in Ghanaian children. We show that the CXCR3 chemokine
ligands IP-10 and Mig (CXCL9) were highly induced in the brains of mice with
murine cerebral malaria caused by Plasmodium berghei ANKA. Mice deficient in
CXCR3 were markedly protected against cerebral malaria and had far fewer T cells
in the brain compared with wild-type mice. In competitive transfer experiments,
CXCR3-deficient CD8(+) T cells were 7-fold less efficient at migrating into the
infected brains than wild-type CD8(+) T cells. Adoptive transfer of wild-type
CD8(+) effector T cells restored susceptibility of CXCR3-deficient mice to
cerebral malaria and also restored brain proinflammatory cytokine and chemokine
production and recruitment of T cells, independent of CXCR3. Mice deficient in
IP-10 or Mig were both partially protected against cerebral malaria mortality when infected with P. berghei ANKA. Brain immunohistochemistry revealed Mig staining of endothelial cells, whereas IP-10 staining was mainly found in neurons. These data demonstrate that CXCR3 on CD8(+) T cells is required for T cell recruitment into the brain and the development of murine cerebral malaria and suggest that the CXCR3 ligands Mig and IP-10 play distinct, nonredundant roles in the pathogenesis of this disease.

**Proc Natl Acad Sci U S A. 2008 Mar 18;105(11):4301-5.**

Correctly folded Pfs48/45 protein of Plasmodium falciparum elicits malaria transmission-blocking immunity in mice.


Departments of Molecular Biology and Medical Microbiology, Nijmegen Center for Molecular Life Sciences, Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands.

Malaria kills >1 million people each year, in particular in sub-Saharan Africa. Although asexual forms are directly responsible for disease and death, sexual stages account for the transmission of Plasmodium parasites from human to the mosquito vector and therefore the spread of the parasite in the population. Development of a malaria vaccine is urgently needed to reduce morbidity and mortality. Vaccines against sexual stages of Plasmodium falciparum are meant to decrease the force of transmission and consequently reduce malaria burden. Pfs48/45 is specifically expressed in sexual stages and is a well established transmission-blocking (TB) vaccine candidate. However, production of correctly folded recombinant Pfs48/45 protein with display of its TB epitopes has been a major challenge. Here, we show the production of a properly folded Pfs48/45 C-terminal fragment by simultaneous coexpression with four periplasmic folding catalysts in Escherichia coli. This C-terminal fragment fused to maltose binding protein was produced at medium scale with >90% purity and a stability over at least a 9-month period. It induces uniform and high antibody titers in mice and elicits functional TB antibodies in standard membrane feeding assays in 90% of the immunized mice. Our data provide a clear perspective on the clinical development of a Pfs48/45-based TB malaria vaccine.

**Proc Natl Acad Sci U S A. 2008 Mar 4;105(9):3262-7.**

Cloning and identification of an oxytocin/vasopressin-like receptor and its ligand from insects.

Stafflinger E, Hansen KK, Hauser F, Schneider M, Cazzamali G, Williamson M, Grimmelikhuijzen CJ.

Center for Functional and Comparative Insect Genomics, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark.

More than 20 years ago, an oxytocin/vasopressin-like peptide, CLITNCPRGamide, was isolated from the locust, Locusta migratoria [Proux JP, et al. (1987) Identification of an arginine vasopressin-like diuretic hormone from Locusta migratoria. Biochem Biophys Res Commun 149:180-186]. However, no similar peptide could be identified in other insects, nor could its prohormone be cloned, or its physiological actions be established. Here, we report that the recently sequenced genome from the red flour beetle Tribolium castaneum contains a gene coding for an oxytocin/vasopressin-like peptide, identical to the locust peptide, which we named inotocin (for insect oxytocin/vasopressin-like peptide) and a gene coding for an inotocin G protein-coupled receptor (GPCR). We cloned the Tribolium inotocin prohormone and the inotocin GPCR and expressed the GPCR in CHO cells.
This GPCR is strongly activated by low concentrations of inotocin (EC(50), 5 \times 10^{-9} \text{ M}), demonstrating that it is the inotocin receptor. Quantitative RT-PCR (qPCR) showed that in adult Tribolium, the receptor is mainly expressed in the head and much less in the hindgut and Malpighian tubules, suggesting that the inotocin/receptor couple does not play a role in water homeostasis. Surprisingly, qPCR also showed that the receptor is 30x more expressed in the first larval stages than in adult animals. The inotocin/receptor couple can also be found in the recently sequenced genome from the parasitic wasp Nasonia vitripennis but not in any other holometabolous insect with a completely sequenced genome (12 Drosophila species, the malaria mosquito Anopheles gambiae, the yellow fever mosquito Aedes aegypti, the silk worm Bombyx mori, and the honey bee Apis mellifera), suggesting that this neuropeptide system is confined to basal holometabolous insects. Furthermore, we identified an oxytocin/vasopressin-like peptide and receptor in the recently sequenced genome from the water flea Daphnia pulex (Crustacea). To our knowledge, this is the first report on the molecular cloning of an oxytocin/vasopressin-like receptor and its ligand from arthropods.

48: Public Health. 2008 Mar 19

**Effect of a community-based delivery of intermittent preventive treatment of malaria in pregnancy on treatment seeking for malaria at health units in Uganda.**

Mbonye AK, Schultz Hansen K, Bygbjerg IC, Magnussen P.

Department of Community Health, Ministry of Health, Box 7272, Kampala, Uganda.

BACKGROUND: The impact of intermittent preventive treatment (IPTp) on malaria in pregnancy is well known. However, in countries where this policy is implemented, poor access and low compliance have been widely reported. Novel approaches are needed to deliver this intervention. OBJECTIVE: To assess whether traditional birth attendants, drug-shop vendors, community reproductive health workers and adolescent peer mobilizers can administer IPTp with sulphadoxine-pyrimethamine (SP) to pregnant women, reach those at greatest risk of malaria, and increase access and compliance with IPTp. STUDY DESIGN: An intervention study compared the delivery of IPTp in the community with routine delivery of IPTp at health units. The primary outcome measures were the proportion of adolescents and primigravidae accessed, and the proportion of women who received two doses of SP. The study also assessed the effect of the intervention on access to malaria treatment, antenatal care, other services and related costs. RESULTS: More women (67.5%) received two doses of SP through the community approach compared with health units (39.9%; \text{P}<0.0001). Women who accessed IPTp in the community were at an earlier stage of pregnancy (21.0 weeks of gestation) than women who accessed IPTp at health units (23.1 weeks of gestation; \text{P}<0.0001). However, health units were visited by a higher proportion of primigravidae (23.6% vs 20.0%; \text{P}<0.04) and adolescents (28.4% vs 25.0%; \text{P}<0.03). Generally, women who accessed IPTp at health units made more visits for malaria treatment (2.6 (1.0-4.7) vs 1.8 (1.4-2.2); \text{P}<0.03). At recruitment, more women who accessed IPTp at health units sought malaria treatment compared with those who accessed IPTp in the community (56.9% vs 49.2%). However, at delivery, a high proportion of women who accessed IPTp in the community had sought malaria treatment (70.3%), suggesting the possibility that the novel approach had a positive impact on care seeking for malaria. Similarly, utilization of antenatal care, insecticide-treated nets and delivery care by women in the community was high. The total costs per woman receiving two doses of SP for IPTp were 4093 Uganda shillings (US$ 2.3) for women who accessed IPTp at health units, and 4491 Uganda shillings (US$ 2.6) for women who accessed IPTp in the community. CONCLUSION: The community approach was effective for the delivery of IPTp, although women still accessed and benefited from malaria treatment and other services at health units. However, the costs for accessing malaria treatment and other services are high and could be a limiting factor in mitigating the burden of malaria in Uganda.
When to seek health care: A duration analysis for malaria patients in Nepal.

Sharma VR. Department of Economics, University of Colorado Denver, 1380 Lawrence Street, Suite 460, Campus Box 181, Post Box 173364, Denver, CO 80217-3364, United States.

We find that the log-normal distribution of care-seeking time - the number of days from the onset of symptoms of malaria to when a patient seeks treatment from a provider - best described the treatment-seeking behavior of malaria patients in rural areas of two districts of Nepal. The care-seeking rate, or the probability of seeking care, was low on the first day of the symptoms; it increased sharply over the first five days and then gradually declined. Since at the time of the research there was a system of malaria workers taking monthly surveillance rounds of each house to detect and treat malaria cases, patients, instead of traveling to a provider for care, generally waited for malaria workers to arrive at home when the wait for malaria workers was short. But, the probability of seeking care on any day rose if the wait was longer. Women generally tended to wait longer for the malaria workers in order to receive treatment at home. Patient's age, household size, education, and the type of malaria species infecting the patient had no significant effect on care-seeking rate. Given an assumption that a wait of 100 days for a malaria worker would effectively represent total absence of surveillance program, the estimated model predicted higher care-seeking rates under no surveillance program than under the monthly surveillance program.

Dihydrofolate reductase I164L mutations in Plasmodium falciparum isolates: clinical outcome of 14 Kenyan adults infected with parasites harbouring the I164L mutation.


Centers for Disease Control and Prevention, Division of Parasitic Diseases, Malaria Branch, Atlanta, GA, USA; Kenya Medical Research Institute, Centre for Vector Biology and Control Research, Kisumu, Kenya.

Recently, Plasmodium falciparum bearing dihydrofolate reductase (DHFR) I164L was isolated from Africa. Quadruple mutations containing I164L confer high-level resistance to antifolate antimalarials. We prospectively measured the effect of co-trimoxazole (CTX) prophylaxis on P. falciparum antifolate resistance development among HIV-infected persons. HIV-positive patients with CD4 cell count <350 cells/μl (n=692) received CTX; HIV-positive patients with CD4 cell count ≥350 cells/μl (n=336) and HIV-negative patients (n=132) received multivitamins. Malaria microscopy-positive samples (n=413) and selected microscopy-negative/PCR-positive samples (n=76) were analysed for DHFR mutations at baseline and during six months follow up. We identified I164L in 14 patients. Seven were malaria microscopy-positive: two failed sulfadoxine-pyrimethamine (SP). Among seven microscopy-negative/PCR-positive patients, none developed patent infections with I164L. I164L was not associated with high-level SP resistance or poor outcome among adults living where malaria is highly endemic. Surveillance to monitor spread of I164L is critical, especially among children and pregnant women, who are potentially a source for I164L amplification.

Performance and reliability of the SYBR Green I based assay for the routine monitoring of susceptibility of Plasmodium falciparum clinical isolates.
Rason MA, Randriantsoa T, Andrianantenaina H, Ratsimbasoa A, Menard D.
Malaria Unit Research, Institut Pasteur de Madagascar, BP 1274, Antananarivo 101, Madagascar.

The performance and the reliability of a SYBR Green I fluorescence-based assay to assess drug susceptibility in routine monitoring were evaluated in 138 Plasmodium falciparum clinical samples. Blood samples were studied for susceptibility to four antimalarial drugs by the SYBR Green I based assay, with the traditional [(3)H]-hypoxanthine isotopic assay as a reference. The two methods were observed to have similar geometric means of IC50s and IC90s, and high correlation (r=0.93 for IC50s and r=0.94 for IC90s) for the drugs tested. The strength of agreement estimated by using concordance coefficient correlation was from almost perfect to substantial for IC50s. Our data demonstrate (i) the reliability of a simple, rapid and easy to use fluorescence-based assay for the routine monitoring of susceptibility of P. falciparum clinical isolates, and (ii) the possible switch from the traditional in vitro drug sensitivity assay to the SYBR Green I method, because previous data acquired by the isotopic assay were comparable with those obtained by the SYBR Green I method. We conclude that this assay will provide an easier method for testing drug susceptibility of malaria parasites, especially in malaria-endemic countries, where there is massive implementation of new artemisinin-based combination therapies.


A randomised trial to assess the efficacy and safety of chlorproguanil/dapsone + artesunate for the treatment of uncomplicated Plasmodium falciparum malaria.

London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK.

We tested the efficacy and safety of chlorproguanil/dapsone co-administered with artesunate (CD+A) for the treatment of uncomplicated Plasmodium falciparum malaria in children compared with amodiaquine+sulfadoxine/pyrimethamine (AQ+SP) at two different sites in Rwanda. The trial was open label and 800 patients were randomly assigned to AQ+SP (n=400) or CD+A (n=400). Patients were hospitalised for 3 days and then followed-up weekly until Day 28 after treatment. Clinical and parasitological outcomes were recorded. Results showed that neither treatment was adequately efficacious. At one site, the adequate clinical and parasitological response (ACPR), PCR-adjusted, was 73.3% in the CD+A arm and 87.8% in the AQ+SP arm (P<0.001), and at the second site the ACPR, PCR-adjusted, was 70.5% in the CD+A arm and 38.1% in the AQ+SP arm (P<0.001). The combination CD+A is considered an alternative to, or replacement for, SP in Africa because CD has been shown to be effective in patients for whom SP treatment has failed and, with its short half-life, it is expected to exert less selection pressure for resistant parasites than SP. However, the results of this trial indicate that in an area of high SP resistance, CD+A may not be the best choice. [ClinicalTrials.gov identifier: NCT00461578].

52: Trends Parasitol. 2008 Mar 18

Simplified antimalarial therapeutic monitoring: using the day-7 drug level?

White NJ, Stepniewska K, Barnes K, Price RN, Simpson J.
Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand; Centre for Vaccinology and Tropical Medicine, Churchill Hospital, Oxford OX3 7LJ, UK.
The blood concentration profiles of most antimalarial drugs vary considerably between patients. The interpretation of antimalarial drug trials evaluating efficacy and effectiveness would be improved considerably if the exposure of the infecting parasite population to the antimalarial drug treatment could be measured. Artemisinin combination treatments are now recommended as first-line drugs for the treatment of falciparum malaria. Measurement of the blood, serum or plasma concentration of the slowly eliminated partner antimalarial drug on day 7 of follow-up is simpler and might be a better determinant of therapeutic response than the area under the concentration-time curve. Measurement of the day-7 drug level should be considered as a routine part of antimalarial drug trials.

53: Trop Med Int Health. 2008 Mar 24

Estimates of the burden of malaria morbidity in Africa in children under the age of 5 years.

Roca-Feltrer A, Carneiro I, Armstrong Schellenberg JR.

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK.

Objective To estimate the direct burden of malaria among children younger than 5 years in sub-Saharan Africa (SSA) for the year 2000, as part of a wider initiative on burden estimates. Methods A systematic literature review was undertaken in June 2003. Severe malaria outcomes (cerebral malaria, severe malarial anaemia and respiratory distress) and non-severe malaria data were abstracted separately, together with information on the characteristics of each study and its population. Population characteristics were also collated at a national level. A meta-regression model was used to predict the incidence of malaria fevers at a national level. For severe outcomes, results were presented as median rates as data were too sparse for modelling. Results For the year 2000, an estimated 545 000 (uncertainty interval: 105 000-1 750 000) children under the age of 5 in SSA experienced an episode of severe malaria for which they were admitted to hospital. A total of 24 000 (interquartile range: 12 000-37 000) suffered from persistent neurological deficits as a result of cerebral malaria. The number of malaria fevers associated with high parasite density in under-5s in SSA in 2000 was estimated as 115 750 000 (uncertainty interval: 91 243 000-257 957 000). Conclusion Our study predicts a lower burden than previous estimates of under-5 malaria morbidity in SSA. As there is a lack of suitable data to enable comprehensive estimates of annual malaria incidence, we describe the information needed to improve the validity of future estimates.

Trop Med Int Health. 2008 Mar 12

High efficacy of two artemisinin-based combinations (artemether-lumefantrine and artesunate plus amodiaquine) for acute uncomplicated malaria in Ibadan, Nigeria.

Falade CO, Ogundele AO, Yusuf BO, Ademowo OG, Ladipo SM.

Department of Clinical Pharmacology, University College Hospital, Ibadan, Nigeria.

Objective To test the hypothesis that artesunate plus amodiaquine (ASAQ) is as effective as artemether-lumefantrine (AL) in the treatment of acute uncomplicated malaria in Nigerian children. Methods In an open label, randomized controlled clinical trial, children aged 6 months to 10 years were randomized to receive artesunate (4 mg/kg daily) plus amodiaquine (10 mg/kg daily) or AL (5-14 kg, one tablet; 15-24 kg, two tablets and 25-34 kg, three tablets twice daily). Both drug regimens were given for 3 days and follow-up was for 28 days. Results A total of 132 children (66 in each group) were randomized to receive either ASAQ or AL. Day 28 cure rates in the per protocol (PP) population were 93% for ASAQ and 95% for...
AL (OR = 0.71, 95% CI = 0.12-3.99, rho = 0.66). Using Kaplan-Meier product-limit estimates of failure, the median survival time for ASAQ was 21 days and for AL 28 days (P = 0.294). PCR corrected day 28 cure rate for PP populations were 98.4% for ASAQ and 100% for AL. Both drugs were well-tolerated. Conclusion ASAQ is as effective as AL and both combinations were efficacious and safe.


Longitudinal analyses of immune responses to Plasmodium falciparum derived peptides corresponding to novel blood stage antigens in coastal Kenya.

Agak GW, Bejon P, Fegan G, Gicheru N, Villard V, Kajava AV, Marsh K, Corradin G.

Department of Biochemistry, University of Lausanne, Chemin des Boveresses 155, 1066-Epalinges, Switzerland.

We have recently described 95 predicted alpha-helical coiled-coil peptides derived from putative Plasmodium falciparum erythrocytic stage proteins. Seventy peptides recognized with the highest level of prevalence by sera from three endemic areas were selected for further studies. In this study, we sequentially examined antibody responses to these synthetic peptides in two cohorts of children at risk of clinical malaria in Kilifi district in coastal Kenya, in order to characterize the level of peptide recognition by age, and the role of anti-peptide antibodies in protection from clinical malaria. Antibody levels from 268 children in the first cohort (Chonyi) were assayed against 70 peptides. Thirty-nine peptides were selected for further study in a second cohort (Junju). The rationale for the second cohort was to confirm those peptides identified as protective in the first cohort. The Junju cohort comprised of children aged 1-6 years old (inclusive). Children were actively followed up to identify episodes of febrile malaria in both cohorts. Of the 70 peptides examined, 32 showed significantly (p<0.05) increased antibody recognition in older children and 40 showed significantly increased antibody recognition in parasitaemic children. Ten peptides were associated with a significantly reduced odds ratio (OR) for an episode of clinical malaria in the first cohort of children and two of these peptides (LR146 and AS202.11) were associated with a significantly reduced OR in both cohorts. LR146 is derived from hypothetical protein PFB0145c in PlasmoDB. Previous work has identified this protein as a target of antibodies effective in antibody dependent cellular inhibition (ADCI). The current study substantiates further the potential of protein PFB0145c and also identifies protein PF11_0424 as another likely target of protective antibodies against P. falciparum malaria.