

Malaria Bulletin: A Compendium of Current Literature

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Abstracts

Acta Trop. 2008 Aug 8.

Competitive interactions between larvae of the malaria mosquitoes *Anopheles arabiensis* and *Anopheles gambiae* under semi-field conditions in western Kenya.

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The present paper reports the occurrence of competition between larvae of the malaria mosquito sibling species *Anopheles arabiensis* and *An. gambiae* under ambient conditions in western Kenya. Larvae of both species were reared at the same density outdoors in single-species and mixed-species populations (species ratio 1:1) in transparent cups that floated in small and large semi-natural pools, which experienced different diurnal variations in water temperature. In a second experiment, both species were reared at similar densities and under the same food conditions in trays in either single-species or mixed-species

populations at different proportions (species ratio 1:1, 1:3 or 3:1). Competition affected the development rate of both species in an opposite way: the development time of larvae of *An. arabiensis* increased whereas the development time of larvae of *An. gambiae* decreased in the presence of its sibling species. In small pools larvae developing in mixed-species populations experienced a higher mortality than larvae reared in single-species populations, whereas no such effect was observed in the large pools. In both species the time to pupation was longer and emerging females were larger in the small pools. Larval mortality of *An. arabiensis* was lower in the small pools compared to the large pools, whereas *An. gambiae* showed the opposite trend. Overall *An. arabiensis* showed reduced development rates, higher mortality rates and emerged with a larger body size compared to *An. gambiae*. The implication of these competitive interactions between larvae of *An. arabiensis* and *An. gambiae* under semi-field conditions needs to be considered in the design and implementation of programmes that aim to reduce malaria transmission as competition may alter the species composition in the field.

Antimicrob Agents Chemother. 2008 Sep;52(9):3085-91.

First assessment in humans of the safety, tolerability, pharmacokinetics, and ex vivo pharmacodynamic antimalarial activity of the new artemisinin derivative artemisone.

Nagelschmitz J, Voith B, Wensing G, Roemer A, Fugmann B, Haynes RK, Kotecka BM, Rieckmann KH, Edstein MD.

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In preclinical studies, artemisone (BAY 44-9585), a new artemisinin derivative, was shown to possess enhanced efficacy over artesunate, and it does not possess the neurotoxicity characteristic of the current artemisinins. In a phase I program with double-blind, randomized, placebo-controlled, single and multiple ascending oral-dose studies, we evaluated the safety, tolerability, pharmacokinetics, and ex vivo pharmacodynamic antimalarial activity of artemisone. Single doses (10, 20, 30, 40, and 80 mg) and multiple doses (40 and 80 mg daily for 3 days) of artemisone were administered orally to healthy subjects. Plasma concentrations of artemisone and its metabolites were measured by liquid chromatography/tandem mass spectrometry (LC/MS-MS). Artemisone was well tolerated, with no serious adverse events and no clinically relevant changes in laboratory and vital parameters. The pharmacokinetics of artemisone over the 10- to 80-mg range demonstrated dose linearity. After the single 80-mg dose, artemisone had a geometric mean maximum concentration of 140.2 ng/ml (range, 86.6 to 391.0), a short elimination half-life ($t(1/2)$) of 2.79 h (range, 1.56 to 4.88), a high oral clearance of 284.1 liters/h (range, 106.7 to 546.7), and a large volume of distribution of 14.50 liters/kg (range, 3.21 to 51.58). Due to artemisone's short $t(1/2)$, its pharmacokinetics were comparable after single and multiple dosing. Plasma samples taken after multiple dosing showed marked ex vivo pharmacodynamic antimalarial activities against two multidrug-resistant *Plasmodium falciparum* lines. Artemisone equivalent concentrations measured by bioassay revealed higher activity than artemisone measured by LC/MS-MS, confirming the presence of active metabolites. Comparable to those of other artemisinin's, artemisone's $t(1/2)$ is well suited for artemisinin-based combination therapy for the treatment of *P. falciparum* malaria.

Antimicrob Agents Chemother. 2008 Sep;52(9):3414-7.

Cell-penetrating peptide TP10 shows broad-spectrum activity against both *Plasmodium falciparum* and *Trypanosoma brucei brucei*.

Arrighi RB, Ebikeme C, Jiang Y, Ranford-Cartwright L, Barrett MP, Langel U, Faye

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Malaria and trypanosomiasis are diseases which afflict millions and for which novel therapies are urgently required. We have tested two well-characterized cell-penetrating peptides (CPPs) for antiparasitic activity. One CPP, designated TP10, has broad-spectrum antiparasitic activity against *Plasmodium falciparum*, both blood and mosquito stages, and against blood-stage *Trypanosoma brucei*.

Antimicrob Agents Chemother. 2008 Sep;52(9):3221-8.

Chemical target validation studies of aminopeptidase in malaria parasites using alpha-aminoalkylphosphonate and phosphonopeptide inhibitors.

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During its intraerythrocytic phase, the most lethal human malarial parasite, *Plasmodium falciparum*, digests host cell hemoglobin as a source of some of the amino acids required for its own protein synthesis. A number of parasite endopeptidases (including plasmepsins and falcipains) process the globin into small peptides. These peptides appear to be further digested to free amino acids by aminopeptidases, enzymes that catalyze the sequential cleavage of N-terminal amino acids from peptides. Aminopeptidases are classified into different evolutionary families according to their sequence motifs and preferred substrates. The aminopeptidase inhibitor bestatin can disrupt parasite development, suggesting that this group of enzymes might be a chemotherapeutic target. Two bestatin-susceptible aminopeptidase activities, associated with gene products belonging to the M1 and M17 families, have been described in blood-stage *P. falciparum* parasites, but it is not known whether one or both are required for parasite development. To establish whether inhibition of the M17 aminopeptidase is sufficient to confer antimalarial activity, we evaluated 35 aminoalkylphosphonate and phosphonopeptide compounds designed to be specific inhibitors of M17 aminopeptidases. The compounds had a range of activities against cultured *P. falciparum* parasites with 50% inhibitory concentrations down to 14 μ M. Some of the compounds were also potent inhibitors of parasite aminopeptidase activity, though it appeared that many were capable of inhibiting the M1 as well as the M17 enzyme. There was a strong correlation between the potencies of the compounds against whole parasites and against the enzyme, suggesting that M17 and/or M1 aminopeptidases may be valid antimalarial drug targets.

Antimicrob Agents Chemother. 2008 Aug 18.

Synthesis, antimalarial activity and intracellular targets of MEFAS, a new hybrid compound derived from mefloquine and artesunate.

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A new synthetic antimalarial drug - a salt derived from two antimalarial molecules, mefloquine (MQ) and artesunate (AS), here named MEFAS - has been tested for its pharmacological activity. AS+MQ hydrochloride combinations are currently used in areas with drug resistant *Plasmodium falciparum* parasites; although AS clears parasiteamia in shorter time periods than any other antimalarial drug, it does not cure infected patients; in addition, MQ causes side effects being rather expensive, important problems considering that malaria mostly affects populations in poor countries. We here show that MEFAS is more effective than AS+MQ combinations, tested in parallel at different mass proportions, against *P. falciparum* (W2 chloroquine-resistant and 3D7 chloroquine-sensitive) in vitro and in mice infected with *P. berghei* promoting cure of this infection. MEFAS tested against HepG2 hepatoma cells exhibited lower toxicity than the antimalarials AS and MQ alone or combined. Possible targets of MEFAS have been studied by confocal microscopy using fluorescent probes (Fluo-4 AM and BCECF-AM) in *P. falciparum* synchronous culture of W2 infected red blood cells. Dynamic images show that MEFAS exhibited intracellular action increasing cytoplasmic Ca(2+) at 1.0 ng/ml. This effect was also observed in the presence of tapsigargin, an inhibitor of SERCA, suggesting an intracellular target distinct from endoplasmic reticulum (ER). Trophozoites loaded with BCECF-AM, when treated with MEFAS, were still able to mobilize protons from the digestive vacuole (DV), altering the pH gradient. However, in the presence of Bafilomycin A1, an inhibitor of the H(+)-pump from acidic compartments of eukaryotic cells, MEFAS had no action on DV. In conclusion, ER and DV are intracellular targets for MEFAS in *Plasmodium* sp., suggesting two modes of action of this new salt. Our data support MEFAS as a candidate for treating human malaria.

BMC Genomics. 2008 Aug 25;9(1):398.

Detection of genome wide polymorphisms in the AT rich *Plasmodium falciparum* genome using a high density microarray.

Jiang H, Yi M, Mu J, Zhang L, Ivens A, Klimczak LJ, Huyen Y, Stephens RM, Su XZ.

ABSTRACT: BACKGROUND: Genetic mapping is a powerful method to identify mutations that cause drug resistance and other phenotypic changes in the human malaria parasite *Plasmodium falciparum*. For efficient mapping of a target gene, it is often necessary to genotype a large number of polymorphic markers. Currently, a community effort is underway to collect single nucleotide polymorphisms (SNP) from the parasite genome. Here we evaluate polymorphism detection accuracy of a high-density 'tiling' microarray with 2.56 million probes by comparing single feature polymorphisms (SFP) calls from the microarray with known SNP among parasite isolates. **RESULTS:** We found that probe GC content, SNP position in a probe, probe coverage, and signal ratio cutoff values were important factors for accurate detection of SFP in the parasite genome. We established a set of SFP calling parameters that could predict mSFP (SFPs called by multiple overlapping probes) with high accuracy (greater than or equal to 94%) and identified 121,087 mSFP genome-wide from five parasite isolates including 40,354 unique mSFP (excluding those from multi-gene families) and ~18,000 new mSFP, producing a genetic map with an average of one unique mSFP per 570 bp. Genomic copy number variation (CNV) among the parasites was also cataloged and compared. **CONCLUSION:** A large number of mSFP were discovered from the *P. falciparum* genome using a high density microarray, most of which were in clusters of highly polymorphic genes at chromosome ends. Our method for accurate mSFP detection and the mSFP identified will greatly facilitate large scale studies of genome variation in the *P. falciparum* parasite and provide useful resources for mapping important parasite traits.

The pathophysiology of malarial anaemia: where have all the red cells gone?

Kai OK, Roberts DJ.

ABSTRACT: Malarial anaemia is an enormous public health problem in endemic areas and occurs predominantly in children in the first 3 years of life. Anaemia is due to both a great increase in clearance of uninfected cells and a failure of an adequate bone marrow response. Odhiambo, Stoute and colleagues show how the age distribution of malarial anaemia and the haemolysis of red blood cells may be linked by an age-dependent increase in the capacity of red blood cells to inactivate complement components absorbed or deposited directly on to the surface of the red blood cell. In this commentary, we discuss what has been established about the role of complement deposition on the surface of red blood cells in the pathology of malarial anaemia, how genetic polymorphisms of the complement control proteins influence the outcome of malaria infection and how the findings of Odhiambo, Stoute and colleagues and others shed light on the puzzling age distribution of different syndromes.

Increased deposition of C3b on red cells with low CR1 and CD55 in a malaria-endemic region of western Kenya: implications for the development of severe anemia.

Odhiambo CO, Otieno W, Adhiambo C, Odera MM, Stoute JA.

ABSTRACT: BACKGROUND: Severe anemia due to *Plasmodium falciparum* malaria is a major cause of mortality among young children in western Kenya. The factors that lead to the age-specific incidence of this anemia are unknown. Previous studies have shown an age-related expression of red cell complement regulatory proteins, which protect erythrocytes from autologous complement attack and destruction. Our primary objective was to determine whether in a malaria-endemic area red cells with low levels of complement regulatory proteins are at increased risk for complement (C3b) deposition in vivo. Secondly, we studied the relationship between red cell complement regulatory protein levels and hemoglobin levels. **METHODS:** Three hundred and forty-two life-long residents of a malaria-holoendemic region of western Kenya were enrolled in a cross-sectional study and stratified by age. We measured red cell C3b, CR1, CD55, and immune complex binding capacity by flow cytometry. Individuals who were positive for malaria were treated and blood was collected when they were free of parasitemia. Analysis of variance was used to identify independent variables associated with the %C3b-positive red cells and the hemoglobin level. **RESULTS:** Individuals between the ages of 6 and 36 months had the lowest red cell CR1, highest %C3b-positive red cells, and highest parasite density. Malaria prevalence also reached its peak within this age group. Among children [less than or equal to]24 months of age the %C3b-positive red cells was usually higher in individuals who were treated for malaria than in uninfected individuals with similarly low red cell CR1 and CD55. The variables that most strongly influenced the %C3b-positive red cells were age, malaria status, and red cell CD55 level. Although it did not reach statistical significance, red cell CR1 was more important than red cell CD55 among individuals treated for malaria. The variables that most strongly influenced the hemoglobin level were age, the %C3b-positive red cells, red cell CR1, and red cell CD55. **CONCLUSIONS:** Increasing malaria prevalence among children >6 to [less than or equal to]36 months of age in western Kenya, together with low red cell CR1 and CD55 levels, results in increased C3b deposition on red cells and low hemoglobin. The strong contribution of age to C3b deposition suggests that there are still additional unidentified age-related factors that increase the susceptibility of red cells to C3b deposition and destruction.

Bull Entomol Res. 2008 Aug 21:1-9.

Cryptic species within *Anopheles longipalpis* from southern Africa and phylogenetic comparison with members of the *An. funestus* group.

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House-resting *Anopheles* mosquitoes are targeted for vector control interventions; however, without proper species identification, the importance of these *Anopheles* to malaria transmission is unknown. *Anopheles longipalpis*, a non-vector species, has been found in significant numbers resting indoors in houses in southern Zambia, potentially impacting on the utilization of scarce resources for vector control. The identification of *An. longipalpis* is currently based on classical morphology using minor characteristics in the adult stage and major ones in the larval stage. The close similarity to the major malaria vector *An. funestus* led to investigations into the development of a molecular assay for identification of *An. longipalpis*. Molecular analysis of *An. longipalpis* from South Africa and Zambia revealed marked differences in size and nucleotide sequence in the second internal transcribed spacer (ITS2) region of ribosomal DNA between these two populations, leading to the conclusion that more than one species was being analysed. Phylogenetic analysis showed the Zambian samples aligned with *An. funestus*, *An. vaneedeni* and *An. parensis*, whereas the South African sample aligned with *An. lesoni*, a species that is considered to be more closely related to the Asian *An. minimus* subgroup than to the African *An. funestus* subgroup. Species-specific primers were designed to be used in a multiplex PCR assay to distinguish between these two cryptic species and members of the *An. funestus* subgroup for which there is already a multiplex PCR assay.

Cell Host Microbe. 2008 Aug 14;4(2):179-87.

Platelet factor 4 mediates inflammation in experimental cerebral malaria.

Srivastava K, Cockburn IA, Swaim A, Thompson LE, Tripathi A, Fletcher CA, Shirk EM, Sun H, Kowalska MA, Fox-Talbot K, Sullivan D, Zavala F, Morrell CN.

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Cerebral malaria (CM) is a major complication of *Plasmodium falciparum* infection in children. The pathogenesis of CM involves vascular inflammation, immune stimulation, and obstruction of cerebral capillaries. Platelets have a prominent role in both immune responses and vascular obstruction. We now demonstrate that the platelet-derived chemokine, platelet factor 4 (PF4)/CXCL4, promotes the development of experimental cerebral malaria (ECM). *Plasmodium*-infected red blood cells (RBCs) activated platelets independently of vascular effects, resulting in increased plasma PF4. PF4 or chemokine receptor CXCR3 null mice had less severe ECM, including decreased T cell recruitment to the brain, and platelet depletion or aspirin treatment reduced the development of ECM. We conclude that *Plasmodium*-infected RBCs can directly activate platelets, and platelet-derived PF4 then contributes to immune activation and T cell trafficking as part of the pathogenesis of ECM.

Exp Parasitol. 2008 Sep;120(1):113-7.

Plasmodium yoelii: novel rhoptry proteins identified within the body of merozoite rhoptries in rodent Plasmodium malaria.

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The biogenesis, organization and function of the rhoptries are not well understood. Antisera were prepared to synthetic peptides prepared as multiple antigenic peptides (MAPs) obtained from a Plasmodium yoelii merozoite rhoptry proteome analysis. The antisera were used in immunofluorescence and immunoelectron microscopy of schizont-infected erythrocytes. Twenty-seven novel rhoptry proteins representing proteases, metabolic enzymes, secreted proteins and hypothetical proteins, were identified in the body of the rhoptries by immunoelectron microscopy. The merozoite rhoptries contain a heterogeneous mixture of proteins that may initiate host cell invasion and establish intracellular parasite development.

Exp Parasitol. 2008 Sep;120(1):29-38.

Plasmodium falciparum: food vacuole localization of nitric oxide-derived species in intraerythrocytic stages of the malaria parasite.

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Nitric oxide (NO) has diverse biological functions. Numerous studies have documented NO's biosynthetic pathway in a wide variety of organisms. Little is known, however, about NO production in intraerythrocytic Plasmodium falciparum. Using diaminorhodamine-4-methyl acetoxymethylester (DAR-4M AM), a fluorescent indicator, we obtained direct evidence of NO and NO-derived reactive nitrogen species (RNS) production in intraerythrocytic P. falciparum parasites, as well as in isolated food vacuoles from trophozoite stage parasites. We preliminarily identified two gene sequences that might be implicated in NO synthesis in intraerythrocytic P. falciparum. We showed localization of the protein product of one of these two genes, a molecule that is structurally similar to a plant nitrate reductase, in trophozoite food vacuole membranes. We confirmed previous reports on the antiproliferative effect of NOS (nitric oxide synthase) inhibitors in P. falciparum cultures; however, we did not obtain evidence that NOS inhibitors had the ability to inhibit RNS production or that there is an active NOS in mature forms of the parasite. We concluded that a nitrate reductase activity produce NO and NO-derived RNS in or around the food vacuole in P. falciparum parasites. The food vacuole is a critical parasitic compartment involved in hemoglobin degradation, heme detoxification and a target for antimalarial drug action. Characterization of this relatively unexplored synthetic activity could provide important clues into poorly understood metabolic processes of the malaria parasite.

Infect Immun. 2008 Sep;76(9):4332-44.

Inhibition of malaria parasite development by a cyclic peptide that targets the vital parasite protein SERA5.

Fairlie WD, Spurck TP, McCoubrie JE, Gilson PR, Miller SK, McFadden GI, Malby R, Crabb BS, Hodder AN.

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The serine repeat antigen (SERA) proteins of the malaria parasites *Plasmodium* spp. contain a putative enzyme domain similar to that of papain family cysteine proteases. In *Plasmodium falciparum* parasites, more than half of the SERA family proteins, including the most abundantly expressed form, SERA5, have a cysteine-to-serine substitution within the putative catalytic triad of the active site. Although SERA5 is required for blood-stage parasite survival, the occurrence of a noncanonical catalytic triad casts doubt on the importance of the enzyme domain in this function. We used phage display to identify a small (14-residue) disulfide-bonded cyclic peptide (SBP1) that targets the enzyme domain of SERA5. Biochemical characterization of the interaction shows that it is dependent on the conformation of both the peptide and protein. Addition of this peptide to parasite cultures compromised development of late-stage parasites compared to that of control parasites or those incubated with equivalent amounts of the carboxymethylated peptide. This effect was similar in two different strains of *P. falciparum* as well as in a transgenic strain where the gene encoding the related serine-type parasitophorous vacuole protein SERA4 was deleted. In compromised parasites, the SBP1 peptide crosses both the erythrocyte and parasitophorous vacuole membranes and accumulates within the parasitophorous vacuole. In addition, both SBP1 and SERA5 were identified in the parasite cytosol, indicating that the plasma membrane of the parasite was compromised as a result of SBP1 treatment. These data implicate an important role for SERA5 in the regulation of the intraerythrocytic development of late-stage parasites and as a target for drug development.

Infect Immun. 2008 Sep;76(9):3924-31.

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

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Splenic microarchitecture is substantially altered during acute malaria infections, which may affect the development and regulation of immune responses. Here we investigated whether engagement of host Toll-like receptor 2 (TLR2), TLR4, TLR9, and the adaptor protein MyD88 is required for induction of the changes and whether antibody responses are modified when immunization takes place during the period of splenic disruption. The alterations in splenic microarchitecture were maximal shortly after the peak of parasitemia and were not dependent on engagement of TLR2, TLR4, or TLR9, and they were only minimally affected by the absence of the MyD88 adaptor molecule. Although germinal centers were formed in infected mice, they did not contain the usual light and dark zones. Immunization of mice with chicken gamma globulin 2 weeks prior to acute *Plasmodium chabaudi* infection did not affect the quantity or avidity of the immunoglobulin G antibody response to this antigen. However, immunization at the same time as the primary *P. chabaudi* infection resulted in a clear transient reduction in antibody avidity in the month following immunization. These data

suggest that the alterations in splenic structure, particularly the germinal centers, may affect the quality of an antibody response during a malaria infection and could impact the development of immunity to malaria or to other infections or immunizations given during a malaria infection.

Infect Immun. 2008 Aug 18.

Enhanced TLR responsiveness associated with MAPK activation in Plasmodium falciparum-infected children.

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Acute Plasmodium falciparum infection is associated with strongly up-regulated cytokine responses that are at least partly the result of activation of Toll-like receptors (TLR). Whether and how TLR expression/responsiveness changes upon malarial infection is, however, currently not well understood. To assess this, we examined expression of TLRs and used the TLR ligands LPS and Pam3Cys to stimulate peripheral blood mononuclear cells (PBMC) from Ghanaian schoolchildren who live in a rural area where P. falciparum is endemic. Expression of TLR2 was higher and responses to its ligand, Pam3Cys, were enhanced in P. falciparum-infected children compared to their uninfected counterparts. In cells from the same children, stimulation Pam3Cys resulted in higher p38 mitogen activated protein kinase (MAPK) activation and higher cytokine production. In vitro experiments confirmed that pre-incubation of PBMC with P. falciparum-infected red blood cells enhanced responsiveness to TLR ligands. Taken together the data indicate that P. falciparum-infected children in malaria-endemic areas have an altered innate immune system, which might be important for the balance between immunity and pathology when new infections are encountered or when novel vaccines are introduced.

Infect Immun. 2008 Aug 18.

Identification and characterization of the Plasmodium yoelii PyP140/RON4 protein, an orthologue of Toxoplasma gondii RON4; the cysteine-rich domain does not protect against lethal parasite challenge infection.

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Previously we identified a Plasmodium yoelii YM 140 kDa merozoite protein, designated PyP140, which formed a complex with Apical Membrane Antigen 1 (AMA1). Furthermore, we had produced a nonprotective monoclonal antibody (mAb), 48F8 that immunoprecipitated metabolically labeled PyP140 and localized the protein to the merozoite's apical end and less frequently to the merozoite surface by immunofluorescence (IFA). Here, using mAb 48F8 we have identified the pyp140 gene by screening a P. yoelii lambda-ZAP cDNA expression library. The pyp140 cDNA covers approximately ninety percent of the putative open reading frame (ORF) of PY02159 from the P. yoelii NL genome sequencing project. Analysis of the complete gene identified the presence of two introns. The ORF encodes a 102,407 Da protein with an amino terminal signal sequence, a series of three unique types of repeats

and a cysteine rich region. The binding site of mAb 48F8 was also identified. A Blast search with the deduced amino acid sequence shows a significant similarity with the *Toxoplasma gondii* RON4 protein, *P. falciparum* RON4 protein and the sequence is highly conserved in other *Plasmodium* species. We produced the cysteine rich domain of PyP140/RON4 using the *Pichia pastoris* expression system and characterized biochemically and biophysically the recombinant protein. BALB/c mice immunized with the protein formulated in oil-in-water adjuvants produced antibodies that recognize parasitized erythrocytes by IFA and native PyP140/RON4 by immunoblot but failed to protect against a lethal *P. yoelii* YM infection. Our results show that PyP140/RON4 is located within the rhoptries or micronemes. It may associate in part with AMA1, but the conserved cysteine-rich domain does not appear to elicit inhibitory antibodies, a finding that is supported by the marked sequence conservation in this protein within *Plasmodium* spp, suggesting that it is not under immune pressure.

Int J Parasitol. 2008 Aug 8.

A study on pathogenicity and mosquito transmission success in the rodent malaria parasite *Plasmodium chabaudi adami*.

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We investigated the parasitology, pathogenicity (virulence) and infectivity to mosquitoes of blood infections in mice, of two strains, DS and DK, of the rodent malaria parasite *Plasmodium chabaudi adami*. Blood infections of DS were found to be highly pathogenic; the asexual parasites in these infections were fast-growing and showed no evidence of selectivity in their infection of host erythrocytes. In contrast to DS, blood infections of DK were much less pathogenic; the asexual parasites were slower-growing and showed a moderate degree of selectivity to a subset of erythrocytes which were not reticulocytes. In both DS and DK infections, infectivity to mosquitoes was highest before the peak of asexual parasitaemia had occurred; usually this did not coincide with the time when gametocyte numbers in the blood were highest. Infections with the pathogenic DS strain in CBA mice produced fewer gametocytes than did the less pathogenic DK strain. The DS strain infections in both CBA and C57 mice were also significantly much less infective to mosquitoes than the DK strain. Investigations by others on the related rodent malaria parasite subspecies, *Plasmodium chabaudi chabaudi*, have indicated that the mosquito infectivity of blood infections in mice tended to be higher in the more pathogenic (virulent) and lower in the less pathogenic strains of this parasite subspecies. This is the converse of the finding of the present investigation of blood infections of *P. c. adami* in mice in which a more pathogenic, or virulent, strain (DS) of these parasites was significantly much less infective to mosquitoes than was a less pathogenic strain (DK).

Int J Parasitol. 2008 Aug 6.

Imaging effector functions of human cytotoxic CD4(+) T cells specific for *Plasmodium falciparum* circumsporozoite protein.

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Malaria vaccines, comprised of irradiated *Plasmodium falciparum* sporozoites or a synthetic peptide containing T and B cell epitopes of the circumsporozoite protein (CSP), elicit multifunctional cytotoxic and non-cytotoxic CD4(+) T cells in immunised volunteers. Both lytic and non-lytic CD4(+)T cell clones recognised

a series of overlapping epitopes within a 'universal' T cell epitope EYLNKIQNSLSTEWSPCSVT of CSP (NF54 isolate) that was presented in the context of multiple DR molecules. Lytic activity directly correlated with T cell receptor (TCR) functional avidity as measured by stimulation indices and recognition of naturally occurring variant peptides. CD4(+) T cell-mediated cytotoxicity was contact-dependent and did not require de novo synthesis of cytotoxic mediators, suggesting a granule-mediated mechanism. Live cell imaging of the interaction of effector and target cells demonstrated that CD4(+) cytotoxic T cells recognise target cells with their leading edge, reorient their cytotoxic granules towards the zone of contact, and form a stable immunological synapse. CTL attacks induced chromatin condensation, nuclear fragmentation and formation of apoptotic bodies in target cells. Together, these findings suggest that CD4(+) CTLs trigger target cell apoptosis via classical perforin/granzyme-mediated cytotoxicity, similar to CD8(+) CTLs, and these multifunctional sporozoite- and peptide-induced CD4(+) T cells have the potential to play a direct role as effector cells in targeting the exoerythrocytic forms within the liver.

J Acquir Immune Defic Syndr. 2008 Sep 1;49(1):55-60.

Imported Malaria in HIV-Infected Patients Enrolled in the ANRS CO4 FHDH Study.

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From the *INSERM, U720, Paris, F-75013 France; daggerUPMC Univ Paris 06, UMR S270, F-75013, Paris, France; double daggerAssistance Publique-Hôpitaux de Paris, Groupe hospitalier Bichat Claude Bernard, Service de parasitologie, Paris, F-75018 France; section signRouen University Hospital, Service de maladies infectieuses et tropicales, Rouen, France; ||Assistance Publique-Hôpitaux de Paris, Groupe hospitalier Tenon, Service de maladies infectieuses et tropicales, Paris, F-75020 France; paragraph signCentre Hospitalier de Tourcoing, Service universitaire des maladies infectieuses et du voyage, Tourcoing, France; #Assistance Publique-Hôpitaux de Paris, Groupe hospitalier Pitié-Salpêtrière, Service de maladies infectieuses et tropicales, Paris, F-75013 France; and **Assistance Publique-Hôpitaux de Paris, Groupe hospitalier Bichat Claude Bernard, Service de maladies infectieuses et tropicales, Paris, F-75018 France.

BACKGROUND:: To describe episodes of imported malaria in human immunodeficiency virus type 1-infected patients and to study the risk factors for severe *Plasmodium falciparum* malaria. **METHODS::** Patients enrolled in the French Hospital Database on HIV who were diagnosed with a first episode of malaria between 1996 and 2003 were included. The severity of *P. falciparum* imported malaria was graded with World Health Organization criteria. Geographic areas were classified according to *P. falciparum* chemoresistance. Risk factors for severe malaria were identified with logistic regression. **RESULTS::** We studied 190 patients infected by *P. falciparum* in 178 cases. All but four of the patients were infected in sub-Saharan Africa, and half were returning from a country with a high *P. falciparum* chloroquine resistance. Their median age was 37.5 years, and 57% came from a country endemic with malaria. The median CD4 cell count was 299/mm, and the median plasma human immunodeficiency virus type 1 RNA load was 4.5 log₁₀ copies/mL. Sixty-five (36.5%) episodes of *P. falciparum* malaria were severe. Severe imported malaria was associated with CD4 cells/mm <350 (odds ratio = 2.58; 95% confidence interval: 1.19 to 5.57). The risk of severe malaria was lower in patients returning from a country with a high prevalence of chemoresistance (odds ratio = 0.50; 95% confidence interval: 0.25 to 0.99). **CONCLUSIONS::** Severe imported malaria in human immunodeficiency virus type 1-infected patients is associated with decreased CD4 cell count. The risk seems lower when *P. falciparum* infection was acquired in areas of high prevalence of chemoresistance.

Accumulation of CVIET Pfcrt allele of Plasmodium falciparum in placenta of pregnant women living in an urban area of Dakar, Senegal.

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Objectives Chemoprophylaxis is recommended during pregnancy to reduce the risk of placental infection. However, in areas with increasing drug resistance, it can trigger selection of resistant parasites in the placenta and increase the frequency of placental malaria. The objective of this study was to analyse the selection of drug-resistant parasites in the placenta in an area where chloroquine was still recommended as prophylaxis. Patients and methods We analysed the polymorphism of parasites from matched placental and venous blood samples at the time of delivery from women in Dakar. Polymorphism of the isolates was studied using nested PCR typing of MSA1 and MSA2 genes, and full sequence of PfcRT exon 2. Results Of 692 women recruited at delivery, 72 had placental malaria. Two Pfcrt exon 2 genotypes were found, and 86% of the placentas had monoallelic CVIET infection compared with 39% that had peripheral blood infection. Mixed parasite populations of CVIET/CVMNK occurred in 53% of the peripheral blood samples but only in 7% of the infected placentas. This selection of CVIET in placenta was not related to a decreased polymorphism of the parasites, as a large diversity of MSA1 and MSA2 was found in both placenta and venous blood. This diversity confirms that a multiplicity of circulation isolates can occur at low parasite transmission. msp1 and msp2 genotyping revealed mostly distinct populations of parasites in venous and placental blood. Conclusions These data suggest that, even in low transmission areas, diverse parasite populations can accumulate in the placenta during pregnancy despite strong selection at the PfcRT locus due to chemoprophylaxis with chloroquine.

J Infect Dis. 2008 Sep 1;198(5):772-780.

Variants in the Toll-Like Receptor Signaling Pathway and Clinical Outcomes of Malaria.

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Background. Malaria is one of the most significant infectious diseases in the world and is responsible for a large proportion of infant deaths. Toll-like receptors (TLRs), key components of innate immunity, are central to countering infection. Variants in the TLR-signaling pathway are associated with susceptibility to infectious diseases. Methods. We genotyped single nucleotide polymorphisms (SNPs) of the genes associated with the TLR-signaling pathway in patients with mild malaria and individuals with asymptomatic Plasmodium infections by means of polymerase chain reaction. Results. Genotype distributions for the TLR-1 I602S differed significantly between patients with mild malaria and persons with asymptomatic infection. The TLR-1 602S allele was associated with an odds ratio (OR) of 2.2 ([Formula: see text]; [Formula: see text]) for malaria among patients with mild malaria due to any

Plasmodium species and 2.1 ([Formula: see text]; [Formula: see text]) among patients with mild malaria due to Plasmodium falciparum only. The TLR-6 S249P SNP showed an excess of homozygotes for the TLR-6 249P allele in asymptomatic persons, compared with patients with mild malaria due to any Plasmodium species (OR 2.1; 95% confidence interval [CI], 1.1-4.2; [Formula: see text]; [Formula: see text]), suggesting that the TLR-6 249S allele may be a risk factor for malaria (OR, 2.0; 95% CI, 1.1-3.7; [Formula: see text]; [Formula: see text]). The TLR-9 -1486C allele showed a strong association with high parasitemia ([Formula: see text]).

Conclusions. Our findings indicate that the TLR-1 and TLR-6 variants are significantly associated with mild malaria, whereas the TLR-9-1486C/T variants are associated with high parasitemia. These discoveries may bring additional understanding to the pathogenesis of malaria.

J Infect Dis. 2008 Aug 27.

Sickle Cell Trait is Associated with a Delayed Onset of Malaria: Implications for Time-to-Event Analysis in Clinical Studies of Malaria.

Crompton PD, Traore B, Kayentao K, Doumbo S, Ongoiba A, Diakite SA, Krause MA, Doumtabe D, Kone Y, Weiss G, Huang CY, Doumbia S, Guindo A, Fairhurst RM, Miller LH, Pierce SK, Doumbo OK.

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Background. The World Health Organization (WHO) recently recommended that the time to first malaria episode serve as the primary end point in phase III malaria vaccine trials—the first of which will be held in Africa. Although common red blood cell (RBC) polymorphisms such as sickle hemoglobin (HbS) are known to protect against malaria in Africa, their impact on this end point has not been investigated.

Methods. A longitudinal study of 225 individuals aged 2-25 years was conducted in Mali. The association between common RBC polymorphisms and the time to first malaria episode was evaluated.

Results. Among children aged 2-10 years, sickle cell trait (HbAS) was associated with a 34-day delay in the median time to first malaria episode ([Formula: see text]). Cox regression analysis showed that greater age (hazard ratio [HR], 0.87 [95% CI, 0.80-0.94]; [Formula: see text]), HbAS (HR, 0.48 [95% CI, 0.26-0.91]; [Formula: see text]), and asymptomatic parasitemia at enrollment (HR, 0.35 [95% CI, 0.14-0.85]; [Formula: see text]) were associated with decreased malaria risk.

Conclusion. Given the delay in the time to first malaria episode associated with HbAS, it would be advisable for clinical trials and observational studies that use this end point to include Hb typing in the design of studies conducted in areas where HbAS is prevalent.

J Infect Dis. 2008 Aug 21.

Acquisition of Invasion-Inhibitory Antibodies Specific for the 19-kDa Fragment of Merozoite Surface Protein 1 in a Transmigrant Population Requires Multiple Infections.

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Antibodies against the 19 kDa C-terminal fragment of merozoite surface protein 1 (MSP1(19)) are a major component of the invasion-inhibitory response in individuals immune to malaria. We report here the acquisition of MSP1(19)-specific invasion-inhibitory antibodies in a group of transmigrants who experienced their sequential malaria infections during settlement in an area of Indonesia where malaria is highly endemic. We used 2 transgenic *Plasmodium falciparum* parasite lines that expressed either endogenous MSP1(19) or the homologous region from *P. chabaudi* to measure the MSP1(19)-specific invasion-inhibitory antibodies. The results revealed that the acquisition of MSP1(19)-specific invasion-inhibitory antibodies required 2 or more *P. falciparum* infections. In contrast, enzyme-linked immunosorbent assays on the same serum samples showed that MSP1(19)-specific antibodies are present after the first malaria infection. This delay in the acquisition of functional antibodies by residents of areas where malaria is endemic is consistent with the observation that multiple malaria infections are required before clinical immunity is acquired.

J Infect Dis. 2008 Aug 15;198(4):602-8.

Recovery of endothelial function in severe falciparum malaria: relationship with improvement in plasma L-arginine and blood lactate concentrations.

Yeo TW, Lampah DA, Gitawati R, Tjitra E, Kenangalem E, McNeil YR, Darcy CJ, Granger DL, Weinberg JB, Lopansri BK, Price RN, Duffull SB, Celermajer DS, Anstey NM.

International Health Division, Menzies School of Health Research and Charles Darwin University, Darwin, Australia.

BACKGROUND: Severe malaria is characterized by microvascular obstruction, endothelial dysfunction, and reduced levels of L-arginine and nitric oxide (NO). L-Arginine infusion improves endothelial function in moderately severe malaria. Neither the longitudinal course of endothelial dysfunction nor factors associated with recovery have been characterized in severe malaria. **METHODS:** Endothelial function was measured longitudinally in adults with severe malaria (n = 49) or moderately severe malaria (n = 48) in Indonesia, using reactive hyperemia peripheral arterial tonometry (RH-PAT). In a mixed-effects model, changes in RH-PAT index values in patients with severe malaria were related to changes in parasitemia, lactate, acidosis, and plasma L-arginine concentrations. **RESULTS:** Among patients with severe malaria, the proportion with endothelial dysfunction fell from 94% (46/49 patients) to 14% (6/42 patients) before discharge or death (P < .001). In severe malaria, the median time to normal endothelial function was 49 h (interquartile range, 20-70 h) after the start of antimalarial therapy. The mean increase in L-arginine concentrations in patients with severe malaria was 11 micromol/L/24 h (95% confidence interval [CI], 9-13 micromol/L/24 h), from a baseline of 49 micromol/L (95% CI, 37-45 micromol/L). Improvement of endothelial function in patients with severe malaria correlated with increasing levels of L-arginine (r = 0.56; P = .008) and decreasing levels of lactate (r = -0.44; P = .001). **CONCLUSIONS:** Recovery of endothelial function in severe malaria is associated with recovery from hypoargininemia and lactic acidosis. Agents that can improve endothelial NO production and endothelial function, such as L-arginine, may have potential as adjunctive therapy early during the course of severe malaria.

Efficacy of intermittent preventive treatment versus chloroquine prophylaxis to prevent malaria during pregnancy in Benin.

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BACKGROUND: In West Africa, treatment for the prevention of malaria during pregnancy has recently changed from chloroquine (CQ) prophylaxis to intermittent preventive treatment (IPTp). We assessed the benefits of IPTp with respect to those of CQ, using a before-after study. **METHODS:** CQ efficacy was evaluated during a cross-sectional survey conducted in Benin between April 2004 and April 2005. IPTp efficacy was assessed using data from an ongoing clinical trial to compare sulfadoxine-pyrimethamine with mefloquine that began in the same maternity clinics during July 2005; the present analysis is limited to women who delivered between November 2005 and November 2006. Treatment assignments were not unblinded. We compared the efficacy of the 2 strategies against low birth weight and placental infection by performing multiple logistic regressions. **RESULTS:** A total of 1699 women (1090 in the CQ group and 609 in the IPTp group) who delivered live singletons were analyzed. Characteristics of women in the CQ group were similar to those of women in the IPTp group. We showed that women in the IPTp group had a significantly decreased risk of delivering an infant with a low birth weight (adjusted odds ratio [aOR], 0.54; 95% confidence interval [CI], 0.38-0.78) and placental infection (aOR, 0.15; 95% CI, 0.09-0.24). **CONCLUSION:** We clearly evidenced that IPTp is substantially more beneficial than CQ for the prevention of malaria during pregnancy.

J Infect Dis. 2008 Aug 13.

VAR2CSA Expression on the Surface of Placenta-Derived Plasmodium falciparum-Infected Erythrocytes.

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Malaria remains a major threat, in sub-Saharan Africa primarily, and the most deadly infections are those with *Plasmodium falciparum*. Pregnancy-associated malaria is a clinically important complication of infection; it results from a unique interaction between proteoglycans in the placental intervillous space and parasite antigens. Both placental and chondroitin sulphate A-selected parasites have high-level transcripts of a unique var gene named var2csa. However, VAR2CSA has not been consistently found by proteomic analysis of placental parasites. Contrary to this, we found VAR2CSA expressed on the surface of infected erythrocytes from placenta. Importantly, this was achieved with cross-reactive antibodies against VAR2CSA.

J Theor Biol. 2008 Aug 13.

Optimal control strategy of malaria vector using genetically modified mosquitoes.

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The development of transgenic mosquitoes that are resistant to diseases may provide a new and effective weapon of diseases control. Such an approach relies on transgenic mosquitoes being able to survive and compete with wild-type populations. These transgenic mosquitoes carry a specific code that inhibits the plasmodium evolution in its organism. It is said that this characteristic is hereditary and consequently the disease fades away after some time. Once transgenic mosquitoes are released, interactions between the two populations and inter-specific mating between the two types of mosquitoes take place. We present a mathematical model that considers the generation overlapping and variable environment factors. Based on this continuous model, the malaria vector control is formulated and solved as an optimal control problem, indicating how genetically modified mosquitoes should be introduced in the environment. Numerical simulations show the effectiveness of the proposed control.

Malar J. 2008 Aug 27;7(1):164.

Integrated vector management: the Zambian experience.

Chanda E, Masaninga F, Coleman M, Sikaala C, Katebe C, Macdonald M, Baboo KS, Govere J, Manga L.

ABSTRACT: BACKGROUND: The Zambian Malaria Control Programme with the Roll Back Malaria (RBM) partners have developed the current National Malaria Strategic Plan (NMSP 2006-2011) which focuses on prevention based on the Integrated Vector Management (IVM) strategy. The introduction and implementation of an IVM strategy was planned in accordance with the World Health Organization (WHO) steps towards IVM implementation namely Introduction Phase, Consolidation Phase and Expansion Phase. Achievements IVM has created commitment for Legal and Regulatory policy review, monitoring, Research and a strong stewardship by the chemical suppliers. It has also leveraged additional resources, improved inter-sectoral collaboration, capacity building and enhanced community participation which facilitated a steady scaling up in coverage and utilisation of key preventive interventions. Thus, markedly reducing malaria incidence and case fatalities in the country. CONCLUSION: Zambia has successfully introduced, consolidated and expanded IVM activities. Resulting in increased coverage and utilization of interventions and markedly reducing malaria-related morbidity and mortality while ensuring a better protection of the environment.

Malar J. 2008 Sep 2;7(1):169.

Plasmodium falciparum gametocyte sex ratios in children with acute, symptomatic, uncomplicated infections treated with amodiaquine.

Sowunmi A, Balogun ST, Gbotosho GO, Happi CT.

ABSTRACT: BACKGROUND: Amodiaquine is frequently used as a partner drug in combination therapy or in some setting as monotherapy, but little is known about its effects on gametocyte production and sex ratio and its potential influence on transmission in Africa. The effects of amodiaquine on sexual stage parasites and gametocyte sex ratio, and the factors associated with a male-biased sex ratio were evaluated in 612 children with uncomplicated Plasmodium falciparum malaria who were treated with amodiaquine during the period 2000 - 2006 in an endemic

area. METHODS: Clinical, parasitological and laboratory parameters were evaluated before treatment and during follow-up for 28-42 days, and according to standard methods. Gametocyte sex ratio was defined as the proportion of peripheral gametocytes that are male. RESULTS: Clinical recovery from illness occurred in all children. Gametocytaemia was detected in 66 patients (11%) before treatment and in another 56 patients (9%) after treatment. Gametocyte densities were significantly higher by days 3-7 following treatment compared with pre-treatment ($P < 0.0001$). Overall, mean gametocyte sex ratio increased significantly during follow-up and over the study periods from 2000-2006 ($P < 0.001$ in both cases), but was female-biased at enrolment throughout the study periods. Absence of fever, a haematocrit $< 25\%$, asexual parasitaemia $> 20,000/uL$, gametocytaemia $< 18/uL$, and enrolment in 2006 were associated with a male-biased sex ratio pre-treatment. Anaemia and high parasitaemia were independent predictors of gametocyte maleness 7 days post treatment. CONCLUSION: Amodiaquine may significantly increase gametocyte carriage, density and sex ratio, and may potentially influence transmission. It is possible that anaemia could have contributed to the increased sex ratio. These findings may have implications for malaria control efforts in Africa.

Malar J. 2008 Aug 28;7(1):168.

Multiple insecticide resistance mechanisms involving metabolic changes and insensitive target sites selected in anopheline vectors of malaria in Sri Lanka.

Perera MD, Hemingway J, Karunaratne SP.

ABSTRACT: BACKGROUND: The current status of insecticide resistance and the underlying resistance mechanisms were studied in the major vector of malaria, *Anopheles culicifacies*, and the secondary vector, *Anopheles subpictus* in five districts (Anuradhapura, Kurunegala, Moneragala, Puttalam and Trincomalee) of Sri Lanka. Eight other anophelines, *Anopheles annularis*, *Anopheles barbirostris*, *Anopheles jamesii*, *Anopheles nigerrimus*, *Anopheles peditaeniatus*, *Anopheles tessellatus*, *Anopheles vagus* and *Anopheles varuna* from Anuradhapura district were also tested. METHODS: Adult females were exposed to the WHO discriminating dosages of DDT, malathion, fenitrothion, propoxur, lambda-cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, permethrin and etofenprox. The presence of metabolic resistance by esterase, glutathione S-transferase (GST) and monooxygenase-based mechanisms, and the sensitivity of the acetylcholinesterase target site were assessed using synergists, and biochemical, and metabolic techniques. RESULTS: All the anopheline species had high DDT resistance. All *An. culicifacies* and *An. subpictus* populations were resistant to malathion, except *An. culicifacies* from Kurunegala, where there was no malathion carboxylesterase activity. Kurunegala and Puttalam populations of *An. culicifacies* were susceptible to fenitrothion. All the *An. culicifacies* populations were susceptible to carbamates. Both species were susceptible to the discriminating dosages of cypermethrin and cyfluthrin, but had different levels of resistance to other pyrethroids. Of the 8 other anophelines, only *An. nigerrimus* and *An. peditaeniatus* were resistant to all the insecticides tested, probably due to their high exposure to the insecticides used in agriculture. *An. vagus* showed some resistance to permethrin. Esterases, GSTs and monooxygenases were elevated in both *An. culicifacies* and *An. subpictus*. AChE was most sensitive to insecticides in Kurunegala and Trincomalee *An. culicifacies* populations and highly insensitive in the Trincomalee *An. subpictus* population. CONCLUSIONS: The complexity of the resistance segregating in these field populations underlines the need for new molecular tools to identify the genomic diversity, differential upregulation and different binding specificities of resistance conferring genes, and the presence of different subspecies with different vectorial capacities.

Reduced paediatric hospitalizations for malaria and febrile illness patterns following implementation of community-based malaria control programme in rural Rwanda.

Sievers AC, Lewey J, Musafiri P, Franke MF, Bucyibaruta BJ, Stulac SN, Rich ML, Karema C, Daily JP.

ABSTRACT: **BACKGROUND:** Malaria control is currently receiving significant international commitment. As part of this commitment, Rwanda has undertaken a two-pronged approach to combating malaria via mass distribution of insecticide-treated bed nets and distribution of antimalarial medications by community health workers. This study attempted to measure the impact of these interventions on paediatric hospitalizations for malaria and on laboratory markers of disease severity. **METHODS:** A retrospective analysis of hospital records pre- and post-community-based malaria control interventions at a district hospital in rural Rwanda was performed. The interventions took place in August 2006 in the region served by the hospital and consisted of mass ITN distribution and community health workers antimalarial medication disbursement. The study periods consisted of the December-February high transmission seasons pre- and post-rollout. The record review examined a total of 551 paediatric admissions to identify 1) laboratory-confirmed malaria, defined by thick smear examination, 2) suspected malaria, defined as fever and symptoms consistent with malaria in the absence of an alternate cause, and 3) all-cause admissions. To define the impact of the intervention on clinical markers of malaria disease, trends in admission peripheral parasitaemia and haemoglobin were analyzed. To define accuracy of clinical diagnoses, trends in proportions of malaria admissions which were microscopy-confirmed before and after the intervention were examined. Finally, to assess overall management of febrile illnesses antibiotic use was described. **RESULTS:** Of the 551 total admissions, 268 (48.6%) and 437 (79.3%) were attributable to laboratory-confirmed and suspected malaria, respectively. The absolute number of admissions due to suspected malaria was smaller during the post-intervention period (N=150) relative to the pre-intervention period (N=287), in spite of an increase in the absolute number of hospitalizations due to other causes during the post-intervention period. The percentage of suspected malaria admissions that were laboratory-confirmed was greater during the pre-intervention period (80.4%) relative to the post-intervention period (48.1%, prevalence ratio [PR]: 1.67; 95% CI: 1.39 - 2.02; chi-squared p-value<0.0001). Among children admitted with laboratory-confirmed malaria, the risk of high parasitaemia was higher during the pre-intervention period relative to the post-intervention period (age-adjusted PR: 1.62; 95% CI: 1.11 - 2.38; chi-squared p-value=0.004), and the risk of severe anaemia was more than twofold greater during the pre-intervention period (age-adjusted PR: 2.47; 95% CI: 0.84 - 7.24; chi-squared p-value=0.08). Antibiotic use was common, with 70.7% of all children with clinical malaria and 86.4% of children with slide-negative malaria receiving antibacterial therapy. **CONCLUSIONS:** This study suggests that both admissions for malaria and laboratory markers of clinical disease among children may be rapidly reduced following community-based malaria control efforts. Additionally, this study highlights the problem of over-diagnosis and over-treatment of malaria in malaria-endemic regions, especially as malaria prevalence falls. More accurate diagnosis and management of febrile illnesses is critically needed both now and as fever aetiologies change with further reductions in malaria.

Malar J. 2008 Aug 27;7(1):166.

The economic burden of malaria on the household in south-central Vietnam.

Morel CM, Thang ND, Xa NX, Hung LX, Thuan LK, Ky PV, Erhart A, Mills AJ, D'Alessandro U.

ABSTRACT: BACKGROUND: Each year, several thousand cases of malaria occur in south-central Vietnam. Evidence from elsewhere suggests that malaria can have an economic impact on the household as the illness prevents households from completing their normal, physically demanding, productive duties such as tending crops and animals. The economic impact of malaria on households was explored within the Raglay ethnic minority living in the mountainous and forested area of south-central Vietnam (Ninh Thuan Province). **METHODS:** Two-hundred fifty-one malaria patients were identified and interviewed in an exit survey at Community Health Centres. The same patient sample was then re-interviewed in a household survey two to four weeks later. Survey data were complemented by approximately 40 informal discussions with health workers, vendors, patients, and community leaders. **RESULTS:** Each episode of malaria was estimated to cost the patient's household an average of 11.79USD (2005 prices), direct costs for travel and treatment representing 6% of the total while the remainder was loss in annual income. **CONCLUSIONS:** Whilst government provision of malaria treatment keeps the direct costs relatively low, the overall loss in income due to illness can still be significant given the poverty amongst this population, especially when multiple cases of malaria occur annually within the same household.

Malar J. 2008 Aug 27;7(1):165.

Unforeseen misuses of bed nets in fishing villages along Lake Victoria.

Minakawa N, Dida GO, Sonye GO, Futami K, Kaneko S.

ABSTRACT: BACKGROUND: To combat malaria, the Kenya Ministry of Health and nongovernmental organizations (NGOs) have distributed insecticide-treated nets (ITNs) for use over beds, with coverage for children under five years of age increasing rapidly. Nevertheless, residents of fishing villages have started to use these bed nets for drying fish and fishing in Lake Victoria. This study investigated the extent of bed net misuse in fishing villages. **METHODS:** Seven fishing villages along the lake were surveyed to estimate how widely bed nets were being used for fishing and drying fish. Villagers were asked why they used the bed nets for such purposes. **RESULTS:** In total, 283 bed nets were being used for drying fish. Of these, 239 were long-lasting insecticidal bed nets (LLIN) and 44 were non-long-lasting insecticidal bed nets (NLLIN). Further, 72 of the 283 bed nets were also being used for fishing. The most popular reasons were because the bed nets were inexpensive or free and because fish dried faster on the nets. LLINs were preferred to NLLINs for fishing and drying fish. **CONCLUSION:** There is considerable misuse of bed nets for drying fish and fishing. Many villagers are not yet fully convinced of the effectiveness of LLINs for malaria prevention. Such misuses may hamper the efforts of NGOs and governmental health organizations.

Malar J. 2008 Aug 25;7(1):163.

Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms.

Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, Della Torre A.

ABSTRACT: BACKGROUND: SINES (Short Interspersed Elements) are homoplasmy-free and co-dominant genetic markers which are considered to represent useful tools for population genetic studies, and could help clarifying the speciation processes

ongoing within the major malaria vector in Africa, *Anopheles gambiae* s.s. Here, we report the results of the analysis of the insertion polymorphism of a nearly 200 bp-long SINE (SINE200) within genome areas of high differentiation (i.e. "speciation islands") of M and S *A. gambiae* molecular forms. METHODS: A SINE-PCR approach was carried out on thirteen SINE200 insertions in M and S females collected along the whole range of distribution of *A. gambiae* s.s. in sub-Saharan Africa. Ten specimens each for *Anopheles arabiensis*, *Anopheles melas*, *Anopheles quadriannulatus* A and 15 M/S hybrids from laboratory crosses were also analysed. RESULTS: Eight loci were successfully amplified and were found to be specific for *A. gambiae* s.s.: 5 on 2L chromosome and one on X chromosome resulted monomorphic, while two loci positioned respectively on 2R (i.e. S200 2R12D) and X (i.e. S200 X6.1) chromosomes were found to be polymorphic. S200 2R12D was homozygote for the insertion in most S-form samples, while intermediate levels of polymorphism were shown in M-form, resulting in an overall high degree of genetic differentiation between molecular forms ($F_{st}=0.46$ $p<0.001$) and within M-form ($F_{st}=0.46$ $p<0.001$). The insertion of S200 X6.1 was found to be fixed in all M- and absent in all S-specimens. This led to develop a novel easy-to-use PCR approach to straightforwardly identify *A. gambiae* molecular forms. This novel approach allows to overcome the constraints associated with markers on the rDNA region commonly used for M and S identification. In fact, it is based on a single copy and irreversible SINE200 insertion and, thus, is not subjected to peculiar evolutionary patterns affecting rDNA markers, e.g. incomplete homogenization of the arrays through concerted evolution and/or mixtures of M and S IGS-sequences among the arrays of single chromatids. CONCLUSIONS: The approach utilized allowed to develop new easy-to-use co-dominant markers for the analysis of genetic differentiation between M and S-forms and opens new perspectives in the study of the speciation process ongoing within *A. gambiae*.

Malar J. 2008 Aug 25;7(1):162.

Malaria incidence in Limpopo Province, South Africa, 1998-2007.

Gerritsen AA, Kruger P, Schim van der Loeff MF, Grobusch MP.

ABSTRACT: BACKGROUND: Malaria is endemic in the low-altitude areas of the northern and eastern parts of South Africa with seasonal transmission. The aim of this descriptive study is to give an overview of the malaria incidence and mortality in Limpopo Province for the seasons 1998-1999 to 2006-2007 and to detect trends over time and place. METHODS: Routinely collected data on diagnosed malaria cases and deaths were available through the provincial malaria information system. In order to calculate incidence rates, population estimates (by sex, age and district) were obtained from Statistics South Africa. The Chi squared test for trend was used to detect temporal trends in malaria incidence over the seasons, and a trend in case fatality rate (CFR) by age group. The Chi squared test was used to calculate differences in incidence rate and CFR between both sexes and in incidence by age group. RESULTS: In total, 58,768 cases of malaria were reported, including 628 deaths. The mean incidence rate was 124.5 per 100,000 person-years and the mean CFR 1.1% per season. There was a decreasing trend in the incidence rate over time ($p < 0.001$), from 173.0 in 1998-1999 to 50.9 in 2006-2007. The CFR was fairly stable over the whole period. The mean incidence rate in males was higher than in females (145.8 versus 105.6; $p < 0.001$); the CFR (1.1%) was similar for both sexes. The incidence rate was lowest in 0-4 year olds (78.3), it peaked at the ages of 35-39 years (172.8), and decreased with age from 40 years (to 84.4 for those [greater than or equal to] 60 years). The CFR increased with increasing age (to 3.8% for those [greater than or equal to] 60 years). The incidence rate varied widely between districts; it was highest in Vhembe (328.2) and lowest in Sekhukhune (5.5). CONCLUSIONS: Information from this study may serve as baseline data to determine the course and distribution of malaria in Limpopo province over time. In the study period there was a decreasing trend in the incidence rate. Furthermore, the study addresses the need for better data over a range of epidemic-prone settings.

Malar J. 2008 Aug 22;7(1):161.

The M18 aspartyl aminopeptidase of Plasmodium falciparum binds to human erythrocyte spectrin in vitro.

Lauterbach SB, Coetzer TL.

ABSTRACT: **BACKGROUND:** During erythrocytic schizogony, Plasmodium falciparum interacts with the human erythrocyte membrane when it enters into, grows within and escapes from the erythrocyte. An interaction between the P. falciparum M18 aspartyl aminopeptidase (PfM18AAP) and the human erythrocyte membrane protein spectrin was recently identified using phage display technology. In this study, recombinant (r) PfM18AAP was characterized and the interaction between the enzyme and spectrin, as well as other erythrocyte membrane proteins, analyzed. **METHODS:** rPfM18AAP was produced as a hexahistidine-fusion protein in Escherichia coli and purified using magnetic bead technology. The pI of the enzyme was determined by two-dimensional gel electrophoresis and the number of subunits in the native enzyme was estimated from Ferguson plots. The enzymatic activity over a pH and temperature range was tested by a coupled enzyme assay. Blot overlays were performed to validate the spectrin-PfM18AAP interaction, as well as identify additional interactions between the enzyme and other erythrocyte membrane proteins. Sequence analysis identified conserved amino acids that are expected to be involved in cofactor binding, substrate cleavage and quaternary structure stabilization. **RESULTS:** rPfM18AAP has a molecular weight of ~67 kDa and the enzyme separated as three entities with pI 6.6, 6.7 and 6.9. Non-denaturing gel electrophoresis indicated that rPfM18AAP aggregated into oligomers. An in vitro coupled enzyme assay showed that rPfM18AAP cleaved an N-terminal aspartate from a tripeptide substrate with maximum enzymatic activity at pH 7.5 and 37C. The spectrin-binding region of PfM18AAP is not found in Homo sapiens, Saccharomyces cerevisiae and other Plasmodium species homologues. Amino acids expected to be involved in cofactor binding, substrate cleavage and quaternary structure stabilization, are conserved. Blot overlays with rPfM18AAP against spectrin and erythrocyte membrane proteins indicated that rPfM18AAP binds to spectrin, as well as to protein 4.1, protein 4.2, actin and glyceraldehyde 3-phosphate dehydrogenase. **CONCLUSIONS:** Studies characterizing rPfM18AAP showed that this enzyme interacts with erythrocyte spectrin and other membrane proteins. This suggests that, in addition to its proposed role in haemoglobin digestion, PfM18AAP performs other functions in the erythrocyte host and can utilize several substrates, which highlights the multifunctional role of malaria enzymes.

Malar J. 2008 Aug 22;7(1):160.

Improving community health worker use of malaria rapid diagnostic tests in Zambia: package instructions, job aid and job aid-plus-training.

Harvey SA, Jennings L, Chinyama M, Masaninga F, Mulholland K, Bell DR.

ABSTRACT: **BACKGROUND:** Introduction of artemisinin combination therapy (ACT) has boosted interest in parasite-based malaria diagnosis, leading to increased use of rapid diagnostic tests (RDTs), particularly in rural settings where microscopy is limited. With donor support, national malaria control programmes are now procuring large quantities of RDTs. The scarcity of health facilities and trained personnel in many sub-Saharan African countries means that limiting RDT use to such facilities would exclude a significant proportion of febrile cases. RDT use by volunteer community health workers (CHWs) is one alternative, but most sub-Saharan African countries prohibit CHWs from handling blood, and little is known about CHW ability to use RDTs safely and effectively. This Zambia-based study was designed to determine: (i) whether Zambian CHWs could prepare and interpret RDTs accurately and safely using manufacturer's instructions alone; (ii) whether simple, mostly pictorial instructions (a "job aid") could raise

performance to adequate levels; and (iii) whether a brief training programme would produce further improvement. METHODS: The job aid and training programme were based on formative research with 32 CHWs in Luangwa District. The study team then recruited three groups of CHWs in Chongwe and Chibombo districts. All had experience treating malaria based on clinical diagnosis, but only six had prior RDT experience. Trained observers used structured observation checklists to score each participant's preparation of three RDTs. Each also read 10 photographs showing different test results. The first group (n=32) was guided only by manufacturer's instructions. The second (n=21) used only the job aid. The last (n=26) used the job aid after receiving a three-hour training. RESULTS: Mean scores, adjusted for education, age, gender and experience, were 57% of 16 RDT steps correctly completed for group 1, 80% for group 2, and 92% for group 3. Mean percentage of test results interpreted correctly were 54% (group 1), 80% (group 2), and 93% (group 3). All differences were statistically significant ($p < 0.05$). CONCLUSIONS: Manufacturer's instructions like those provided with the RDTs used in this study are insufficient to ensure safe and accurate use by CHWs. However, well-designed instructions plus training can ensure high performance. More study is underway to determine how well this performance holds up over time.

Malar J. 2008 Aug 21;7(1):159.

Spatial prediction of Plasmodium falciparum prevalence in Somalia.

Noor AM, Clements AC, Gething PW, Moloney G, Borle M, Shewchuk T, Hay SI, Snow RW.

ABSTRACT: BACKGROUND: Maps of malaria distribution are vital for optimal allocation of resources for anti-malarial activities. There is a lack of reliable contemporary malaria maps in endemic countries in sub-Saharan Africa. This problem is particularly acute in low malaria transmission countries such as those located in the horn of Africa. METHODS: Data from a national malaria cluster sample survey in 2005 and routine cluster surveys in 2007 were assembled for Somalia. Rapid diagnostic tests were used to examine the presence of Plasmodium falciparum parasites in finger-prick blood samples obtained from individuals across all age-groups. Bayesian geostatistical models, with environmental and survey covariates, were used to predict continuous maps of malaria prevalence across Somalia and to define the uncertainty associated with the predictions. RESULTS: For analyses the country was divided into north and south. In the north, the month of survey, distance to water, precipitation and temperature had no significant association with P. falciparum prevalence when spatial correlation was taken into account. In contrast, all the covariates, except distance to water, were significantly associated with parasite prevalence in the south. The inclusion of covariates improved model fit for the south but not for the north. Model precision was highest in the south. The majority of the country had a predicted prevalence of < 5%; areas with [greater than or equal to] 5% prevalence were predominantly in the south. CONCLUSIONS: The maps showed that malaria transmission in Somalia varied from hypo- to meso-endemic. However, even after including the selected covariates in the model, there still remained a considerable amount of unexplained spatial variation in parasite prevalence, indicating effects of other factors not captured in the study. Nonetheless the maps presented here provide the best contemporary information on malaria prevalence in Somalia.

Malar J. 2008 Aug 20;7(1):158.

Establishment of a large semi-field system for experimental study of African malaria vector ecology and control in Tanzania.

Ferguson HM, Ng'habi KR, Walder T, Kadungula D, Moore SJ, Lyimo I, Russell TL, Urassa H, Mshinda H, Killeen GF, Knols BG.

ABSTRACT: BACKGROUND: Medical entomologists increasingly recognize that the ability to make inferences between laboratory experiments of vector biology and epidemiological trends observed in the field is hindered by a conceptual and methodological gap occurring between these approaches which prevents hypothesis-driven empirical research from being conducted on relatively large and environmentally realistic scales. The development of Semi-Field Systems (SFS) has been proposed as the best mechanism for bridging this gap. Semi-field systems are defined as enclosed environments, ideally situated within the natural ecosystem of a target disease vector and exposed to ambient environmental conditions, in which all features necessary for its life cycle completion are present. Although the value of SFS as a research tool for malaria vector biology is gaining recognition, only a few such facilities exist worldwide and are relatively small in size (< 100 m²). **METHODS:** The establishment of a 625 m² state-of-the-art SFS for large-scale experimentation on anopheline mosquito ecology and control within a rural area of southern Tanzania, where malaria transmission intensities are amongst the highest ever recorded, is described. **RESULTS:** A greenhouse frame with walls of mosquito netting and a polyethylene roof was mounted on a raised concrete platform at the Ifakara Health Institute. The interior of the SFS was divided into four separate work areas that have been set up for a variety of research activities including mass-rearing for African malaria vectors under natural conditions, high throughput evaluation of novel mosquito control and trapping techniques, short-term assays of host-seeking behaviour and olfaction, and longer-term experimental investigation of anopheline population dynamics and gene flow within a contained environment that simulates a local village domestic setting. **CONCLUSIONS:** The SFS at Ifakara was completed and ready for use in under two years. Preliminary observations indicate that realistic and repeatable observations of anopheline behaviour are obtainable within the SFS, and that habitat and climatic features representative of field conditions can be simulated within it. As work begins in the SFS in Ifakara and others around the world, the major opportunities and challenges to the successful application of this tool for malaria vector research and control are discussed.

Malar J. 2008 Aug 18;7(1):157.

Phagocytosis of haemozoin (malarial pigment) enhances metalloproteinase-9 activity in human adherent monocytes: Role of IL-1beta and 15-HETE.

Prato M, Gallo V, Giribaldi G, Arese P.

ABSTRACT: BACKGROUND: It has been shown previously that human monocytes fed with haemozoin (HZ) or trophozoite-parasitized RBCs displayed increased matrix metalloproteinase-9 (MMP-9) enzyme activity and protein/mRNA expression and increased TNF production, and showed higher matrix invasion ability. The present study utilized the same experimental model to analyse the effect of phagocytosis of: HZ, delipidized HZ, beta-haematin (lipid-free synthetic HZ) and trophozoites on production of IL-1beta and MMP-9 activity and expression. The second aim was to find out which component of HZ was responsible for the effects. **METHODS:** Native HZ freshly isolated from *Plasmodium falciparum* (Palo Alto strain, Mycoplasma-free), delipidized HZ, beta-haematin (lipid-free synthetic HZ), trophozoites and control meals such as opsonized non-parasitized RBCs and inert latex particles, were fed to human monocytes. The production of IL-1beta by differently fed monocytes, in presence or absence of specific MMP-9 inhibitor or anti-hIL-1beta antibodies, was quantified in supernatants by ELISA. Expression of IL-1beta was analysed by quantitative real-time RT-PCR. MMP-9 activity and protein expression were quantified by gelatin zymography and Western blotting. **RESULTS:** Monocytes fed with HZ or trophozoite-parasitized RBCs generated increased amounts of IL-1beta and enhanced enzyme activity (in cell supernatants) and protein/mRNA expression (in cell lysates) of monocyte MMP-9. The latter appears to be causally related to enhanced IL-1beta production, as enhancement of both expression and enzyme activity were abrogated by anti-hIL-1beta Abs. Upregulation of IL-1beta and MMP-9 were absent in monocytes fed with

beta-haematin or delipidized HZ, indicating a role for HZ-attached or HZ-generated lipid components. 15-HETE (15(S,R)-hydroxy-6,8,11,13-eicosatetraenoic acid) a potent lipoperoxidation derivative generated by HZ from arachidonic acid via haem-catalysis was identified as one mediator possibly responsible for increase of both IL-1beta production and MMP-9 activity. CONCLUSION: Results indicate that specific lipoperoxide derivatives generated by HZ may play a role in modulating production of IL-1beta and MMP-9 expression and activity in HZ/trophozoite-fed human monocytes. Results may clarify aspects of cerebral malaria pathogenesis, since MMP-9, a metalloproteinase able to disrupt the basal lamina is possibly involved in generation of hallmarks of cerebral malaria, such as blood-brain barrier endothelium dysfunction, localized haemorrhages and extravasation of phagocytic cells and parasitized RBCs into brain tissues.

Malar J. 2008 Aug 18;7(1):156.

Dry season ecology of Anopheles gambiae complex mosquitoes in The Gambia.

Jawara M, Pinder M, Drakeley CJ, Nwakanma DC, Jallow E, Bogh C, Lindsay SW, Conway DJ.

ABSTRACT: BACKGROUND: Malaria in The Gambia is highly seasonal, with transmission occurring as *Anopheles gambiae* s.l. populations expand during and immediately after a single annual rainy season that lasts from June to October. There has been very limited investigation of the ecology of vectors during the dry season, when numbers are very limited and distributions may be restricted. METHODS: Weekly adult mosquito collections (pyrethrum spray, light trap, and search collections from rooms, as well as light trap collections from animal shelters, abandoned wells and grain stores), and artificial sentinel breeding site surveys were performed in four villages near the upper tidal and partially saline part of the Gambia River in the last four months of an annual dry season (March to June). Mosquito species were identified by morphological and DNA analysis, and ELISA assays were performed to test for *Plasmodium falciparum* sporozoites and human blood meal components. RESULTS: Adults of *An. gambiae* s.l. were collected throughout the period, numbers increasing towards the end of the dry season when humidity was increasing. Adult collections were dominated by *An. melas* (86%), with *An. gambiae* s.s. (10%) and *An. arabiensis* (3%) also present throughout. Most females collected in room search and spray collections contained blood meals, but most from light traps were unfed. None of the females tested (n=1709) contained sporozoites. Larvae (mostly *An. gambiae* s.s.) were recovered from artificial sentinel breeding sites in the two villages that had freshwater pools. These two villages had the highest proportions of *An. gambiae* s.s. adults, and experienced the most substantial increase in proportions of *An. gambiae* s.s. after the onset of rains. CONCLUSION: During the dry season population minimum, *An. melas* was the predominant vector species, but differences among villages in availability of fresh-water breeding sites correlate with egg laying activity and relative numbers of *An. gambiae* s.s. adults, and with the increase in this species immediately after the beginning of the rains. Local variation in dry season vector persistence is thus likely to influence spatial heterogeneity of transmission intensity in the early part of the rainy season.

Malar J. 2008 Aug 16;7(1):155.

Acquisition of naturally occurring antibody responses to recombinant protein domains of Plasmodium falciparum erythrocyte membrane protein 1.

Mackintosh CL, Christodoulou Z, Mwangi TW, Kortok M, Pinches R, Williams TN, Marsh K, Newbold CI.

ABSTRACT: BACKGROUND: Antibodies targeting variant antigens expressed on the surface of *Plasmodium falciparum* infected erythrocytes have been associated with

protection from clinical malaria. The precise target for these antibodies is unknown. The best characterized and most likely target is the erythrocyte surface-expressed variant protein family Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1). METHODS: Using recombinant proteins corresponding to five domains of the expressed A4 var gene, A4 PfEMP1, the naturally occurring antibody response was assessed, by ELISA, to each domain in serum samples obtained from individuals resident in two communities of differing malaria transmission intensity on the Kenyan coast. Using flow cytometry, the correlation in individual responses to each domain with responses to intact A4-infected erythrocytes expressing A4 PfEMP1 on their surface as well as responses to two alternative parasite clones and one clinical isolate was assessed.. RESULTS: Marked variability in the prevalence of responses between each domain and between each transmission area was observed, as was a strong correlation between age and reactivity with some but not all domains. Individual responses to each domain varied strikingly, with some individuals showing reactivity to all domains and others with no reactivity to any, this was apparent at all age groups. Evidence for possible cross-reactivity in responses to the domain DBL4gamma was found. CONCLUSIONS: Individuals acquire antibodies to surface expressed domains of a highly variant protein. The finding of potential cross-reactivity in responses to one of these domains is an important initial finding in the consideration of potential vaccine targets.

Malar J. 2008 Aug 9;7:154.

Different methodological approaches to the assessment of in vivo efficacy of three artemisinin-based combination antimalarial treatments for the treatment of uncomplicated falciparum malaria in African children.

Ashley EA, Pinoges L, Turyakira E, Dorsey G, Checchi F, Bukirwa H, van den Broek I, Zongo I, Urruta PP, van Herp M, Balkan S, Taylor WR, Olliaro P, Guthmann JP.

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BACKGROUND: Use of different methods for assessing the efficacy of artemisinin-based combination antimalarial treatments (ACTs) will result in different estimates being reported, with implications for changes in treatment policy. METHODS: Data from different in vivo studies of ACT treatment of uncomplicated falciparum malaria were combined in a single database. Efficacy at day 28 corrected by PCR genotyping was estimated using four methods. In the first two methods, failure rates were calculated as proportions with either (1a) reinfections excluded from the analysis (standard WHO per-protocol analysis) or (1b) reinfections considered as treatment successes. In the second two methods, failure rates were estimated using the Kaplan-Meier product limit formula using either (2a) WHO (2001) definitions of failure, or (2b) failure defined using parasitological criteria only. RESULTS: Data analysed represented 2926 patients from 17 studies in nine African countries. Three ACTs were studied: artesunate-amodiaquine (AS+AQ, N = 1702), artesunate-sulphadoxine-pyrimethamine (AS+SP, N = 706) and artemether-lumefantrine (AL, N = 518). Using method (1a), the day 28 failure rates ranged from 0% to 39.3% for AS+AQ treatment, from 1.0% to 33.3% for AS+SP treatment and from 0% to 3.3% for AL treatment. The median [range] difference in point estimates between method 1a (reference) and the others were: (i) method 1b = 1.3% [0 to 24.8], (ii) method 2a = 1.1% [0 to 21.5], and (iii) method 2b = 0% [-38 to 19.3]. The standard per-protocol method (1a) tended to overestimate the risk of failure when compared to alternative methods using the same endpoint definitions (methods 1b and 2a). It either overestimated or underestimated the risk when endpoints based on parasitological rather than clinical criteria were applied. The standard method was also associated with a 34% reduction in the number of patients evaluated compared to the number of patients enrolled. Only 2% of the sample size was lost when failures were classified on the first day of parasite recurrence and survival analytical methods were used. CONCLUSION: The primary purpose of an in vivo study should be

to provide a precise estimate of the risk of antimalarial treatment failure due to drug resistance. Use of survival analysis is the most appropriate way to estimate failure rates with parasitological recurrence classified as treatment failure on the day it occurs.

Malar J. 2008 Aug 7;7:153.

Gains in awareness, ownership and use of insecticide-treated nets in Nigeria, Senegal, Uganda and Zambia.

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BACKGROUND: In April 2000, the Roll Back Malaria (RBM) "Abuja Summit" set a target of having at least 60% of pregnant women and children under five use insecticide-treated nets (ITNs). Thereafter, programmes were implemented to create demand, reduce taxes and tariffs, spur the commercial market, and reach vulnerable populations with subsidized ITNs. Using national ITN monitoring data from the USAID-sponsored AED/NetMark project, this article examines the extent to which these activities were successful in increasing awareness, ownership, and use of nets and ITNs. **METHODS:** A series of surveys with standardized sampling and measurement methods was used to compare four countries at two points in time. Surveys were conducted in 2000 and again in 2004 (Nigeria, Senegal, Zambia) or 2006 (Uganda). They contained questions permitting classification of each net as untreated, ever-treated or currently-treated (an ITN). Household members as well as nets owned were enumerated so that households, household members, and nets could be used as units of analysis. Several measures of net/ITN ownership, plus RBM ITN use indicators, were calculated. The results show the impact of ITN activities before the launch of massive free net distribution programmes. **RESULTS:** In 2000, treated nets were just being introduced to the public, but four to six years later the awareness of ITNs was nearly universal in all countries but Nigeria, where awareness increased from 7% to 60%. By any measure, there were large increases in ownership of nets, especially treated nets, in all countries. All countries but Nigeria made commensurate gains in the proportion of under-fives sleeping under a net/ITN, and in all countries the proportion of pregnant women sleeping under a net/ITN increased greatly. **CONCLUSION:** A mix of demand creation, a strengthened commercial sector, reduced taxes and tariffs, and programmes making ITNs available at reduced prices resulted in impressive gains in awareness, ownership, and use of nets and ITNs in Nigeria, Senegal, Zambia, and Uganda between 2000 and 2004-2006. None of the countries reached the ambitious Abuja targets for ITN use, but they made substantial progress towards them.

Malar J. 2008 Aug 7;7:152.

Ethnobotanical study of some of mosquito repellent plants in north-eastern Tanzania.

Kweka EJ, Mosha F, Lowassa A, Mahande AM, Kitau J, Matowo J, Mahande MJ, Massenga CP, Tenu F, Feston E, Lyatuu EE, Mboya MA, Mndeme R, Chuwa G, Temu EA.

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BACKGROUND: The use of plant repellents against nuisance biting insects is common and its potential for malaria vector control requires evaluation in areas with different level of malaria endemicity. The essential oils of *Ocimum suave* and *Ocimum kilimandscharicum* were evaluated against malaria vectors in north-eastern Tanzania. **METHODOLOGY:** An ethnobotanical study was conducted at Moshi in

Kilimanjaro region north-eastern Tanzania, through interviews, to investigate the range of species of plants used as insect repellents. Also, bioassays were used to evaluate the protective potential of selected plants extracts against mosquitoes. RESULTS: The plant species mostly used as repellent at night are: fresh or smoke of the leaves of *O. suave* and *O. kilimandscharicum* (Lamiaceae), *Azadirachta indica* (Meliaceae), *Eucalyptus globules* (Myrtaceae) and *Lantana camara* (Verbenaceae). The most popular repellents were *O. kilimandscharicum* (OK) and *O. suave* (OS) used by 67% out of 120 households interviewed. Bioassay of essential oils of the two *Ocimum* plants was compared with citronella and DEET to study the repellence and feeding inhibition of untreated and treated arms of volunteers. Using filter papers impregnated with *Ocimum* extracts, knockdown effects and mortality was investigated on malaria mosquito *Anopheles arabiensis* and *Anopheles gambiae*, including a nuisance mosquito, *Culex quinquefasciatus*. High biting protection (83% to 91%) and feeding inhibition (71.2% to 92.5%) was observed against three species of mosquitoes. Likewise the extracts of *Ocimum* plants induced KD90 of longer time in mosquitoes than citronella, a standard botanical repellent. Mortality induced by standard dosage of 30 mg/m² on filter papers, scored after 24 hours was 47.3% for OK and 57% for OS, compared with 67.7% for citronella. CONCLUSION: The use of whole plants and their products as insect repellents is common among village communities of north-eastern Tanzania and the results indicate that the use of *O. suave* and *O. kilimandscharicum* as a repellent would be beneficial in reducing vector biting. The widespread use of this approach has a potential to complement other control measures.

Mol Cell Biol. 2008 Aug 18.

Distinct malaria parasite sporozoites reveal transcriptional changes that cause differential tissue infection competence in the mosquito vector and mammalian host.

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The malaria parasite sporozoite transmission stage develops and differentiates within parasite oocysts in the *Anopheles* mosquito midgut. Successful inoculation of the parasite into a mammalian host is critically dependent on the sporozoites ability to first infect the mosquito salivary glands. Remarkable changes in the tissue infection competence are observed as the sporozoites transit from the midgut oocysts to the salivary glands. Our microarray analysis shows that when compared to oocyst sporozoites, salivary gland sporozoites up-regulate expression of at least 124 unique genes. Conversely, oocyst sporozoites show up-regulation of at least 47 genes, (up-regulated in oocyst sporozoites, UOS genes) before they infect the salivary glands. Targeted gene deletion of UOS3, encoding a putative transmembrane protein that localizes to the sporozoite secretory organelles rendered oocyst sporozoites unable to infect the mosquito salivary glands but maintained the parasites liver infection competence. This phenotype demonstrates the significance of differential UOS expression. Thus, the UIS-UOS gene classification provides a framework to elucidate infectivity and transmission success of *Plasmodium* sporozoites on a whole genome scale. Genes identified herein might represent targets for vector-based transmission blocking strategies (UOS) as well as strategies that prevent mammalian host infection (UIS).

Mol Microbiol. 2008 Aug 27.

Reverse genetics screen identifies six proteins important for malaria development in the mosquito.

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Transmission from the vertebrate host to the mosquito vector represents a major population bottleneck in the malaria life cycle that can successfully be targeted by intervention strategies. However, to date only about 25 parasite proteins expressed during this critical phase have been functionally analysed by gene disruption. We describe the first systematic, larger scale generation and phenotypic analysis of *Plasmodium berghei* knockout (KO) lines, characterizing 20 genes encoding putatively secreted proteins expressed by the ookinete, the parasite stage responsible for invasion of the mosquito midgut. Of 12 KO lines that were generated, six showed significant reductions in parasite numbers during development in the mosquito, resulting in a block in transmission of five KOs. While expression data, time point of essential function and mutant phenotype correlate well in three KOs defective in midgut invasion, in three KOs that fail at sporulation, maternal inheritance of the mutant phenotype suggests that essential function occurs during ookinete formation and thus precedes morphological abnormalities by several days.

Mol Microbiol. 2008 Aug 18.

Polymorphisms within PfMDR1 alter the substrate specificity for antimalarial drugs in *Plasmodium falciparum*.

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Resistance to several antimalarial drugs has been associated with polymorphisms within the P-glycoprotein homologue (Pgh-1, PfMDR1) of the human malaria parasite *Plasmodium falciparum*. Pgh-1, coded for by the gene *pfmdr1*, is predominately located at the membrane of the parasite's digestive vacuole. How polymorphisms within this transporter mediate altered antimalarial drug responsiveness has remained obscure. Here we have functionally expressed *pfmdr1* in *Xenopus laevis* oocytes. Our data demonstrate that Pgh-1 transports vinblastine, an established substrate of mammalian MDR1, and the antimalarial drugs halofantrine, quinine, and chloroquine. Importantly, polymorphisms within Pgh-1 alter the substrate specificity for the antimalarial drugs. Wildtype Pgh-1 transports quinine and chloroquine, but not halofantrine, whereas polymorphic Pgh-1 variants, associated with altered drug responsiveness, transport halofantrine but not quinine and chloroquine. Our data further suggest that quinine acts as an inhibitor of Pgh-1. Our data are discussed in terms of the model that Pgh-1 mediates, in a variant-specific manner, import of certain drugs into the *P. falciparum* digestive vacuole, and that this contributes to accumulation of, and susceptibility to, the drug in question.

Nat Med. 2008 Aug 31.

Attenuated Plasmodium yoelii lacking purine nucleoside phosphorylase confer protective immunity.

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Malaria continues to devastate sub-Saharan Africa owing to the emergence of drug resistance to established antimalarials and to the lack of an efficacious vaccine. Plasmodium species have a unique streamlined purine pathway in which the dual specificity enzyme purine nucleoside phosphorylase (PNP) functions in both purine recycling and purine salvage. To evaluate the importance of PNP in an in vivo model of malaria, we disrupted PyPNP, the gene encoding PNP in the lethal Plasmodium yoelii YM strain. P. yoelii parasites lacking PNP were attenuated and cleared in mice. Although able to form gametocytes, PNP-deficient parasites did not form oocysts in mosquito midguts and were not transmitted from mosquitoes to mice. Mice given PNP-deficient parasites were immune to subsequent challenge to a lethal inoculum of P. yoelii YM and to challenge from P. yoelii 17XNL, another strain. These in vivo studies with PNP-deficient parasites support purine salvage as a target for antimalarials. They also suggest a strategy for the development of attenuated nontransmissible metabolic mutants as blood-stage malaria vaccine strains.

Parasite Immunol. 2008 Sep;30(9):482-6.

Protective immunity induced by daily bites from irradiated mosquitoes infected with Plasmodium yoelii.

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SUMMARY Individuals in malaria endemic regions do not develop fully protective immune responses against Plasmodium liver stage infections. In high transmission areas, individuals can be exposed to more than two infective mosquito bites daily. Their exposure to Plasmodium sporozoites, therefore, is in the form of small and frequent doses. This is very different from individuals studied in controlled immunization trials where the delivery of large numbers of radiation-attenuated sporozoites in a limited number of doses can induce sterile protective immunity. Using irradiated mosquitoes infected with Plasmodium yoelii 17XNL, we tested whether daily bites from a few mosquitoes can induce a protective immune response in mice. This immunization strategy successfully induced a protective response, preventing the development of liver stages when mice were challenged with nonirradiated sporozoites. These results provide further support for the development of liver stage vaccines. They are also a call for further study into why fully protective responses against the liver stage are not seen in individuals from endemic regions.

Parasitol Res. 2008 Sep 2.

Genetic characterization, distribution and prevalence of avian pox and avian malaria in the Berthelot's pipit (Anthus berthelotii) in Macaronesia.

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Exotic pathogens have been implicated in the decline and extinction of various native-island-bird species. Despite the fact that there is increasing concern about the introduction of diseases in island ecosystems, little is known about parasites in the islands of Macaronesia. We focus on Berthelot's pipit (*Anthus berthelotii*), an endemic and widespread Macaronesian bird species, using a combination of field studies and molecular techniques to determine: (1) the range and prevalence of avian pox and malaria in Berthelot's pipits throughout the species' distribution, (2) the genetic characterization of both parasites in order to ascertain the level of host specificity. We sampled 447 pipits across the 12 islands inhabited by this species. Overall, 8% of all individuals showed evidence of pox lesions and 16% were infected with avian malaria, respectively. We observed marked differences in the prevalence of parasites among islands both within and between archipelagos. Avian pox prevalence varied between 0-54% within and between archipelagos and avian malaria prevalence varied between 0-64% within and between archipelagos. The diversity of pathogens detected was low: only two genetic lineages of avian malaria and one lineage of avian pox were found to infect the pipit throughout its range. Interestingly, both avian malaria parasites found were *Plasmodium* spp. that had not been previously reported in the Macaronesian avifauna (but that had been observed in the lesser kestrel *Falco naumannii*), while the avian pox was a host specific lineage that had previously been reported on two of the Canary Islands.

Parasitol Res. 2008 Sep;103(4):751-63.

Ribozyme cleavage of *Plasmodium falciparum* gyrase A gene transcript affects the parasite growth.

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Deoxyribonucleic acid (DNA) gyrase is an important enzyme that facilitates the movement of replication and transcription complexes through DNA by creating negative supercoils ahead of the complex. Its presence in *Plasmodium falciparum* is now established and considered a good drug target since it is absent in the human host. The sequence of *P. falciparum* gyrase A subunit was analyzed for its messenger ribonucleic acid (mRNA) folding as well as target accessibility for ribozymes. The four GUC triplet sites identified at 334, 491, 1907, and 2642 nucleotide positions of the Gyrase A mRNA were also accessible to oligos by RNase H assay. Site GUC(491) was optimally accessible followed by GUC(1907), GUC(334), and GUC(2642) sites. Ribozymes were produced against all these sites and tested for their in vitro transcript cleavage potentials where RZ(491) showed the maximum cleavage rate. Therefore, this ribozyme (RZ(491)) was chemically synthesized albeit with modifications so as to make it resistant against ribonuclease attack. The modified ribozyme retained its cleavage potential and was able to inhibit the *P. falciparum* parasite growth up to 49.54% and 74.77% at 20 and 30 μ M ribozyme concentrations, respectively, as compared to the untreated culture. However, up to 20% and 24.32% parasite growth inhibition was observed at the same ribozyme concentrations of 20 and 30 μ M when compared with control ribozyme-treated cultures. This ribozyme as well as other targets identified here can be investigated further to develop the effective chemotherapeutic agents against malaria.

Mutations in PFCRT K76T do not correlate with sulfadoxine-pyrimethamine-amodiaquine failure in Pikine, Senegal.

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In 2003, the high level of chloroquine (CQ) treatment failure for uncomplicated *Plasmodium falciparum* malaria cases has led Senegal to adopt a new combination therapy with sulfadoxine-pyrimethamine and amodiaquine (SP-AQ). From September through November 2004, we used the 14-day World Health Organization follow-up protocol to assess the therapeutic response in patients with uncomplicated *P. falciparum* malaria in an area of high prevalence of pfcrt T76 mutant allele and SP resistance mutations. Of the 82 patients who were recruited, 68 (82.9%) completed follow-up. The response of the patients to treatment was adequate clinical response for 63 out of 68 patients (92.6%), while five (7.4%) clinical failures were recorded, four early treatment failures, and one late treatment failure. The prevalence of the pfcrt T76 allele at day 0 was 59.5%. The two-sided Fisher's exact test did not show an association between pfcrt T76 allele and treatment failure ($p = 0.167$). The transitory treatment is effective and safe. However, the presence of high levels of mutant alleles points out the need to closely monitor the new therapeutic regimen.

Parasitol Res. 2008 Aug 5.

Screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* (Hübner), *Sylepta derogata* (F.) and *Anopheles stephensi* (Liston).

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Plant extracts, especially botanical insecticides, are currently studied more and more because of the possibility of their use in plant protection. Biological activity of five solvent plant extracts were studied using fourth instar larvae of gram pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae), cotton leaf roller *Sylepta derogata* (Lepidoptera: Pyralidae) and malaria vector *Anopheles stephensi* (Diptera: Culicidae). Antifeedant and larvicidal activity of acetone, chloroform, ethyl acetate, hexane and methanol peel, leaf and flower extracts of *Citrus sinensis*, *Ocimum canum*, *Ocimum sanctum* and *Rhinacanthus nasutus* were used in this study. During preliminary screening, the extracts were tested at 1,000 ppm concentration. The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in peel chloroform extract of *C. sinensis*, flower methanol extract of *O. canum* against the larvae of *H. armigera* (LC(50) = 65.10, 51.78, LC(90) = 277.39 and 218.18 ppm), peel methanol extract of *C. sinensis*, flower ethyl acetate extract of *O. canum* and leaf acetone extract of *O. sanctum* against the larvae of *S. derogata* (LC(50) = 20.27, 58.21, 36.66, LC(90) = 113.15, 285.70 and 668.02 ppm), peel methanol extract of *C. sinensis*, leaf and flower ethyl acetate extracts of *O. canum* against the larvae of *A. stephensi* (LC(50) = 95.74, 101.53, 28.96, LC(90) = 303.20, 492.43 and 168.05 ppm), respectively. These results suggest that the chloroform and methanol extract of *C. sinensis*, ethyl acetate flower extracts of *O. canum* and acetone extract of *O. sanctum* have the potential to be used as an ideal eco-friendly approach for the control of the agricultural pests *H. armigera*, *S. derogata* and medically important vector *A. stephensi*.

Parasitology. 2008 Aug 11:1-10.

Towards a comprehensive simulation model of malaria epidemiology and control.

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SUMMARY Planning of the control of *Plasmodium falciparum* malaria leads to a need for models of malaria epidemiology that provide realistic quantitative prediction of likely epidemiological outcomes of a wide range of control strategies. Predictions of the effects of control often ignore medium- and long-term dynamics. The complexities of the *Plasmodium* life-cycle, and of within-host dynamics, limit the applicability of conventional deterministic malaria models. We use individual-based stochastic simulations of malaria epidemiology to predict the impacts of interventions on infection, morbidity, mortality, health services use and costs. Individual infections are simulated by stochastic series of parasite densities, and naturally acquired immunity acts by reducing densities. Morbidity and mortality risks, and infectiousness to vectors, depend on parasite densities. The simulated infections are nested within simulations of individuals in human populations, and linked to models of interventions and health systems. We use numerous field datasets to optimise parameter estimates. By using a volunteer computing system we obtain the enormous computational power required for model fitting, sensitivity analysis, and exploration of many different intervention strategies. The project thus provides a general platform for comparing, fitting, and evaluating different model structures, and for quantitative prediction of effects of different interventions and integrated control programmes.

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Host control of malaria infections: constraints on immune and erythropoietic response kinetics.

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The two main agents of human malaria, *Plasmodium vivax* and *Plasmodium falciparum*, can induce severe anemia and provoke strong, complex immune reactions. Which dynamical behaviors of host immune and erythropoietic responses would foster control of infection, and which would lead to runaway parasitemia and/or severe anemia? To answer these questions, we developed differential equation models of interacting parasite and red blood cell (RBC) populations modulated by host immune and erythropoietic responses. The model immune responses incorporate both a rapidly responding innate component and a slower-responding, long-term antibody component, with several parasite developmental stages considered as targets for each type of immune response. We found that simulated infections with the highest parasitemia tended to be those with ineffective innate immunity even if antibodies were present. We also compared infections with dyserythropoiesis (reduced RBC production during infection) to those with compensatory erythropoiesis (boosted RBC production) or a fixed basal RBC production rate. Dyserythropoiesis tended to reduce parasitemia slightly but at a cost to the host of aggravating anemia. On the other hand, compensatory erythropoiesis tended to reduce the severity of anemia but with enhanced parasitemia if the innate response was ineffective. For both parasite species, sharp transitions between the schizont and the merozoite stages of development (i.e., with standard

deviation in intra-RBC development time (≤ 2.4 h) were associated with lower parasitemia and less severe anemia. Thus tight synchronization in asexual parasite development might help control parasitemia. Finally, our simulations suggest that *P. vivax* can induce severe anemia as readily as *P. falciparum* for the same type of immune response, though *P. vivax* attacks a much smaller subset of RBCs. Since most *P. vivax* infections are nonlethal (if debilitating) clinically, this suggests that *P. falciparum* adaptations for countering or evading immune responses are more effective than those of *P. vivax*.

PLoS ONE. 2008 Aug 13;3(8):e2940.

Phase 1 trial of AMA1-C1/Alhydrogel plus CPG 7909: an asexual blood-stage vaccine for Plasmodium falciparum malaria.

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BACKGROUND: Apical Membrane Antigen 1 (AMA1), a polymorphic merozoite surface protein, is a leading blood-stage malaria vaccine candidate. This is the first reported use in humans of an investigational vaccine, AMA1-C1/Alhydrogel, with the novel adjuvant CPG 7909. **METHODS:** A phase 1 trial was conducted at the University of Rochester with 75 malaria-naïve volunteers to assess the safety and immunogenicity of the AMA1-C1/Alhydrogel+CPG 7909 malaria vaccine. Participants were sequentially enrolled and randomized within dose escalating cohorts to receive three vaccinations on days 0, 28 and 56 of either 20 microg of AMA1-C1/Alhydrogel+564 microg CPG 7909 (n = 15), 80 microg of AMA1-C1/Alhydrogel (n = 30), or 80 microg of AMA1-C1/Alhydrogel+564 microg CPG 7909 (n = 30). **RESULTS:** Local and systemic adverse events were significantly more likely to be of higher severity with the addition of CPG 7909. Anti-AMA1 immunoglobulin G (IgG) were detected by enzyme-linked immunosorbent assay (ELISA), and the immune sera of volunteers that received 20 microg or 80 microg of AMA1-C1/Alhydrogel+CPG 7909 had up to 14 fold significant increases in anti-AMA1 antibody concentration compared to 80 microg of AMA1-C1/Alhydrogel alone. The addition of CPG 7909 to the AMA1-C1/Alhydrogel vaccine in humans also elicited AMA1 specific immune IgG that significantly and dramatically increased the in vitro growth inhibition of homologous parasites to levels as high as 96% inhibition. **CONCLUSION/SIGNIFICANCE:** The safety profile of the AMA1-C1/Alhydrogel+CPG 7909 malaria vaccine is acceptable, given the significant increase in immunogenicity observed. Further clinical development is ongoing. **TRIAL REGISTRATION:** ClinicalTrials.gov NCT00344539.

PLoS ONE. 2008 Aug 6;3(8):e2861.

A randomised trial of an eight-week, once weekly primaquine regimen to prevent relapse of plasmodium vivax in Northwest Frontier Province, Pakistan.

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BACKGROUND: Vivax malaria remains a major cause of morbidity in the subtropics. To undermine the stability of the disease, drugs are required that prevent relapse and provide reservoir reduction. A 14-day course of primaquine (PQ) is effective but cannot safely be used in routine practice because of its interaction with glucose-6-phosphate dehydrogenase (G6PD) deficiency for which testing is seldom available. Safe and effective use of PQ without the need for

G6PD testing would be ideal. The efficacy and safety of an 8-week, once weekly PQ regimen was compared with current standard treatment (chloroquine alone) and a 14-day PQ regimen. METHODS AND PRINCIPAL FINDINGS: 200 microscopically confirmed *Plasmodium vivax* patients were randomly assigned to either once weekly 8-week PQ (0.75 mg/kg/week), once weekly 8-week placebo, or 14-day PQ (0.5mg/kg/day) in North West Frontier Province, Pakistan. All patients were treated with a standard chloroquine dose and tested for G6PD deficiency. Deficient patients were assigned to the 8-week PQ group. Failure was defined as any subsequent episode of vivax malaria over 11 months of observation. There were 22/71 (31.0%) failures in the placebo group and 1/55 (1.8%) and 4/75 (5.1%) failures in the 14-day and 8-week PQ groups, respectively. Adjusted odds ratios were: for 8-week PQ vs. placebo-0.05 (95%CI: 0.01-0.2, p<0.001) and for 14-day PQ vs. placebo-0.01 (95%CI: 0.002-0.1, p<0.001). Restricted analysis allowing for a post-treatment prophylactic effect confirmed that the 8-week regimen was superior to current treatment. Only one G6PD deficient patient presented. There were no serious adverse events. CONCLUSIONS: A practical radical treatment for vivax malaria is essential for control and elimination of the disease. The 8-week PQ course is more effective at preventing relapse than current treatment with chloroquine alone. Widespread use of the 8-week regimen could make an important contribution to reservoir reduction or regional elimination where G6PD testing is not available. TRIAL REGISTRATION: ClinicalTrials.gov NCT00158587.

PLoS Pathog. 2008 Aug 22;4(8):e1000135.

Viral paratransgenesis in the malaria vector *Anopheles gambiae*.

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Paratransgenesis, the genetic manipulation of insect symbiotic microorganisms, is being considered as a potential method to control vector-borne diseases such as malaria. The feasibility of paratransgenic malaria control has been hampered by the lack of candidate symbiotic microorganisms for the major vector *Anopheles gambiae*. In other systems, densovirus (DNVs) are attractive agents for viral paratransgenesis because they infect important vector insects, can be genetically manipulated and are transmitted to subsequent generations. However, *An. gambiae* has been shown to be refractory to DNV dissemination. We discovered, cloned and characterized the first known DNV (AgDNV) capable of infection and dissemination in *An. gambiae*. We developed a flexible AgDNV-based expression vector to express any gene of interest in *An. gambiae* using a two-plasmid helper-transducer system. To demonstrate proof-of-concept of the viral paratransgenesis strategy, we used this system to transduce expression of an exogenous gene (enhanced green fluorescent protein; EGFP) in *An. gambiae* mosquitoes. Wild-type and EGFP-transducing AgDNV virions were highly infectious to *An. gambiae* larvae, disseminated to and expressed EGFP in epidemiologically relevant adult tissues such as midgut, fat body and ovaries and were transmitted to subsequent mosquito generations. These proof-of-principle data suggest that AgDNV could be used as part of a paratransgenic malaria control strategy by transduction of anti-*Plasmodium* peptides or insect-specific toxins in *Anopheles* mosquitoes. AgDNV will also be extremely valuable as an effective and easy-to-use laboratory tool for transient gene expression or RNAi in *An. gambiae*.

PLoS Pathog. 2008 Aug 8;4(8):e1000121.

Temperature shift and host cell contact up-regulate sporozoite expression of *Plasmodium falciparum* genes involved in hepatocyte infection.

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Plasmodium sporozoites are deposited in the skin by Anopheles mosquitoes. They then find their way to the liver, where they specifically invade hepatocytes in which they develop to yield merozoites infective to red blood cells. Relatively little is known of the molecular interactions during these initial obligatory phases of the infection. Recent data suggested that many of the inoculated sporozoites invade hepatocytes an hour or more after the infective bite. We hypothesised that this pre-invasive period in the mammalian host prepares sporozoites for successful hepatocyte infection. Therefore, the genes whose expression becomes modified prior to hepatocyte invasion would be those likely to code for proteins implicated in the subsequent events of invasion and development. We have used *P. falciparum* sporozoites and their natural host cells, primary human hepatocytes, in in vitro co-culture system as a model for the pre-invasive period. We first established that under co-culture conditions, sporozoites maintain infectivity for an hour or more, in contrast to a drastic loss in infectivity when hepatocytes were not included. Thus, a differential transcriptome of salivary gland sporozoites versus sporozoites co-cultured with hepatocytes was established using a pan-genomic *P. falciparum* microarray. The expression of 532 genes was found to have been up-regulated following co-culture. A fifth of these genes had no orthologues in the genomes of Plasmodium species used in rodent models of malaria. Quantitative RT-PCR analysis of a selection of 21 genes confirmed the reliability of the microarray data. Time-course analysis further indicated two patterns of up-regulation following sporozoite co-culture, one transient and the other sustained, suggesting roles in hepatocyte invasion and liver stage development, respectively. This was supported by functional studies of four hitherto uncharacterized proteins of which two were shown to be sporozoite surface proteins involved in hepatocyte invasion, while the other two were predominantly expressed during hepatic parasite development. The genome-wide up-regulation of expression observed supports the hypothesis that the shift from the mosquito to the mammalian host contributes to activate quiescent salivary gland sporozoites into a state of readiness for the hepatic stages. Functional studies on four of the up-regulated genes validated our approach as one means to determine the repertoire of proteins implicated during the early events of the Plasmodium infection, and in this case that of *P. falciparum*, the species responsible for the severest forms of malaria.

PLoS Pathog. 2008 Aug 8;4(8):e1000118.

An erythrocyte vesicle protein exported by the malaria parasite promotes tubovesicular lipid import from the host cell surface.

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Plasmodium falciparum is the protozoan parasite that causes the most virulent of human malarial. The blood stage parasites export several hundred proteins into their host erythrocyte that underlie modifications linked to major pathologies of the disease and parasite survival in the blood. Unfortunately, most are 'hypothetical' proteins of unknown function, and those that are essential for parasitization of the erythrocyte cannot be 'knocked out'. Here, we combined bioinformatics and genome-wide expression analyses with a new series of transgenic and cellular assays to show for the first time in malaria parasites that microarray read out from a chemical perturbation can have predictive value. We thereby identified and characterized an exported *P. falciparum* protein resident in a new vesicular compartment induced by the parasite in the erythrocyte. This protein, named Erythrocyte Vesicle Protein 1 (EVP1), shows

novel dynamics of distribution in the parasite and intraerythrocytic membranes. Evidence is presented that its expression results in a change in TVN-mediated lipid import at the host membrane and that it is required for intracellular parasite growth, but not invasion. This exported protein appears to be needed for the maintenance of an essential tubovesicular nutrient import pathway induced by the pathogen in the host cell. Our approach may be generalized to the analysis of hundreds of 'hypothetical' *P. falciparum* proteins to understand their role in parasite entry and/or growth in erythrocytes as well as phenotypic contributions to either antigen export or tubovesicular import. By functionally validating these unknowns, one may identify new targets in host-microbial interactions for prophylaxis against this major human pathogen.

Public Health. 2008 Sep;122(9):923-32.

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

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

Rural Remote Health. 2008 Jul-Sep;8(3):956.

Malaria and pond-based rainwater harvesting linkages in the fringes of central highland Ethiopia.

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INTRODUCTION: Several studies have unravelled the linkages between malaria and macro-sized water bodies (lakes, dams, irrigations) in various parts of tropical Africa. However, those findings cannot be extrapolated to areas where micro-sized rainwater harvesting (RWH) ponds are dominant. This article reveals the linkages between malaria and RWH in some parts of central Ethiopia where micro-level irrigation is practised. METHODOLOGY: A descriptive study was conducted in five sample districts of Amhara and Oromia states. Systematic random sampling was employed to select 300 households. Data were collected using household survey,

focus group discussion and key informant interview techniques. RESULTS: The launch of RWH in the surveyed area, coupled with warming regional temperatures, has created breeding pools for mosquitoes and a longer malaria transmission period. The location of RWH ponds, the type of pond covers in use, and the limitations of some government policies are among the factors respondents believe have led to an expansion of malaria incidence in recent years. Users and non-users of RWH varied on the malaria-RWH nexus, which could be attributed to RWH-induced socioeconomic differences. CONCLUSIONS: Given the growing need for micro-level irrigated agriculture to feed a rapidly growing tropical population, coupled with a predicted warming of global and regional air temperatures, this study suggests a need for further investigation on a broader scale.

Soc Sci Med. 2008 Sep;67(5):854-62.

The persistent problem of malaria: Addressing the fundamental causes of a global killer.

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Despite decades of global eradication and control efforts and explosive global economic development, malaria is the most important vector-borne disease of our day, killing more people today than 40 years ago and affecting millions worldwide, particularly poor residents of tropical regions. Global eradication efforts from the 1950s through the 1980s largely failed, leaving vector and parasite resistance in their wake. The persistence of malaria and the magnitude of its effects call for an action paradigm that links the traditional proximal arenas of intervention with malaria's fundamental causes by addressing the environmental, economic, and political dimensions of risk. We explore the more distal determinants of malaria burden that create underlying vulnerabilities, evaluating malaria risk as a function of socioeconomic context, environmental conditions, global inequality, systems of health care provision, and research. We recommend that future action to combat malaria be directed by a broad-spectrum approach that meaningfully addresses both the proximal and fundamental causes of this disease.

Soc Sci Med. 2008 Sep;67(5):696-707.

'He is now like a brother, I can even give him some blood' - Relational ethics and material exchanges in a malaria vaccine 'trial community' in The Gambia.

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This paper explores social relations within the 'trial community' (staff and volunteers) of a Malaria Vaccine Trial (MVT), implemented by the Medical Research Council (MRC) in The Gambia between 2001 and 2004. It situates ethical concerns with medical research within the everyday life of scientific fieldwork. Based upon discussions with volunteers and staff, we explore processes of mediation between scientific project and study population, and between formal ethics, local ethical debates and everyday practice. We observe that material contact and substantial transactions, notably of blood and medicine, are central to the construction of the MVT. These transactions are guided by a concrete and relational form of ethics, which contrasts with the abstract and vertical formal ethical principles underwriting the scientific study protocol. The success of the MVT owed much to these kinship-like ethics. One possible conclusion from these observations is that research ethics should be understood, not just as a quasi-legal frame but also as an open, searching movement, much in the same way that kinship is not merely a juridical institution and a prescriptive frame of

rules, but a network made through relational work. However, this conclusion raises new problems: by contrasting formal, abstract principles to intimate, immediate relations, and economic justice to personal morality, we accept that the order of medical research is moved further out of the public and political, and into the domains of either quasi-legal claims or of private morality. Irrespective of the undeniable importance of clear-cut rules and of good face-to-face relations, a third essential foundation of medical research ethics is the democratically constituted public sphere, including equitable health services, and transparent institutions to facilitate open debate and regulate particular interests. Ultimately, the ethics of global science can rely neither on principles nor trust but requires citizenship and democratic government.

Soc Sci Med. 2008 Sep;67(5):708-20.

Taking social relationships seriously: Lessons learned from the informed consent practices of a vaccine trial on the Kenyan Coast.

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Individual informed consent is a key ethical obligation for clinical studies, but empirical studies show that key requirements are often not met. Common recommendations to strengthen consent in low income settings include seeking permission from community members through existing structures before approaching individuals, considering informed consent as a process rather than a single event, and assessing participant understanding using questionnaires. In this paper, we report on a qualitative study exploring community understanding and perceptions of a malaria vaccine trial (MVT) conducted in a rural setting on the Kenyan Coast. The MVT incorporated all of the above recommendations into its information-giving processes. The findings support the importance of community level information-giving and of giving information on several different occasions before seeking final individual consent. However, an emerging issue was that inter-personal interactions and relationships between researchers and community members, and within the community, play a critical role in participants' perceptions of a study, their decisions to consent or withdraw, and their advice to researchers on study practicalities and information to feedback at the end of the trial. These relationships are based on and continually tested by information-giving processes, and by context specific concerns and interests that can be difficult to predict and are well beyond the timescale and reach of single research activities. On the basis of these findings, we suggest that the current move towards increasingly ambitious and stringent formal standards for information-giving to individuals be counter-balanced with greater attention to the diverse social relationships that are essential to the successful application of these procedures. This may be assisted by emphasising respecting communities as well as persons, and by recognising that current guidelines and regulations may be an inadequate response to the complex, often unpredictable and ever shifting ethical dilemmas facing research teams working 'in the field'.

Trans R Soc Trop Med Hyg. 2008 Sep;102(9):861-7.

Adherence and efficacy of supervised versus non-supervised treatment with artemether/lumefantrine for the treatment of uncomplicated Plasmodium falciparum malaria in Bangladesh: a randomised controlled trial.

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As artemether/lumefantrine is now deployed as the first-line treatment for

uncomplicated falciparum malaria in Bangladesh, information on its efficacy and adherence to its use is important. A randomised controlled non-inferiority trial comparing directly observed treatment (DOT) and non-directly observed treatment (NDOT) was conducted in 320 patients with uncomplicated falciparum malaria in Bandarban Hill Tract District, Bangladesh. Both regimens showed similar high levels of PCR-corrected 42-day parasitological and clinical cure rates (99.3% in the NDOT group and 100% in the DOT group; $P=0.49$). Survival analysis for the time to recurrence of infection showed no difference between treatment groups (log rank, $P=0.98$). Adherence, as assessed by counting remaining tablets and oral interviews, was 93% in the NDOT group and was confirmed by Day 7 lumefantrine concentrations. Adherence was independent of educational level. Patients with plasma lumefantrine concentrations $<280\text{ng/ml}$ at Day 7 were at greater risk for re-infection (relative risk 5.62; $P=0.027$). The efficacy of artemether/lumefantrine for the treatment of uncomplicated falciparum malaria in Bangladesh is high and is similar for DOT and NDOT. Adherence to therapy is high.

Trans R Soc Trop Med Hyg. 2008 Sep;102(9):868-74.

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

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

Trans R Soc Trop Med Hyg. 2008 Aug 27.

Using malarial retinopathy to improve the classification of children with cerebral malaria.

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The mechanisms leading to death in cerebral malaria (CM) remain unclear. We compared clinical and laboratory data among children with CM, categorized by ocular fundus findings, to elucidate differences that suggest different underlying pathological processes. From 1999-2005, standard examinations, treatment and record keeping were used for children with a clinical diagnosis of CM. Children were divided into ocular subgroups: normal fundus (N), malarial retinopathy (R), or papilloedema alone (P) and appropriate statistical tests were used to compare clinical and laboratory findings among groups. Eight hundred and eighty children who had eye examinations within 6h of admission were included in the analysis. The groups differed significantly in case-fatality rates: Group P, 44.4% (95% CI 25.3-63.2), Group R, 18.0% (95% CI 15.6-22.3) and Group N, 7.0%

(95% CI 4.2-9.8). There were also significant differences among the groups in blood pressure, prevalence of deep breathing, haematocrit, parasite density, platelet concentration and, among survivors, hours taken to recover from coma. Differences among groups suggest that different underlying pathophysiological processes are operating in children with CM defined by existing criteria. Our proposed classification, by improving the specificity of diagnosis, would enhance consistency among different study sites and prove useful in future research studies.

Trans R Soc Trop Med Hyg. 2008 Aug 19.

Predictors of antimalarial treatment failure in an area of unstable malaria transmission in eastern Sudan.

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The factors that identify patients at risk of malaria treatment failure were evaluated in an area of unstable malaria transmission in eastern Sudan. We analyzed data from 471 patients who had been enrolled in six previous clinical antimalarial trials for uncomplicated *Plasmodium falciparum* malaria. Thirty-four (7.3%) had treatment failure (crude). In logistic regression models, an age of ≤ 5 years (odds ratio [OR]=3.7; 95% CI 1.5-8.6; P=0.002) and parasitaemia that took 3 days to clear (OR=2.4; 95% CI 1.0-5.9; P=0.04) were found to be predictors for treatment failure. Presenting temperature (OR=1.4; 95% CI 0.9-2.2; P=0.1), level of parasitaemia (OR=1.0; 95% CI 1.0-1.0; P=0.8) and presence of gametocytes (OR=0.3; 95% CI 0.9-1.2; P=0.1) were not associated with treatment failure. Thus, these factors might be used to identify those in whom treatment might fail in the future.

Trans R Soc Trop Med Hyg. 2008 Aug 14.

Malaria and HIV co-infection in pregnancy in sub-Saharan Africa: impact of treatment using antimalarial and antiretroviral agents.

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Malaria and HIV infection represent severe public health problems in sub-Saharan Africa, and pregnant women are at increased risk because the two diseases intersect in pregnancy, causing adverse perinatal outcome. As access to antiretroviral drugs is increasing in the sub-region, and new combinations of antimalarial drugs are being implemented while more are being evaluated, there is potential for interactions between these therapies. In this report, the impact of treatment using antimalarial and antiretroviral agents in pregnant women with malaria and HIV co-infection was reviewed, using scientific publications identified through a Medline Entrez-Pubmed search with reference to sub-Saharan Africa. The safety and operational feasibility of use of antimalarial and antiretroviral agents to treat co-infected pregnant women were evaluated. Although use of these therapies was shown to improve the health of pregnant women with co-infection, low adherence, poor-quality drugs, resource scarcity, lack of infrastructure and inadequate treatment in sub-Saharan Africa continue to hamper treatment outcome. The absence of studies on interaction between antimalarials and antiretrovirals, as well as mounting evidence of treatment failure due to drug resistance and adverse drug reactions, in most parts of sub-Saharan Africa, make the establishment of new guidelines for the prevention of malaria and HIV infection during pregnancy imperative.

Trends Parasitol. 2008 Sep;24(9):396-400.

Mosquitocidal vaccines: a neglected addition to malaria and dengue control strategies.

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The transmission of vector-borne diseases is dependent upon the ability of the vector to survive for longer than the period of development of the pathogen within the vector. One means of reducing mosquito lifespan, and thereby reducing their capacity to transmit diseases, is to target mosquitoes with vaccines. Here, the principle behind mosquitocidal vaccines is described, their potential impact in malaria and dengue control is modeled and the current research that could make these vaccines a reality is reviewed. Mosquito genome data, combined with modern molecular techniques, can be exploited to overcome the limited advances in this field. Given the large potential benefit to vector-borne disease control, research into the development of mosquitocidal vaccines deserves a high profile.

Trends Parasitol. 2008 Sep;24(9):406-10.

Knowlesi malaria: newly emergent and of public health importance?

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Several questions on public health impact have arisen from the discovery of a large focus of the simian malaria parasite, *Plasmodium knowlesi*, in the human population. *P. knowlesi* malaria is not newly emergent and was overlooked until molecular tools to distinguish between *P. knowlesi* and the morphologically similar *Plasmodium malariae* became available. *Knowlesi* malaria is a zoonosis that is widely distributed in Southeast Asia and can be fatal. Information on *knowlesi* malaria should be included in medical and public health guidelines to encourage the accurate diagnosis and treatment of patients, and monitor the incidence and distribution of cases. A complete emergence of *P. knowlesi* into the human population could be overwhelming and, although challenging, the prevention of this situation deserves serious consideration.

Trends Parasitol. 2008 Sep;24(9):392-395.

Malaria vaccines: immunity, models and monoclonal antibodies.

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Although experts in the field have agreed on the malaria vaccine technology roadmap that should be followed (<http://www.malariavaccineroadmap.net/>), the path towards an effective malaria vaccine remains littered with intellectual and practical pot-holes. The animal models that are currently available are problematic, and current understanding of the exact mechanisms and targets of protective immune responses is incomplete. However, recent technological advances might help overcome some of these hurdles.

Trends Parasitol. 2008 Aug 27.

TRAP-like protein of Plasmodium sporozoites: linking gliding motility to host-cell traversal.

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To reach its final destination in the liver, the sporozoite (the stage of the malaria parasite that is transmitted by the mosquito vector) needs to glide through tissues and traverse host cells. Although the molecular bases of these behaviors are typically considered separately, two recent reports suggest the first molecular link between the two via a novel protein called 'TRAP-like protein'.

Trends Parasitol. 2008 Aug 27.

Control to elimination: implications for malaria research.

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Recent reports indicate that a high level of malaria control can be achieved with existing control tools once their use has been scaled up. This has led to renewed interest in the possibility of malaria elimination, an approach that is now supported by several influential organisations. An increasing focus on elimination requires a review of priorities within the malaria research agenda. The development of drugs and vaccines with a strong transmission-blocking potential becomes increasingly important. Novel approaches to surveillance will be necessary to ensure that once elimination has been achieved, it is not threatened by a rapid reintroduction of malaria from neighbouring areas.

Trends Parasitol. 2008 Aug 22.

Cuban parasitology in review: a revolutionary triumph.

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Although the population of Hispaniola, Cuba's most similar neighbour in the Caribbean, continues to be threatened by parasitic diseases (including malaria), many tropical parasitic infections in Cuba have been eliminated or controlled. However, some parasitic infections remain important in the Cuban population, and the occurrence of vectors and the high possibility of introduction of parasites mean that Cuban diagnosticians must remain alert. Some key aspects of human parasitology in Cuba are reviewed here, including historical information, comparative data from Hispaniola and Jamaica, and how Cuba strives to maintain and improve its control against parasitic infections. Data from recent key novel parasitology research conducted in Cuba are also described.

Vaccine. 2008 Aug 18;26(35):4526-35.

Immunogenicity of a recombinant malaria vaccine candidate, domain I+II of AMA-1 ectodomain, from Indian *P. falciparum* alleles.

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Among the few vaccine candidates under development, apical membrane antigen (AMA-1) of *Plasmodium falciparum* is one of the most promising erythrocyte stage malaria vaccine candidates under consideration. The overall structure of AMA-1 appears to be conserved as compared to other surface proteins, but there are numerous amino acid substitutions identified among different *P. falciparum* isolates. Antisera raised against recombinant AMA-1 or naturally acquired human antibodies were strongly inhibitory only towards homologous parasites. In an attempt to examine the strain specificity of antibodies elicited to AMA-1, we have cloned, expressed and purified two allelic variants of domain I+II of AMA-1 ectodomain from Indian *P. falciparum* isolates in bacteria. One of these is a new haplotype not reported so far and varies in 18 aa positions from the geographically diverse forms 3D7 and 15 from FVO. Refolded proteins were recognized by a conformation specific monoclonal antibody 4G2.dcl1 and hyper immune sera. Immunization of mice and rabbits with the purified proteins using CFA/IFA adjuvant generated high titer polyclonal antibodies. Both the alleles induced high levels of IgG1, IgG2a and IgG2b and a low level of IgG3 in mice. Lymphocyte proliferation assays using splenocytes from immunized mice showed significant proliferative responses and cytokines interleukin-2 (IL-2), IL-4, IL-10 and IFN-gamma presence in the culture supernatants. The anti-AMA-1 rabbit antibodies obtained with both the proteins were active in an in vitro parasite growth invasion/inhibition assay. These results suggest that recombinant AMA-1 domain I+II formulated with CFA/IFA adjuvant elicited cellular and humoral responses and is capable of inducing high titer invasion inhibitory antibodies supporting further development of this vaccine candidate.

Vaccine. 2008 Aug 12;26(34):4338-4344.

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

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

assays. These results provide support for further clinical development of a vaccine for *P. vivax* malaria based on recombinant PvRII.