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**THE LAMBARÉNÉ-ORGAN-DYSFUNCTION SCORE (LODS) IS A SIMPLE CLINICAL PREDICTOR FOR FATAL MALARIA IN AFRICAN CHILDREN**

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*Plasmodium falciparum* malaria accounts for more than a million deaths annually, mostly among young children in sub-Saharan Africa. Identifying those who are likely to die is difficult. Prior studies suggested that quantitative scores (Multi-Organ-Dysfunction Score and simplified Multi-Organ-Dysfunction Score, MODS and sMODS) are useful markers predicting morbidity, but the cohorts were not large enough to detect an association with case fatality. We used stepwise backward logistic regression to select the best predictors out of nine variables evaluated on admission to predict death in 23,800 hospitalised children with *P. falciparum* malaria. The study was conducted from December 2000 to May 2005 in six hospital-based research units (Banjul in The Gambia, Blantyre in Malawi, Kilifi in Kenya, Kumasi in Ghana and Lambaréne and Libreville in Gabon) in a network established to study severe malaria in African children (SMAC). The Lambaréne-Organ-Dysfunction-Score (LODS) counts how many of the three variables coma, prostration and deep breathing are present. A LODS > 0 (OR = 9.6; 95%CI 8.0-11.4) has a sensitivity of 85% to predict death and a LODS < 3 is highly specific for survival (98%). The LODS is a simple clinical predictor for fatal malaria in African children. This score provides a sufficiently accurate and rapid identification of children needing either referral or increased attention.

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**SULFADOXINE-PYRIMETHAMINE VERSUS UNSUPERVISED ARTEMETHER-LUMEFANTRINE VERSUS UNSUPERVISED AMODIAQUINE-ARTESUNATE FIXED-DOSE FORMULATION FOR UNCOMPLICATED FALCIPARUM MALARIA IN BENINESE CHILDREN: A RANDOMIZED EFFECTIVENESS NON-INFERIORITY TRIAL**

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In order to measure the potential impact of the 2004 malaria treatment guidelines in Benin that recommend ACTs (artemisinin-based combination therapies: artemether-lumefantrine as first line therapy and amodiaquine-artesunate as second line therapy) in the management of uncomplicated malaria in young children, we conducted an open randomised non-inferiority trial to compare the effectiveness of sulfadoxine-pyrimethamine (SP) to unsupervised artemether-lumefantrine (AL) and to unsupervised amodiaquine-artesunate fixed-dose formulation (ASAQ). The trial took place in southern Benin in children aged 6 to 60 months with fever or a

history of fever, and a 6-weeks follow-up was performed after treatment. The primary objective was a comparison of day 28 PCR-corrected effectiveness rates. 240 children (48 SP, 96 AL and 96 ASAQ) with a mean age of 26 months were randomized from May to October 2007. Before PCR correction, the intention to treat (ITT) analysis (239 patients) showed day 28 effectiveness rates of 20.8%, 78.1% and 70.5% with SP, AL and ASAQ respectively. After PCR correction, day 28 ITT effectiveness rates were 27.1%, 83.3% and 87.4% respectively. The per protocol analysis (217 patients) showed day 28 effectiveness rates of 21.7%, 88.0% and 76.1% with SP, AL and ASAQ respectively. After PCR correction, day 28 effectiveness rates were 28.3%, 94.0% and 93.2% respectively. Comparisons of SP with ACTs were highly significant in any case, whereas there was no significant difference between AL and ASAQ in the PCR-corrected analyses. The rate of new infections was significantly higher in children treated with ASAQ compared to those treated with AL. Two children treated with SP had to be hospitalized for severe anemia. There was no difference between treatment arms in terms of incidence of adverse events. No severe adverse event was related to a study drug. The potential impact on malaria morbidity and mortality of the replacement of SP by ACTs in this study area could be highly significant.

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**RISK FOR SEVERE DISEASE IN ADULTS WITH FALCIPARUM MALARIA**

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We conducted a clinical study of malaria acquired worldwide in adults in a non-endemic country over a 16 year period to determine risk factors for severe *Plasmodium falciparum* malaria. All patients managed by our unit from 1991 to 2006 with confirmed malaria were prospectively evaluated. Factors predicting disease severity according to a) strict World Health Organisation (WHO) criteria, b) a composite measure of unfavourable outcome and c) length of hospital stay, were identified through logistic-regression analysis. We evaluated 676 episodes; 482 (71%) due to *P. falciparum* and 194 to non-falciparum malaria. Black patients were at significantly reduced risk of developing severe disease, an unfavourable outcome or prolonged stay in hospital compared to Asians or whites. Of six patients with falciparum malaria who died, none were black. Patients with parasitemias  $\geq 2\%$  had odds of severe malaria of 12 times higher than patients with  $<2\%$  parasites. Patients with a history of previous clinical malaria, regardless of ethnicity, were at significantly reduced risk of WHO-definition severe malaria. Ethnicity and parasitemia are important independent risk factors for severe falciparum malaria while a history of previous malaria significantly reduces the risk of severe disease (WHO Criteria). These results have important implications for management guidelines in non endemic countries.

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**ASSESSING THE CARDIAC EFFECTS OF ARTESUNATE (AS) AND AMODIAQUINE (AQ) IN HEALTHY VOLUNTEERS IN A SAFETY AND PK, SINGLE DOSE, RANDOMISED, TWO PHASE CROSS OVER STUDY OF A NEW FIXED DOSE AS/AQ COMBINATION AND LOOSE AS + AQ**

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Evaluating QT prolongation as a risk marker for Torsades de Pointe ventricular tachycardia is an essential step for registering new drugs. AS and AQ are well established, antimalarial drugs but have few cardiac data. AQ may be cardiotoxic in overdose. In a randomized, two phase, pharmacokinetic and safety, cross over study, healthy Malaysian adults received fixed-dose AS/AQ (200 + 540mg) and loose AS+AQ (200 + 600mg) 60 days apart. ECGs were performed at baseline, 1h, 2h, 4h, 24h, Day 60 and repeated at cross-over. The QT interval was corrected using the Fridericia formula (QTcF). Analysis was by ANOVA for repeated measures. There were no statistically significant differences between the two arms regarding the PR, QRS and QTcF intervals over time. The baseline QTcFs were 396 ( $\pm 18$ ) ms for both arms. Mean QTcF (AS+AQ) increased significantly at 2h ( $7 \pm 13$  ms,  $p=0.018$ ) and 4 h ( $7 \pm 11$  ms,  $p=0.008$ ). The only significant change for AS/AQ was a decrease ( $p=0.013$ ) in the QTcF on D60: -14 (-25 to -4) ms. Post baseline, most volunteers had normal QTcFs despite increases or decreases in the QTcF. Only one (male) volunteer had a 'flag' QTcF of 456 ms (AS/AQ +4h, phase 2). Changes in the PR (maximum values = 206 to 211 ms,  $n=2$ ) and QRS (maximum values = 122 and 127 ms,  $n=2$ ) intervals were modest. Heart rates were normal during both phases and trended down over time. The ECG interval changes were small and transient, consistent with natural variation and regression of the mean. PK ECG analyses will be done to determine if a drug effect may be present.

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**INTRAVASCULAR HEMOLYSIS: A NEGLECTED MECHANISM OF NITRIC OXIDE QUENCHING, ENDOTHELIAL DYSFUNCTION AND IMPAIRED PERfusion IN SEVERE FALCIPARUM MALARIA?**

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Hemolysis of infected and uninfected red cells has long been recognized as a significant contributor to malarial anemia, but has not been thought of as a contributor to endothelial dysfunction and activation in severe malaria. In sickle cell disease, hemolysis causes quenching of endothelial nitric oxide (NO) resulting in pulmonary hypertension and endothelial activation. We hypothesized that similar to other hemolytic states, cell free hemoglobin from red cell hemolysis would contribute to quenching of nitric oxide and endothelial dysfunction in severe falciparum malaria. Plasma hemoglobin was measured in adults with moderately severe ( $n=78$ ) and severe malaria ( $n=49$ ), and in healthy controls ( $n=23$ ), and was related

to endothelial function measured using reactive hyperemia-peripheral arterial tonometry (RH-PAT, a measure of endothelial NO bioavailability). Linear regression was used to relate concentrations of plasma hemoglobin with malaria disease severity, lactate, endothelial function and plasma histidine rich protein-2 (HRP2). Plasma cell-free hemoglobin was associated with disease severity, being higher in severe malaria (median 91.7 ng/mL [IQR 53.7-125]) than in moderately severe malaria (median 44.2 ng/mL [IQR 22.2-76]) or healthy controls (median 22.6 ng/mL [IQR 15.8-40.8]). Plasma hemoglobin was independently associated with endothelial dysfunction ( $r=0.33$ ;  $p=0.0001$ ) and increased venous lactate. As well as contributing to anemia in falciparum malaria, hemolysis causes NO quenching and may be a significant contributor to endothelial dysfunction and impaired microvascular perfusion in severe malaria.

**1195**

**PHARMACOKINETIC PROPERTIES OF CHLOROQUINE AND SULFADOXINE-PYRIMETHAMINE IN PREGNANCY**

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Intermittent preventive treatment in pregnancy (IPTp) may reduce maternal and infant morbidity and mortality. However, few pharmacokinetic (PK) and/or safety data exist for antimalarial drugs in pregnancy. Although chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) are used widely and considered safe, PK data is needed to optimize dosing and maximize effectiveness of IPTp. We have, therefore, performed a PK study of CQ + SP in 30 pregnant women and in a control group of 30 age-matched non-pregnant women in Papua New Guinea. All received one dose of SP (1500/75mg: mean 28/1.4mg/kg) and 3 daily doses of CQ (450mg/day: 8.5mg/kg/day). Women were bled up to 16 times over the ensuing 42 days and plasma assayed for CQ, desethylchloroquine (DECQ), S, N-acetyl-sulfadoxine (NA-S), and P by HPLC. From compartmental modeling of concentration-time data, the volume of distribution (Vd), clearance (Cl) and elimination half-lives ( $t_{1/2}e$ ) of CQ, S and P were determined. AUC was determined by non-compartmental analysis. Differences in PK parameters between pregnant and non-pregnant groups were assessed by non-parametric statistical methods. A two-compartment model best-described the CQ data and showed that, compared with non-pregnant controls, pregnant subjects had similar Vd (median 180 vs 156/L/kg in non-pregnant group:  $P=0.5$ ) but significantly more rapid Cl (15 vs 11ml/min/kg:  $P=0.04$ ), shorter  $t_{1/2}e$  (196 vs 236h:  $P=0.03$ ) and lower AUC for both CQ (34 vs 56 $\mu$ g.h/L:  $P<0.01$ ) and DECQ (25 vs 47 $\mu$ g.h/L:  $P<0.01$ ). A one-compartment model best-described the disposition of S showing significantly larger Vd (0.24 vs 0.21/L/kg:  $P<0.01$ ), more rapid Cl (0.022 vs 0.016ml/min/kg:  $P<0.01$ ), shorter  $t_{1/2}e$  (134 vs 161h:  $P=0.03$ ) and lower AUC (22 vs 34g.h/L:  $P<0.01$ ) in pregnant subjects. Data for P will also be presented. Because lower plasma concentrations of CQ, DECQ and S could compromise both curative efficacy and post-treatment prophylactic properties in pregnant patients, IPTp regimens should incorporate higher mg/kg doses than recommended for non-pregnant patients.

**1196**

**CD8+ T CELL RESPONSES IN NONLYMPHOID TISSUE AND PARASITE CONTROL DURING *TRYpanosoma cruzi* INFECTION**

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Chagas disease is caused by persistent infection with *Trypanosoma cruzi*. Amastigotes of this protozoan parasite replicate in host cell cytosol, allowing parasite antigens to be presented via class I major

histocompatibility complex. Thus, CD8+ T cells are critical to the immune response to *T. cruzi*. Though the surface phenotype of CD8+ T cells in both spleen and skeletal muscle is consistent with an effector/effector memory phenotype, most CD8+ T cells from skeletal muscle of *T. cruzi*-infected mice are incapable of interferon- $\gamma$  (IFN $\gamma$ ) production upon ex vivo restimulation, suggesting a functional defect that could promote parasite persistence. To determine if this phenomenon was specific for skeletal muscle, we examined adipose tissue, a recently-identified site of parasite persistence in mice. Like CD8+ T cells infiltrating muscle, those in adipose tissue also display an effector/effector memory phenotype and are poor producers of IFN $\gamma$  after ex vivo restimulation. Thus, we conclude that CD8+ T cells isolated from sites of parasite persistence in chronic *T. cruzi* infection have a phenotype and effector potential that is independent of tissue microenvironment. Despite their apparent low effector, we hypothesize that CD8+ T cell activity in sites of parasite persistence is transient but crucial, given that parasite load is very well controlled in these tissues. In support of this hypothesis, we found that a substantial fraction of CD8+ T cells at the sites of infection express the recent activation marker CD69, indicating that this portion of CD8+ T cells is likely responding to parasite antigen. Moreover, trackable CD8+ T cells from spleens of naïve or *T. cruzi*-infected mice transferred into mice with established infection are incorporated into the ongoing response in recipient tissue, developing into effectors and effector memory T cells. We propose a model in which CD8+ T cells contribute to a dynamic peripheral immune response that maintains control of this continuously replicating parasite.

## 1197

**LEISHMANIA BRAZILIENSIS INTERACTION WITH DENDRITIC CELLS: DISTINCT ROLES FOR TLR2 AND TLR3**

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*Leishmania braziliensis* (*Lb*) is the causative agent of cutaneous and mucosal leishmaniasis (ML) in South America. In humans, ML is a severe and disfiguring form of the disease and is characterized by excessive B and T cell responses to the parasite. In animal models of *Lb* infection, most inbred strains of mice are genetically resistant to infection, showing only a transient period of active disease. We have recently reported the selective activation of murine DCs and up-regulation of several signals (e.g., STATs and ISG15) that are essential for the activation of innate immunity against *Lb* in mice, as reported previously. However, it remains unclear whether pathogen recognition receptors, such as TLRs, are involved in DC activation, and, if so, how these early events lead to the production of proinflammatory cytokines in *Lb*-infected DCs. To address these issues, we generated bone marrow-DCs from MyD88 $^{-/-}$ , TLR2 $^{-/-}$  and TLR3 $^{-/-}$  mice and examined their responsiveness to *Lb* infection. In contrast to wild-type DCs, which were efficiently activated to produce cytokines and to prime naïve CD4+ T cells, the lack of TLR2 expression resulted in a significantly higher expression of MHC class II and co-stimulatory molecules and IL-12p40. As such, *Lb*-infected TLR2 $^{-/-}$  DCs were more competent in priming naïve CD4+ T cells *in vitro* than were the wide-type controls. This enhanced DC function was unique to TLR2 deficiency, because similarly infected MyD88 $^{-/-}$  and TLR3 $^{-/-}$  DCs showed a significant reduction in DC activation and T cell priming. Given that TLR2 is known to negatively regulate signals triggered by exogenous stimuli, we propose that TLR2 and TLR3 may play distinct roles in *Lb* infection and are further testing this hypothesis via *in vitro* and *in vivo* approaches. This study will provide new information on the regulation of innate immunity to *Leishmania* parasites.

## 1198

**TLR INVOLVEMENT DURING EXPERIMENTAL MALARIA: IMPLICATIONS FOR BOTH ENDS OF THE CLINICAL SPECTRUM OF HUMAN DISEASE**

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*Plasmodium berghei* is a murine model for cerebral malaria whilst *P. chabaudi* is used to study hyperparasitaemia and anemia. The first is characterized by early excess inflammation, leading to host immunopathology and death ('hyperresponsive' model). The second lacks the initial inflammatory response, however, death occurs later through uncontrolled parasitaemia ('hyporesponsive' model). We hypothesized that each model represents a pole of the clinical spectrum observed in human disease, and anticipated that TLRs (and their signaling pathways) would be involved in these divergent clinical outcomes. Despite the important role played by TLR2 in GPI signaling, in both models, no differences were observed between infected TLR2 $^{-/-}$  animals compared to wildtype controls (WT). *P. berghei*-infected TLR2 $^{-/-}$  mice did not show increased survival compared to susceptible WT mice; parasitaemias, weight, hematocrit, urine hemoglobin and plasma cytokines were similar between the two groups. Furthermore, as with WT mice, TLR2 $^{-/-}$  animals infected with *P. chabaudi* proved resistant to infection. No difference was observed in any of the measured parameters between the groups. Contrastingly, in both models, infected IRAK4 $^{-/-}$  mice (IRAK4 is a molecule involved in TLR signaling) showed marked differences to WT and TLR2 $^{-/-}$  mice during infection. When infected with *P. berghei*, the survival of IRAK4 $^{-/-}$  mice (>40% at day 14) was highly improved compared to controls (0% by day 9). Parasitaemias and serum cytokine levels were decreased (TNF, IFN-, IL-10, p<0.05, Mann-Whitney U), emphasizing the detrimental role of IRAK4 and TLR signaling in early inflammatory responses to malaria. During *P. chabaudi* infection, however, the survival of IRAK4 $^{-/-}$  animals was significantly lower than WT animals; the mice also had higher parasitaemias (Kruskal-Wallis, p<0.01), greater weight loss and lower serum cytokine levels than wild-type mice (TNF, two-way ANOVA, p<0.05). This demonstrates the key role played by IRAK4 late in infection, required for parasite clearance. Data from our experiments demonstrate that abrogating inflammation at one end of the clinical spectrum (cerebral malaria) is beneficial to the host, whilst, at the other end of the spectrum (hyper-parasitaemia/anemia), it worsens disease. Our findings emphasize the importance of integrated studies in order to fully understand the impact of treatment/interventions on malaria infection.

## 1199

**MOSQUITO RUNX4 IN THE IMMUNE REGULATION OF PPO GENES AND ITS EFFECT ON AVIAN MALARIA INFECTION**

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Melanization is a prominent defense mechanism employed by arthropods, including mosquitoes. Conflicting results have been reported when discerning whether phenoloxidase (PO) activation and melanin synthesis can successfully combat the infections of many bacterial and fungal species in flies and the malaria parasites in mosquitoes. The extensive gene expansion of 10 proPO genes, which encode key enzymes to activate melanization, in the mosquito, *Aedes aegypti*, lead us to hypothesize that mosquito melanization reactions have been diversified for distinct purposes. Thus, we showed that the loss of malaria parasites by ookinete melanization in Cactus-depleted mosquitoes is a distinct mechanism from the melanotic tumor formation resulting from Serpin-2 depletion in the mosquito, *Aedes aegypti*. Furthermore, we report that the parasitic loss in the mosquitoes with Cactus depletion is mediated by RUNX4, the orthologue of *Drosophila* Lozenge and a specific transcriptional activator of immune-inducible proPO genes. Specifically, we found that microbial infection induced four mosquito PPO genes, which are proposed to

be independent of Serpin-2 inhibition. This up-regulation is activated indirectly by the Toll immune pathway and directly by RUNX4. Mosquito RUNX4 specifically bound to the RUNT-binding motifs from the mosquito PPO gene promoters and activated *Drosophila* PPO genes in S2 cells. Concurrent silencing of RUNX4 and Cactus dismissed the activation of immune-inducible PPO genes resulting from Cactus depletion and thus compromised the killing of the avian malaria parasite *Plasmodium gallinaceum*. Our findings reveal the presence of a RUNX4-dependent immune activation of PPO genes under the regulation of the Toll immune pathway and its potential immune role to restrict the parasite development. We will further address the role of RUNX4 and immune-inducible PPOs in the immune response against the malaria parasite.

## 1200

### STIMULATION OF TOLL-LIKE RECEPTOR 2 BY *PLASMODIUM FALCIPARUM* GLYCOSYLPHOSPHATIDYLINOSITOLS ENHANCES MACROPHAGE INTERNALIZATION OF PARASITIZED AND UNINFECTED ERYTHROCYTES

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Toll-like receptors (TLRs) are highly conserved innate sensing receptors that activate host defenses upon detection of microbial products. In the context of malaria, *Plasmodium falciparum* glycosylphosphatidylinositols (*PfGPI*) have been shown to stimulate macrophage cytokine production via TLR2. In addition to their role in inflammation, TLRs have also been characterized as regulators of phagocytosis. *P. falciparum* parasitized erythrocytes (PEs) can be non-opsonically internalized by macrophages in a process predominantly mediated by scavenger receptor CD36. Moreover, uninfected erythrocytes (UEs) are rendered susceptible to macrophage clearance during malaria infection due to surface modifications, and this is believed to contribute to the pathogenesis of severe malarial anemia. We hypothesized that stimulation of macrophage TLR2 by *PfGPI* would enhance innate clearance of PEs as well as malaria-exposed UEs. We first employed a PE model consisting of anti-CD36 antibodies conjugated to human erythrocytes ("anti-CD36 EBABs"). Pre-stimulation of primary human and murine macrophages with *PfGPI* or a synthetic TLR2 agonist (FSL-1) significantly increased uptake of anti-CD36 EBABs in a TLR2-dependent manner. Internalization of *P. falciparum* PEs was similarly enhanced. Fc-mediated phagocytosis of IgG-opsonized PEs was modestly increased by TLR2 activation. Notably, stimulation of macrophage TLR2 enhanced phagocytosis of UEs isolated from *P. falciparum* culture. Thus, in this *in vitro* system, TLR2-mediated macrophage activation enhanced clearance of both *P. falciparum* PEs and malaria-exposed UEs. These data underscore the complexity of the role of TLRs in malaria infection: TLR-enhanced phagocytosis may benefit infected individuals by decreasing parasite burden, but in other contexts may predispose to severe malarial anemia by enhancing UE destruction. Therapeutic targeting of TLR pathways in malaria must be carefully considered.

## 1201

### CONGENITAL CHAGAS DISEASE TRANSMISSION IN SANTA CRUZ, BOLIVIA

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Although Santa Cruz city has never had vectorial transmission, Chagas disease prevalence is high due to migration from endemic rural areas. We conducted a study of congenital Chagas disease in a public hospital. From

Nov. 2006 to June 2007, women presenting for delivery were enrolled in serological screening; *Trypanosoma cruzi* infection was confirmed when specimens were positive by 2 or more serological assays (whole epimastigote and recombinant ELISAs, IFA). Maternal blood specimens were also examined by polymerase chain reaction (PCR) using primers targeting kinetoplast minicircle DNA. For infants of seropositive mothers, we collected cord blood and tissue from the umbilical cord segment proximal to the infant, and peripheral blood specimens at 7, 21 30, 90 and 180 days of life (or until infection was diagnosed). Infant blood specimens were collected in heparinized microhematocrit tubes, centrifuged, and the buffy coat layer examined for the presence of motile trypanostigotes. Cord blood specimens and umbilical tissue were examined by PCR. Of 530 women, 154 (29%) had confirmed positive serology results. Infection prevalence rose by quartile of age: 18.4% (13-18 years), 24.2% (19-23 years), 29.3% (24-29 years) and 46.0% (30-45 years) (Chi square for trend 23.78,  $p < 0.0001$ ). Eight infants were found to have congenital *T. cruzi* infection. Seven were diagnosed by direct examination at 7 (2), 21, 30, 90, 180 and 280 days; no cord blood specimens were positive by direct examination. One infant was diagnosed by serology at 9 months. Seven of 8 infants with confirmed congenital infection had cord blood available for PCR; 5 were positive. Umbilical tissue PCR was positive in 6 of 6 confirmed infected infants. One additional infant whose cord blood specimen was negative by direct examination had positive PCR in cord blood and tissue. Seropositive women with positive PCR were significantly more likely to transmit *T. cruzi* to their infants than those with negative PCR (8/96 PCR-positive vs 0/57 PCR-negative mothers ( $p < 0.05$  by 2-tailed Fishers exact test). Infants were treated as soon as infection was confirmed. The rate of congenital transmission has fallen from 10% in the 1980s to 5% of infants of infected women, consistent with findings of other studies. PCR shows promise for early detection of congenital infection, and to predict which women are at highest risk to transmit *T. cruzi* to their infants.

## 1202

### DIAGNOSTIC ACCURACY OF *LEISHMANIA* OLIGO-C-TEST FOR THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN PERU

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Molecular methods, such as PCR, have become promising tools for the diagnosis of leishmaniasis, both for their high sensitivity and specificity. However, the practical utility of these techniques is limited by their infrastructural requirements and the expertise needed to conduct them. Recently, a simple and rapid dipstick method for the detection of amplified *Leishmania* PCR products was developed (*Leishmania* OligoC-TesT). We estimated the diagnostic accuracy of the *Leishmania* OligoC-Test for diagnosis of cutaneous leishmaniasis (CL) on 61 lesions from 45 consecutive patients presenting to the Leishmaniasis Clinic at the Instituto de Medicina Tropical "Alexander von Humboldt", Peru. Lesions were classified as (i) confirmed CL (50 cases), (ii) suspected CL (2 cases) and non CL (9 cases) based on parasitological detection and leishmanin skin test results. The sensitivity of the *Leishmania* OligoC-Test was 72.5% and 92% on lesion aspirates and scrapings, respectively. Furthermore, we compared the assay with a conventional PCR targeting the kinetoplast DNA (kDNA) and a significant higher sensitivity (94%) was observed with the kDNA PCR on the aspirate samples while no significant difference was observed between both methods on the scraping samples (88%). Positive PCR results were observed in the 9 non CL lesions and the role of PCR in CL diagnosis is discussed. Additionally, 4 patients were tested with the OligoC-TesT in a low-equipped rural hospital laboratory located in the Peruvian central jungle. The test results were concordant to the outcome

of the conventional diagnostic procedures but obtained only 5 hours after initial sample taking. The evaluated assay showed clear advantages as a simple and rapid molecular tool for diagnosis of CL in reference laboratories and in near-to-field hospital settings.

## 1203

**EQUIVALENCE STUDY USING REDUCED DOSES OF ANTIMONY PLUS RECOMBINANT HUMAN GM-CSF COMPARED WITH ANTIMONY IN STANDARD DOSES FOR CUTANEOUS LEISHMANIASIS: A RANDOMIZED, DOUBLE BLIND STUDY**

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The response to recombinant human granulocyte macrophage colony stimulating factor for the treatment of cutaneous leishmaniasis was evaluated. Forty American cutaneous leishmaniasis (ACL) patients with lesions for  $\leq$  60 days were enrolled in a double-blind-randomized-placebo controlled trial. The test group included 20 patients treated with GM-CSF intralesionally injected (200 $\mu$ ) at enrollment, and one week after, associated with parenteral sodium meglumin antimony (20mg/Kg/d) for 10 days. The control group included 20 patients treated with standard antimony (20mg/Kg/d) for 20 days plus saline as placebo. EKG and AST, ALT, urea, creatinine, amylase were performed to evaluate antimony toxicity. The results show that GM-CSF applied intralesionally in conjunction to antimony in reduced time, cures cutaneous leishmaniasis patients in a equivalent time as the full regimen treatment with antimony, 91 +/- 45,69 versus 90,5 +/- 53,2 days, respectively. This study opens the possibility of reducing the treatment of ACL to 10 days which in large population samples might improve adherence to therapy. Moreover, the combined therapy will be important for patients with other conditions that increase the risk of antimony therapy, such as older patients and those with liver, heart and kidney diseases.

## 1204

**A NOVEL AND HIGHLY POTENT CLASS OF COMPOUNDS FOR THE TREATMENT OF TRYpanosomiasis**

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It has been known for some time that trifluralin, an herbicide introduced in the 1960s, exhibits a degree of antiprotozoal activity. This compound is non-toxic and inexpensive to produce, making it a potential lead for an antiparasitic drug discovery program. We initially embarked on a project to make a range of trifluralin analogues, and in particular more water soluble analogues, in an attempt to improve the activity of this class of compound to the point where one may have potential as a commercial drug. Our most recent discovery has been what we refer to as the 3rd Generation Analogues. The compounds described are simple to synthesise and cheap to produce. The best of these compounds exhibited a 4,000-fold increase in activity compared to trifluralin. In particular, they have excellent *in vitro* activity against *Trypanosoma rhodesiense* (40 nM), *T. cruzi* (50 nM), and *Leishmania donovani* (90 nM) and cure *T. rhodesiense* infections in a validated mouse model when given orally. Several representatives of the class are also Ames negative. This work is being undertaken as an antiparasitic drug discovery program funded by the Drugs for Neglected Diseases initiative.

## 1205

**AN2920, A NOVEL OXBORALE, SHOWS *IN VITRO* AND *IN VIVO* ACTIVITY AGAINST *TRYpanosoma brucei***

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*Trypanosoma brucei* is the causative agent of Human African Trypanosomiasis (HAT) a protozoan disease of sub-Saharan Africa. The WHO estimates that approximately 500,000 people suffer from HAT and existing therapies are either ineffective or toxic. Anacor Pharmaceuticals, Inc has been developing small molecule, boron-containing compounds with anti-microbial activities. A novel compound, AN2920, is part of a series of boron-containing molecules with activity against *T. brucei*. AN2920 demonstrates *in vitro* efficacy against *T. brucei brucei* ( $IC_{50}$  = 0.41  $\mu$ M) and *T. b. rhodesiense* ( $IC_{50}$  = 0.53  $\mu$ M). No cytotoxicity was observed at 24 hr when assayed using murine L929 fibroblasts. In a 72-hr L929 cytotoxicity assay the  $IC_{50}$  = 30.3  $\mu$ M. No significant metabolism was observed by mouse liver microsomes and the half life of the compound, tested for 30 min at 1  $\mu$ M in the presence of microsomes, was >350 min. To test *in vivo* efficacy of AN2920 in a mouse model, animals were infected with the laboratory strain of *T. b. brucei* and treated intraperitoneally (IP) for 5 days with 100 mg/kg of AN2920 twice per day (BID). After 4 weeks, 100% survival without parasitemia was observed. Efficacy was also observed against *T. b. gambiense* using this dosing regimen. AN2920 was retested against *T. b. brucei* at 20 mg/kg BID, dosing orally and IP. After 30 days, 33% survival was observed for both dosing routes. AN2920 extended the lives of uncured animals by 2-3 fold beyond untreated controls. Taken together, these results suggest that boron-containing small molecules may be novel chemical entities for treatment of HAT.

## 1206

**SCREENING FDA APPROVED DRUGS FOR ACTIVITY AGAINST *TRYpanosoma cruzi*: LOOKING FOR COMBINATION CHEMOTHERAPY FOR CHAGAS DISEASE**

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*Trypanosoma cruzi* infection remains an important public health problem in Latin America with more than 12 million individuals chronically infected. Current therapy for Chagas disease consists of nifurtimox or benznidazole; both drugs are poorly tolerated and are not fully effective in the chronic stage of the infection. The cost of new drug development is a major impediment to bringing new chemical entities through preclinical and clinical testing for use against neglected diseases. Thus, we are testing FDA approved drugs for anti-*T. cruzi* activity, alone and in combinations. A semi-high throughput screening method was employed using mammalian stage *T. cruzi* grown in murine fibroblast cells with parasite growth quantified by  $\beta$ -galactosidase reporter activity, as reported previously. The Microsource Spectrum collection of 2000 compounds (including >500 FDA approved drugs) was screened in duplicate at a single concentration of 10  $\mu$ M. First pass hits included 356 compounds (17.8%) that inhibited growth by >75%. After excluding compounds that were not drug candidates (alkylating agents, topical drugs, etc.) and were non-toxic to mammalian cells at 10  $\mu$ M, we had a list of 148 compounds (7.4%). Additional testing showed most of these compounds with  $IC_{50}$  activity in the range of 1-10  $\mu$ M and five compounds with submicromolar activity. The active compounds ( $IC_{50}$  < 10  $\mu$ M) fell into a variety of classes including:

antihistamines, selective serotonin reuptake inhibitors, benzodiazepines, tricyclics, and antibiotics. The lab is currently testing combinations of these compounds (with each other and with established anti-*T. cruzi* inhibitors) to search for synergistic combinations. Active combinations will be studied in the murine Chagas disease model to validate the approach of using off-the-shelf compounds for combating a neglected parasitic disease.

## 1207

### ANTILEISHMANIAL ACTIVITY OF SELECTED FDA-APPROVED DRUGS IN A MURINE CUTANEOUS LEISHMANIASIS MODEL

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Current therapeutic options licensed in the U.S. for cutaneous leishmaniasis (CL) are extremely limited. Current intravenous therapies, such as sodium stibogluconate have considerable associated toxicities, and are suboptimal means of treating a self-limited skin disease, albeit a potentially debilitating one. Oral azoles have shown modest efficacy in limited settings. The limited local therapies available are generally suitable only for uncomplicated lesions. There is a need for a safe oral drug for CL. We recently presented a large scale effort to screen already FDA-approved drugs for *in vitro* activity against *Leishmania major*, using infected macrophages. To date 1100 drugs have been screened *in vitro*. We established an *L. major*-infected BALB/c screening model to test drugs with potent *in vivo* activity ( $IC_{50}$  less than 10 mcg/mL). Candidate drugs were first subjected to a rigorous decision matrix to determine suitability for 2-4 weeks of continuous oral therapy, favorable pharmacokinetics, and prior testing *in vivo* or in humans. Drug screening is currently ongoing. We will report on the results of the top 5-10 candidate drugs in the *in vivo* mouse model. Most of the active substances belong to categories of fungicides, antibiotics, anti-asthmatics, antiprotozoals and antidepressants. The intent of our strategy is to accelerate the process of antileishmanial drug development with reduced cost and shortened timelines.

## 1208

### ROLE OF RED CELL COMPLEMENT REGULATORY PROTEINS IN ERYTROPHAGOCYTOSIS DURING *PLASMODIUM CHABAUDI* INFECTION

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*Plasmodium falciparum* malaria accounts for 1-2 million deaths per year, with the majority due to complications such as severe anemia. The pathogenesis of this anemia is not completely understood and cannot be explained solely by the direct destruction of red cells by the parasite. Red cells of children with severe anemia and malaria are deficient in the complement regulatory proteins (CRPs) decay accelerating factor (DAF/CD55) and complement receptor 1 (CR1/CD35). In order to understand the significance of these deficiencies we studied the role of the mouse complement receptor 1 related protein Y (Crry) in red cell protection during infection with *P. chabaudi*. We hypothesized that Crry heterozygous knockout mice (Crry<sup>+/−</sup>) infected with *P. chabaudi* would have more severe anemia than wild-type mice. There were no differences in anemia between knockout and wild-type animals due to compensatory extramedullary hematopoiesis. However, *P. chabaudi*-infected Crry<sup>+/−</sup> mice showed increased erytrophagocytosis compared to wild-type animals, suggesting that complement activation is an important mechanism in this phenomenon. Erytrophagocytosis of uninfected red cells may be important in the development of anemia, as it is a common finding in tissues of malaria-infected patients and animals. Therefore, we are investigating the role of complement in erytrophagocytosis by quantitating C3 deposition on red cells using immunohistochemistry and

by use of complement inhibitors to reverse erytrophagocytosis. Results of these studies will reveal potential therapeutic strategies to diminish uninfected red cell destruction during malaria infection in humans.

## 1209

### ATP DEPLETION OF RED BLOOD CELLS RECAPITULATES THE PHENOTYPE ASSOCIATED WITH PYRUVATE KINASE DEFICIENCY AND PROTECTS AGAINST *PLASMODIUM FALCIPARUM* MALARIA

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The protective effect of pyruvate kinase deficient (PKD) erythrocytes in *Plasmodium falciparum* infection has been demonstrated. There is inhibition of merozoite invasion into PKD erythrocytes, as well as increased phagocytosis of PKD erythrocytes infected with early stages of *P. falciparum* infection. PK deficiency arises from a number of different mutations in the *PKLR* gene leading to impaired enzyme activity. In agreement with previous reports, we found reduced levels of ATP in PKD homozygous erythrocytes compared to normal erythrocytes (31±14%), as well as in PKD heterozygous erythrocytes (64±7%). The inhibition of glycolysis at the level of enolase by fluoride has been used as a model system for inherited erythrocyte pyruvate kinase deficiency. Using normal erythrocytes treated with sodium fluoride at different concentrations, we show that there is a correlation between ATP levels and inhibition of parasite invasion and enhanced phagocytosis of ring-forms. We further observed increased levels of ATP in parasitized PKD erythrocytes and fluoride treated erythrocytes compared to the parasitized normal, G6PD deficient and β-thalassemia erythrocytes. These data suggest that the chemical conversion of normal erythrocytes to reduced-level-ATP erythrocytes will facilitate the investigation of the mechanism of protection of PKD erythrocytes against *P. falciparum*.

## 1210

### AFM STUDY OF THE EXTRACELLULAR AND THE CYTOPLASMIC SURFACES OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTE MEMBRANES

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Infection of human erythrocytes by the protozoan *Plasmodium falciparum* results in dramatic morphological and functional changes of host cells. During the process of maturation, parasites export parasite-expressed proteins, such as PfEMP1 and KHARP, to the host cell membrane thus forming knobs on the host cell surface and thereby stiffening the membrane and causing cytoadherence (cell stickiness) to occur. To investigate the formation of knobs as well as the relationship between knobs and the host cell cytoskeleton, atomic force microscopy (AFM) was used to study both the extracellular and the cytoplasmic surfaces of infected erythrocyte membranes. Although the cytoskeletal structure can be observed from both the extracellular surface and cytoplasmic surface, the AFM images of cytoplasmic surface uncovered more details of the spectrin network. Knobs and their connections or linkages to the spectrin network were clearly observed from the cytoplasmic surface of infected erythrocytes. The size and distribution of knobs viewed from the cytoplasmic surface were similar to those observed from the extracellular surface. While the spectrin network seems quite intact during the trophozoite stage, some breakages of the cytoskeleton are detected at the schizont stage. Furthermore, some internal structures such as transport vesicles, parasite-generated membrane system as well as parasites at the trophozoite stage were also imaged using AFM. Finally, numerous

super-sticky submicrometer crystals were also observed to adhere to the inner surface of the membrane at the schizont stage. This study may help to further understand the internal changes undergone by the host erythrocytes during parasite maturation.

## 1211

### IDENTIFICATION OF A NOVEL FAMILY OF VARIANT SURFACE ANTIGENS IN *PLASMODIUM FALCIPARUM*

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*Plasmodium falciparum* variably expressed surface antigens (VSA) have been proposed as an escape mechanism from the host immune response giving rise to persistent infections in humans. With a portion of the genome still uncharacterized, we sought a means to identify novel VSA that might play a role in pathogenesis. We hypothesized that nucleotide diversity, variant expression, and presence of the Pexel motif could be used to filter the genome into a testable set of candidate VSA. We identified over 93,000 high confidence SNPs across the genome. Most genes demonstrate low pairwise nucleotide diversity ( $\pi$ ), with 85% of the genome having a  $\pi$  value less than  $2.0 \times 10^{-3}$ . To identify novel antigens, we focused on the top 5% of highly diverse genes and further evaluated the 3D7 transcriptome and a set of five patient transcriptomes to identify variably expressed genes. To discriminate genes that are exported to the surface of the infected red blood cell, we also factored the presence of the Pexel motif into our analysis. Candidates that fulfilled our filter criteria could be divided into two groups: a large number of known antigens, and a handful of uncharacterized genes. One small paralogous gene family demonstrated significantly higher nucleotide diversity than other Pexel containing genes, which is consistent with the prediction that they represent a novel family of VSAs. Steady-state transcriptome analysis indicates that these genes are expressed across different parasite lines and are generally up-regulated *in vivo*. To test their antigenicity, we have expressed recombinant protein from these genes and tested their reactivity with antibodies in patient plasma samples from immune patient volunteers from Senegal. Preliminary analysis shows that these proteins are variably recognized in different immune patient samples, supporting our hypothesis that they are variant antigens. We are also determining the cellular localization of these gene products. Nucleotide diversity, along with other bioinformatic parameters, represent a powerful tool for identifying novel genes involved in pathogenesis and predict new targets for vaccine development in various infectious diseases.

## 1212

### CHARACTERIZATION OF NATURALLY ACQUIRED ANTIBODIES TO PFRH DOMAINS AND DETERMINATION OF THEIR FUNCTIONAL INHIBITORY ACTIVITY

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Field studies conducted in malaria-endemic areas contribute to our understanding of naturally acquired immunity to malaria and also aid in identifying potential candidate molecules to select for a vaccine. The invasion of erythrocytes by *Plasmodium falciparum* occurs through multiple receptor-ligand interactions. Members of the PfRh protein

family play a critical role in directing *P. falciparum* parasites to alternative receptors for invasion. These proteins localize to the merozoite surface and are exposed to the blood stream during the process of invasion; however, they remain unstudied for their contribution to the humoral immune response against *Plasmodium*. This study investigates whether plasma from malaria infected individuals in Senegal contains antibodies against domains within the PfRh proteins but also assesses the inhibitory activity of these antibodies with respect to invasion. Sera were collected from malaria infected patients from different areas in Senegal with different endemicities over a period of 4 years (n= 539). Total IgG to recombinant antigens representing the unique domains of the PfRh paralogs, PfRh1, PfRh2a, PfRh2b and PfRh4, were determined by ELISA. Immune reactivity in this population to PfRh2a and PfRh2b was significantly greater (40.8% and 16.1% respectively) compared to PfRh4 and PfRh1 (5.1% and 3.4% respectively). Of positive IgG responses, we have determined IgG subclass and find that IgG1 and IgG3 are predominant. We are determining associations between IgG subclass and age, parasite density, seasonally and sequence polymorphisms. We have also determined immune responses to specific C-terminal regions that distinguish PfRh2a and PfRh2b. In addition to IgG titer we also address the potential inhibitory activity of these antibodies using *P. falciparum* knockout strains lacking PfRh ligands. We are currently performing invasion assays using these parasite lines in the presence of purified IgG in which non-specific inhibitory factors had been removed. The presence of a humoral response to the PfRh proteins together with invasion inhibitory potential will validate these proteins as potential vaccine candidate antigens.

## 1213

### GENOTYPIC DIFFERENCES IN *PLASMODIUM FALCIPARUM* FROM DIFFERENT MALARIAL DISEASE STATES IN CHILDREN FROM UGANDA

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*Plasmodium falciparum* infection leads to widely different clinical conditions in children ranging from cerebral malaria (CM), severe malarial anemia (SMA), uncomplicated malaria (UM) and asymptomatic parasitemia (AP). Studies on parasite and human genetics may help in determining the molecular basis of the diversity of clinical outcomes. We used polymorphic merozoite surface protein 1 and 2 (MSP-1 and 2) and glutamate rich protein (GLURP) DNA markers to genotype *P. falciparum* parasites collected from children with CM, UM and AP from Uganda. In total 94, 88 and 65 samples from children with CM, UM and AP respectively were assayed. Differences in the frequencies of parasite genotypes of *P. falciparum* parasites between CM, UM and AP were determined by  $\chi^2$  tests. Frequencies of one or more alleles from the MSP-1 RO33 and MAD20, MSP-2 FC27 or GLURP allelic families did not differ between children with CM, UM or AP. Children with CM or UM were more likely to have one or more MSP-1 K1 alleles than children with AP (97.8 % vs 83.1%, P = 0.002, and 98.9% vs 83.1%, P = 0.005, respectively), but children with CM and UM did not differ in MSP-1 K1 allele frequency. Children with CM were also more likely to have one or more MSP2-3D7 alleles than children with AP (92.2% vs 75.4%, P = 0.03), but in addition, children with CM were more likely to have one or more MSP-2 3D7 alleles than children with UM (92.6% vs 80.7%, P = 0.02). These study results suggest that MSP2-3D7 genotypes may be overrepresented in children with CM as compared to uncomplicated malaria or asymptomatic parasitemia. This may relate to functional differences conferred by this genotype or to the association of this genotype with an unrelated genetic factors. Analysis with microsatellite markers may allow further characterization of strains associated with increased disease severity. Insight into the structural and functional diversity of genes associated with virulence could reveal new strategies for intervention of malarial disease.

## 1214

**ABO POLYMORPHISM AND PLASMODIUM FALCIPARUM MALARIA**

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Malaria has been a major selective force on red blood cell (RBC) polymorphisms that confer protection to severe disease. Several lines of evidence suggest that the outcome of *Plasmodium falciparum* infection may also be influenced by ABO blood group antigens. Blood type O predominates in malaria endemic regions and has been associated with protection from developing severe and complicated malaria. Although the molecular details of protection has not been fully elucidated, previous studies have demonstrated reduced rosetting in type O RBCs. Based on observations showing enhanced phagocytosis of infected RBCs occurs with other RBC polymorphisms associated with protection, we hypothesized that infected type O RBCs may be more efficiently cleared by the innate immune clearance than type A RBCs. Here we show that primary human macrophages phagocytosed *P. falciparum*-infected type O RBCs more avidly than infected type A RBCs ( $p<0.001$ ). Furthermore, that hemichrome deposition in infected type O RBCs is significantly greater than in infected type A RBCs ( $p<0.05$ ), which may account for enhanced recognition and phagocytosis of type O infected RBCs. Collectively our data suggest that type O individuals may have more proficient clearance of infected RBCs contributing to an overall decrease in parasite burden and a reduction of the number of infected erythrocytes available to bind within the microvascular beds of vital organs. This represents an additional putative mechanism by which blood type O may contribute to protection against severe malaria.

## 1215

**MOLECULAR CHARACTERISATION OF PYRETHROID RESISTANCE IN ANOPHELES FUNESTUS, MALARIA VECTOR IN AFRICA**

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A major QTL *rp1* conferring pyrethroid resistance to the malaria vector *Anopheles funestus*, was previously identified. Here we present a fine-scale mapping of *rp1*, the identification and characterisation of the genes conferring this resistance. 650 F6 and F8 individuals from reciprocal crosses between susceptible and resistant strains were genotyped with SNPs and microsatellite markers for QTL mapping. A BAC clone containing *rp1* was sequenced and annotated. Quantitative PCR were carried out to study the expression pattern of the P450s genes and the *in vitro* interaction of the genes differentially expressed with pyrethroids was assessed. *rp1* was the major QTL explaining 85% of the genetic variance to pyrethroid resistance. Two other QTLs of minor effect *rp2* and *rp3* were detected. Fifteen genes were identified in the 120kb BAC clone containing the *rp1* QTL with a cluster of 10 P450 genes among which CYP6P9 and CYP6P4 were duplicated. These two genes were significantly differentially expressed between susceptible and resistant strains. Enzymes from these genes metabolise pyrethroid *in vitro*. Specific mutations associated with

resistance were identified in CYP6P9 and CYP6P4. For each gene, two A/G SNPs were identified and genotyped for over 650 specimens. The G/G genotypes confer resistance at 100% and these could be used to design of a diagnostic assay to detect this metabolic resistance. In conclusion, CYP6P9 and CYP6P4 are the main genes conferring pyrethroid resistance in the laboratory strain FUMOZ-R. Further studies will be carried out to estimate their contribution in the pyrethroid resistance in field populations.

## 1216

**TOXICITY OF HIGHLY SELECTIVE CARBAMATES TOWARDS THE MALARIA MOSQUITO, *ANOPHELES GAMBIAE***

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Insecticide-treated bednets (ITNs) are an important tool for the management of *Anopheles gambiae*, the major vector of malaria in Africa. Pyrethroids are the only insecticides approved for bednet treatments; however, widespread resistance and lack of alternative chemicals undermine the use of ITNs for mosquito control. Our research focus is to develop highly selective insecticides with high mosquito toxicity and low mammalian toxicity that might be used in parallel with current-use pyrethroids. We report the re-engineering of carbamate insecticides to increase selectivity and mitigate resistance development in *An. gambiae*. Based on mosquito acetylcholinesterase (AChE) protein homology modeling, we have synthesized new carbamates that are highly selective to *An. gambiae* AChE. Anticholinesterase activities of each carbamate were evaluated for both human and mosquito AChEs and compared to those of propoxur (WHO standard for mosquito control), and other conventional carbamate insecticides. We demonstrate novel carbamates of greater selectivity (ca. > 8000-fold) towards *An. gambiae* AChE, compared to 3-fold selectivity with propoxur. The new carbamates have increased potency towards mosquitoes (ca. 60-fold) than that of propoxur. We confirm both intrinsic and contact mosquito toxicity of these carbamates and demonstrate comparable toxicities to that of propoxur, and other conventional carbamates. With such high levels of selectivity, potency and toxicity, these novel carbamates provide valuable leads to developing of alternative mosquitocides for use in insecticide treated bednets and indoor residual sprays. Our findings are important in the search for new mosquito selective-insecticides and the possible use of these carbamates in malaria control programs will be discussed.

## 1217

**COMBINING ORGANOPHOSPHATES AND REPELLENTS ON FABRICS: A PROMISING STRATEGY TO BETTER CONTROL PYRETHROID RESISTANT MOSQUITOES**

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With the spread of pyrethroid resistance in most mosquito vector species and the lack of alternative compounds for public health, the search for new strategies that provide better control of resistant populations has become a priority. A new concept was developed in the laboratory by mixing repellents and non pyrethroid insecticides. Here, this concept was studied for personal and community protection under field conditions in Benin and Burkina Faso, West Africa. Indeed we studied the efficacy of battle-dress and bed nets impregnated with organophosphate(PM)/repellent(DEET or KBR) mixtures, respectively against *Aedes aegypti*, the main dengue and yellow fever vector and *Anopheles gambiae*, the main

malaria vector. First, KBR and PM+KBR impregnated battle-dress allowed better protection against *Ae. aegypti* bites than permethrin impregnated battle-dress. Secondly, results showed evidence of synergism between repellents (DEET or KBR) and pyrimiphos-methyl (PM) on nets in field conditions. PM+DEET and PM+KBR treated nets were as effective as a standard pyrethroid (deltamethrin 25mg/m<sup>2</sup>) against susceptible *An. gambiae* populations and more effective against resistant *An. gambiae* populations. Results also demonstrated that mixtures did select neither *Kdr* allele nor *AcE1<sup>R</sup>* allele. In conclusion, these field trials showed that mixing repellents and organophosphates has the potential to be a good alternative strategy to manage the spread of resistance. However, significant improvements remain to be done to improve residual effect of Insecticide-Repellent Treated fabrics.

## 1218

### DEVELOPMENT OF A NOVEL FORMULATION FOR USE IN INDOOR RESIDUAL SPRAY PROGRAMS

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Fenitrothion wettable powder (WP) is recommended by the World Health Organization (WHO) for Indoor Residual Spraying (IRS) against malaria vectors. However, with the increase in the use of Long Lasting Insecticidal Nets (LLINs) as a low cost and highly effective intervention, IRS has more recently been used in many parts of Africa as a secondary treatment option, or for use in epidemic zones. In some instances, particularly in highly malarious areas, the use of LLINs and IRS together can give very dramatic reductions in incidence. With the focus now turning towards the elimination or eradication rather than control of malaria, the combination of several vector control interventions combined with the administration of effective anti-malarial drugs will become the norm as countries step up their efforts to eliminate this parasite. There is an increasing concern over the development of resistance to pyrethroids, possibly affecting LLIN performance, which are currently all dependent on this insecticide class. To minimize selection pressure the use of pyrethroid-based IRS products is not recommended with LLIN applications. Alternatives to the widespread use of pyrethroid and DDT-based IRS products are clearly needed (*kdr* resistant insects share a common resistance mechanism to DDT and pyrethroids). To meet this need, a novel Sumithion® IRS formulation is being developed. Laboratory trials to evaluate residual efficacy on a range of representative substrate types against *Anopheles* mosquitoes have been conducted. This data, along with interim results of Phase II hut studies being conducted in Benin are presented.

## 1219

### HUMAN ANTIBODY RESPONSE TO *ANOPHELES GAMBIAE* SALIVA: A NEW IMMUNO-EPIDEMIOLOGICAL MARKER TO EVALUATE THE EFFECTIVENESS OF INSECTICIDES TREATED NETS (ITNS)?

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In a way to improve malaria control, many efforts are conducted under WHO recommendations to develop new tool/indicator for malaria control, such as for evaluating the anti-vector strategies. Previous studies

have shown that the evaluation of human antibody (Ab) response to arthropod salivary proteins represent an epidemiological indicator of exposure to vector bites, and especially our team demonstrated that IgG response to whole saliva of *Anopheles gambiae* in exposed individuals represent a marker of the intensity of *Anopheles* exposure. The objective of the present study was to validate whether this immunological marker based on human anti-saliva IgG Ab levels could be one new indicator to evaluate the effectiveness of ITNs use in malaria control programs. One longitudinal study, concerning individuals (n=108, children and adults) living in malaria endemic area in Angola, was performed from March 2005 to October 2006. The studied cohort was followed for parasitological, clinical, entomological and immunological data, each 6 weeks before and after the well-controlled use of Permanet® mosquito nets (Long Lasting Insecticide Net; installation in Feb. 2006). Seasonal variations of anti-saliva IgG Ab levels to *An. gambiae* saliva were observed before and after the installation of ITNs which appeared to be associated with the exposure to *An. gambiae* (evaluated by the classical entomological methods) and the prevalence/intensity of malaria infection. Moreover, a significant decrease of the anti-saliva IgG response was observed after the ITNs use which was correlated with the decrease of malaria parasitemia, the current and referent criteria showing the effectiveness of these ITNs. In a way to identify new tools for malaria control, we have shown that anti-saliva IgG response in exposed individuals could be not only an immuno-epidemiological marker of exposure to *An. gambiae* bites, but also a potential indicator for evaluating the ITNs effectiveness. Several future studies are needed to confirm this hypothesis in other transmission areas and to identify some immunogenic salivary proteins as higher specific markers. Nevertheless, this study represents a first approach to elaborate such new indicators for evaluating the effectiveness of anti-vector strategies, bases on the evaluation of human Ab response to salivary proteins of arthropod vectors.

## 1220

### EFFICACY OF INSECTICIDE TREATED MATERIALS (ITMS) FOR DENGUE CONTROL IN LATIN AMERICA AND ASIA: CLUSTER RANDOMIZED CONTROLLED TRIALS IN VENEZUELA AND THAILAND

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Dengue fever is the fastest spreading arboviral disease worldwide. In the absence of a vaccine, *Aedes aegypti* vector control remains the most effective strategy to prevent dengue transmission. Our initial studies in Latin America indicated that insecticide treated materials (ITMs) can impact on dengue vector populations and potentially on dengue virus transmission. Cluster randomized trials are underway in Venezuela (6000 households in 75 clusters) and Thailand (2000 households in 26 clusters) to further clarify the efficacy of ITMs in suppressing dengue vector populations. These trials incorporate several advances on the earlier studies: first, different types of ITMs are being tested alone and in combination and householders may choose the manner of deployment; secondly, spill-over effects of the interventions into neighboring control areas are monitored by including external control sites; thirdly, efficacy of ITMs for dengue vector control is measured on a large scale for the first time in SE Asia. Both study sites had high entomological indices at baseline (Venezuela average pupae per person index = 0.52, average Breteau index = 15.3; Thailand average pupae per person index = 0.22, average Breteau index = 22.4), and the ITM interventions were adopted and maintained by the population in both sites to a similar extent, although their manner of deployment varied (Venezuela: window curtains and jar covers; Thailand: indoor and window curtains). Although the trials are set to complete in early 2009, preliminary data and analyses will be presented and important

differences affecting the potential applicability of ITM use in Venezuela and Thailand will be discussed.

## 1221

### REDUCED EFFICACY OF PYRETHROID SPACE SPRAYS FOR DENGUE CONTROL IN PYRETHROID RESISTANCE AREA (MARTINIQUE)

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The last 30 years saw a dramatic resurgence of several infectious diseases like Dengue fever and Chikungunya causing major public health problems. Unfortunately, vector control remains extremely difficult to implement because it requires a large budget, skilled staff, commitment, and active community participation. To reduce the infection rate during epidemics, space spraying is the only solution for adult mosquito control. In the Caribbean, insecticide resistance is widely developed in *Aedes aegypti* and may represent a serious obstacle for dengue vector control. In this context, the efficacy of pyrethroid and organophosphate ULV-space sprays was investigated in Martinique (French West Indies) where *Ae. aegypti* previously shown to be resistant to conventional insecticides. WHO cylinder tests showed high level of resistance of a wild-field caught population of *Ae. Aegypti* to deltamethrin ( $RR_{95} = 68$ ) and, in a lesser extend, to pyrethrum ( $RR_{95} = 14$ ) and naled ( $RR_{95} = 12$ ) compared to the susceptible reference strain. A simulated field trial implemented in this locality showed that this resistance can strongly reduce the knock-down effect and mortality of deltamethrin (1 g/ha) and synergized pyrethrins (10 g/ha) applied by thermal fogging. The mortality rates of all pyrethroids were below 60% at 20 m and then dropped below 30% at 30 m. Conversely, the efficacy of naled (114 g/ha) was high against both susceptible and resistant mosquitoes, i.e. mortality and KD effect were above 75% until 50 m. This finding has important implications for dengue vector control and emphasizes the need to develop innovative tools and strategies to maintain effective control of multi resistant *Ae. aegypti* populations.

## 1222

### FATAL OUTBREAK FROM CONSUMING XANTHUM STRUMARIUM SEEDLINGS DURING TIME OF FOOD SCARCITY IN NORTHEASTERN BANGLADESH

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In November 2007 a cluster of deaths was identified at a government hospital in northeastern Bangladesh. Patients presented with unconsciousness, elevated liver enzymes, and a history of repeated vomiting and restlessness. We investigated this outbreak in order to describe the clinical syndrome and determine etiology and risk factors for disease. We defined suspect cases as those experiencing vomiting and probable cases as vomiting and altered mental status in the outbreak areas from 2 to 11 November. We identified cases at hospitals and by house-to-house visits. In-depth interviews detailed illness histories and generated hypotheses about the etiology of disease. We conducted a cohort study in two villages to investigate risk factors for developing vomiting and unconsciousness, with a focus on foods consumed. In total, 81 patients were identified from 11 villages; 24% (19/81)

died. Cases resided in remote areas and were poor; many villagers reported eating only two meals per day. In-depth interviews with 33 cases showed that 31 (94%) had consumed *ghagra shak*, or cocklebur (*Xanthium strumarium*) in the hours before illness onset. Mature *ghagra shak* plants are usually consumed in these villages in small amounts to flavor foods or for medicinal purposes. However, due to severe and late flooding in 2007, villagers relied more upon uncultivated foods such as *ghagra shak*, and consumed very young plants. Persons who ate this plant were 28.9 (95% CI 9.2 to 90.8,  $P < 0.001$ ) times more likely than others to develop vomiting and unconsciousness during the outbreak. Consuming *X. strumarium* has caused similar illness and death in livestock and children in other parts of the world. The toxic agent in the plant is carboxyatractylone, which is found in the seeds and seedlings. Messages advising against consuming seedlings should be communicated broadly across the country and communities at risk for food scarcity should be targeted for food relief. This outbreak provides further example of how poverty and the lack of food security imperils lives.

## 1223

### EFFECT OF READY-TO-USE-THERAPEUTIC FOOD SUPPLEMENTATION ON THE NUTRITIONAL STATUS, MORTALITY AND MORBIDITY OF CHILDREN 6 TO 60 MONTHS IN NIGER: A CLUSTER RANDOMIZED TRIAL

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Ready-to-use-therapeutic foods (RUTF) are becoming an important component of the effective outpatient treatment of severe wasting. Their utility for prevention of wasting, however, has not been evaluated. Further, some findings of adverse health effects due to iron and folic acid supplementation suggest that iron supplementation in settings where the prevalence of malaria and other infectious diseases is high should be proceeded with cautiously. We evaluate the effect of a 3-month preventative supplementary feeding using RUTF on the nutritional status, mortality and morbidity of children 6 to 60 months. A cluster randomized trial of 12 villages in Maradi, Niger. Six villages were randomized to intervention and 6 to no intervention. Villages were visited monthly from August 2006 to March 2007. All children in the study villages between 6 and 60 mo of age were eligible for recruitment. The monthly distribution consisted of one packet per day of RUTF (PlumpyNut®, 500kcal/day) to each eligible child with weight-for-height  $\geq 80\%$  of the NCHS reference median from August to October 2006. Our main outcome was change in weight-for-height Z (WHZ) score according to the WHO Standards and incidence of wasting ( $WHZ < -2$ ) over 8 months of follow up. The adjusted overall effect of the intervention on WHZ change was 0.18 Z (95% CI: 0.09, 0.27) over 8 mo. This effect was strongest in children 24 mo or younger at baseline. The intervention resulted in a 36% (95% CI: 20% - 49%) reduction in the incidence of wasting and a 57% (95% CI: 43% - 68%) reduction in the incidence of severe wasting. There was no evidence of increased risk of malaria associated with RUTF supplementation. There was a non-significant 49% reduction in mortality associated with the intervention. In conclusion, in a setting of acute food insecurity, short-term preventative supplementation with RUTF reduced the decline in WHZ and incidence of wasting and severe wasting. This study suggests that this product fortified with 11.5 mg of iron/100g did not aggravate malaria but further research is needed.

**1224****PATHOGENESIS OF HAEMORRHAGE ASSOCIATED WITH DENGUE INFECTION IN ADULTS IN VIETNAM**

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The number of adults with severe dengue disease continues to increase in South-East Asia, as well as in South Asia and Latin American countries. Bleeding manifestations and severe liver involvement appear to be more common in adults than in children and may cause death. To date the pathogenesis of bleeding in dengue infections remains poorly understood, and there is little formal data comparing adult and paediatric patterns of disease. We present data from a prospective study of more than 600 adults representing the full spectrum of dengue disease admitted to a single hospital in Vietnam in 2006-7. Clinical and basic laboratory features will be described, with particular reference to bleeding manifestations and coagulation tests/platelet abnormalities, documented carefully throughout the evolution of the disease, and compared with similar observations from a group of children admitted to the hospital during the same dengue season. In addition to thrombocytopenia, an increase in activated partial thromboplastin time (APTT) and a reduction in fibrinogen levels were the two most consistent abnormalities detected, although typical DIC was unusual. Heparan sulfate (HS), a major constituent of the endothelial surface glycocalyx layer that is a known receptor for the dengue virus, is closely related to the therapeutic anticoagulant agent, heparin, and could function in a similar way to increase the APTT if released into the circulation. HS levels were measured in a subgroup of 200 of the adult dengue patients, and found to be markedly elevated; we will present data correlating HS levels with clinical severity and with the APTT derangement in support of this hypothesis. Secondly *in vivo* mammalian studies of intrinsic permeability indicate that despite its large size fibrinogen leaks from the microvasculature at a similar rate to the much smaller albumin molecule. Albumin leakage increases dramatically in patients with dengue shock syndrome. Using immuno-histochemistry we demonstrate interstitial leakage of fibrinogen in a series of 15 skin biopsies taken from among the adults with DSS, suggesting that leakage rather than consumption accounts for the low fibrinogen levels noted.

**1225****IMPACT OF MASS AZITHROMYCIN TREATMENT ON THE PREVALENCE OF ACTIVE TRACHOMA AND OCULAR CHLAMYDIA TRACHOMATIS IN THE GAMBIA**

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Trachoma, caused by ocular serovars of *Chlamydia trachomatis* (CT), is the leading infectious cause of blindness. Antibiotic treatment is part of the WHO control strategy and The Gambia has qualified for a donation of azithromycin by Pfizer. We report the prevalence of active trachoma and ocular CT before and one year after mass azithromycin treatment in The Gambia. At both baseline and follow-up, children aged 0-9 years in 6 villages were screened for trachoma clinical signs according to the WHO simplified grading system. Ocular specimens were taken from each child's right eye and processed by Amplicor PCR for the second-collected swab of baseline samples, and the first-collected swab of follow-up samples.

Mass treatment was conducted after baseline screening. At baseline 280/1171 children (23.9%) had active trachoma and 35 (3.0%) were PCR positive. At follow-up 207/1175 children (17.6%) had active trachoma and 45 (3.8%) were PCR positive. After treatment the prevalence of active trachoma decreased in all villages although in one village the difference was insignificant ( $p=0.952$ ). There was no evidence of CT infection in 2 villages at follow-up, and only one PCR positive case in 2 other villages. However, 2 villages had higher CT prevalence at follow-up than at baseline. WHO policy is to mass treat annually for 3 years any community where the prevalence of active trachoma in children aged 1-9 years is  $\geq 10\%$ . At baseline, all 6 villages qualified for mass treatment and at follow-up all but one village still had  $\geq 10\%$  prevalence. However, CT prevalence was low indicating that these villages may be unnecessarily receiving treatment, thus wasting scarce resources. The higher CT prevalence at follow-up than at baseline may be explained by low treatment coverage (51.0%) in one village, but not in the other (93.6%). Another explanation is that of cross-border re-infection from Senegal, as has previously been reported. Antibiotic treatment should therefore be conducted over a wide geographical area to limit re-infection from occurring. Investment in the long-term "F" and "E" components of the SAFE strategy is also important if trachoma elimination is to be maintained in The Gambia.

**1226****EXTRA-HEPATIC CYSTIC HYDATID DISEASE: A DIAGNOSTIC DILEMMA?**

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Cystic hydatid disease (CHD), a zoonosis due to the metacestode of the canine tapeworm *Echinococcus granulosus* is endemic in parts of the sheep-rearing areas of the Middle East, including Kuwait. CHD of the liver, with the presenting triad of symptoms of abdominal pain, a palpable mass and jaundice is well documented. However, man being an aberrant host, a variable proportion of the cysts develop extra-hepatically giving rise to non-specific symptoms, resulting in delayed or misdiagnosis. The magnitude of the problem of extra-hepatic cysts, and the consequent difficulties in diagnosis has yet to be defined and may be an underestimate in endemic countries, including Kuwait. We first describe patients presenting with extra-hepatic CHD to show the variety of unusual presentations and the difficulties in laboratory diagnosis. We discuss such cyst/s in the lung in a Saudi female; in the posterior triangle of the neck in a female Kuwaiti and in the breast in another; in the brain and heart in a Syrian male and in the pelvis in a Bangladeshi male. The diagnosis in each was confirmed by serology, presence of scolices and hooks after nucleopore filtration of aspirated fluid and/or histopathology of sectioned cysts. We then show that of a total of 1201 patients, CHD was diagnosed in 198 (16.5%); 155 (78.3%) had cysts in the liver but in 39 (19.7%) they were extra-hepatic. In four patients (2%), cysts were both in the liver and extra-hepatic too. The number of patients with extra-hepatic cysts will vary in each endemic zone depending on the phenotypic and genotypic variability of the parasite. The G1 strain involving dogs/sheep rather than camels/sheep is predominant in this endemic area influencing the site of location of hydatid cysts in man. We elaborate on the mode of transmission which also influences the target organ in each geographic area. Indeed our data may not reflect the true magnitude of the problem as a whole body scan was not performed either on those with hepatic or extra-hepatic cyst. Even with the advent of imaging studies, the availability of serology and the increased use of fine-needle aspiration cytology, we

show that diagnosis of extra-hepatic CHD is fraught with difficulties. Such data are essential for the attending physician to make an informed judgment and to differentiate CHD from masses like tumours, congenital, simple and other cystic lesions which we encountered and enumerate in our extensive list from this geographic locale.

## 1227

### SEROPREVALENCE OF *STRONGYLOIDES* IN NEWLY ARRIVED IMMIGRANTS AND REFUGEES

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*Strongyloides stercoralis* is an intestinal parasite that is highly endemic in tropical countries with reported seroprevalence ranging in immigrants from 1-75% depending on their country of origin, immigration class, and the diagnostic method used. A large proportion of newly arrived immigrants and refugees may be asymptotically infected with *strongyloides* for life. Such chronic infections put these individuals at risk for disseminated disease (associated with high mortality) and that could be prevented through targeted screening or empiric treatment programs. A total of 1294 foreign-born adults ( $\geq 18$  years), having lived  $\leq 5$  years in Canada were recruited from 2 hospitals and 3 clinics in Montreal, between October 2002 and December 2004. Socio-demographic information was collected via a questionnaire. Antibodies to recombinant *Strongyloides stercoralis* NIE antigen [Optical density (OD)  $<0.35$ =negative,  $\geq 0.35-0.45$ =low positive,  $>0.45-0.75$ =moderately positive,  $>0.75$ =high positive] and antibodies to *Brugia malayi* antigen (OD  $<0.3$ =negative,  $\geq 0.3-0.4$ =low positive,  $>0.4-0.7$ =moderately positive,  $>0.7$ =high positive) were detected by ELISA. The mean age was  $33 \pm 8.8$  years (range 18-76), 66% were female, 49% were refugee or refugee claimants and 47% had a university degree. A large portion of the patient population had antibodies to *strongyloides* 27% (95% CI, 22-32%) and ranged in 6 different geographic regions from 17%-41%. A total of 11% (146/1294) of the study population had positive filaria serology and 19% (67/350) of all patients with positive *strongyloides* serology also had positive filaria serology, suggesting some cross reactivity and/or dual infection. In preliminary multivariate analysis, positive *strongyloides* serology was more common in men than women [OR, 95%CI; 1.8, (1.2-2.1)], in refugees vs immigrants [1.4 (1.01-1.9)], in those with moderately positive filaria serology [3.3 (1.9-5.7)], or high positive filaria serology [2.4 (1.01-5.5)] vs those with negative serology, and within several world regions after adjusting for the variables noted above as well as age and several socioeconomic factors. In conclusion, a large proportion of adult immigrants and refugees in the Montreal area are infected with *strongyloides* and are at risk for disseminated disease. These individuals would likely benefit from targeted screening programs and or empiric treatment.

## 1228

### PHENOTYPIC AND GENOTYPIC EVIDENCE OF EMERGING IVERMECTIN RESISTANCE IN ONCHOCERCIASIS

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Onchocerciasis, commonly known as "River blindness" is a disease affecting over 37 million people, primarily in Africa. Ivermectin, the only drug for mass treatment is showing reduced efficacy to adult *Onchocerca volvulus*, the causative agent of the disease. We have carried out a 21

month longitudinal study, on 301 subjects from 10 Ghanaian communities that have received between 7-20 rounds of IVM treatment, to assess the microfilaricidal effect of ivermectin and its impact on adult female worm reproductive status. Nodules were carried out on 140 subjects three months after the last IVM treatment. Embryograms were constructed on all intact female worms. We observed significant differences in these communities in terms of worm burdens, female worm production of various embryonic stages and production of intra-uterine stretched mf. From this data, we have classified communities into three response groups. Three communities showed poor response, two were moderate responders and five communities, including a previously IVM naïve community, were good responders. B tubulin isotype 1 gene has been shown to be linked to IVM selection in *O. volvulus* and also associated with IVM resistance in veterinary nematodes. We have genotyped the full length genomic DNA for β-tubulin of 284 *O. volvulus* adult worms obtained from all response groups. We observed single nucleotide polymorphisms (SNPs) at 21 sites on the entire 3696 bp gene. Of these, seven occurred in exons, one translating into an amino acid change, while 14 occurred in introns; of particular interest were the changes in the first intron. We observed significance differences ( $P < 0.03$ ) between the three response groups at five SNPs occurring in both exons and introns. The phenotypic and genotypic evidence indicates that IVM resistance is being selected and is manifested as a loss of effect of IVM on suppression of parasite reproduction. B-tubulin may be a useful genetic marker for the selection of IVM resistance.

## 1229

### CO-CULTURE WITH *PLASMODIUM FALCIPARUM*-INFECTED RED BLOOD CELLS INDUCES DIFFERENTIATION OF FUNCTIONALLY COMPETENT REGULATORY T CELLS FROM LYMPHOCYTES OF MALARIA-NAÏVE DONORS

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An important aspect of clinical immunity to malaria is the ability to down-regulate inflammatory responses once parasitaemia is under control, in order to avoid host-mediated pathology. Previously, we found that regulatory T cells (Tregs) are upregulated in response to sporozoite challenge in malaria naïve volunteers, leading to reduced pro-inflammatory responses and enhanced parasite growth. More recently we found that natural exposure to malaria may induce a transient increase in the number of functional Tregs: increased percentages and absolute numbers of CD4<sup>+</sup>Foxp3<sup>+</sup>CD127<sup>-low</sup> T cells were found in individuals living in a rural village with stable malaria transmission than in individuals living in an urban area where malaria rarely occurs. Moreover, in the same rural population, levels of FOXP3 mRNA were higher at the end of the malaria transmission season than at the end of the dry season 6 months later. In the present study we demonstrate that functionally active Tregs can be induced *in vitro*, in a dose dependent manner, by co-culture with *P. falciparum* schizont extract or viable parasitized red blood cells. Depletion of CD25<sup>+</sup> T cells prior to co-culture abrogates this effect suggesting either that natural Tregs are the precursors of the induced population or that their presence is required for induction to take place. Tregs express very high levels of Fas (CD95), and low levels of Bcl2 both ex-vivo and after induction *in vitro*, suggesting they are prone to undergo apoptosis. This would explain the transient increase in this population observed *in vivo* after exposure to malaria. Ongoing studies addressing the role of apoptosis in the fate of malaria-induced Tregs *in vitro* will be presented.

**1230****FUNCTIONAL RELATIONSHIP BETWEEN IL-1 $\beta$  PROMOTER HAPLOTYPES (-31C/T AND -511A/G) AND PEDIATRIC SEVERE MALARIAL ANEMIA**

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Interleukin (IL)-1 is an important inflammatory mediator in *Plasmodium falciparum* infections. Although the inflammatory profile associated with protection against severe malarial anemia (SMA) is largely undefined, increased IL-1 $\beta$  production appears to limit parasitemia. Previous studies showed associations between individual IL-1 $\beta$  promoter variants (-31C/T and -511A/G) and malaria disease severity. To further examine the role of IL-1 $\beta$  promoter variants in conditioning malaria disease outcomes, the relationship between -31C/T and -511A/G haplotypes, SMA (Hb<6.0g/dL), high-density parasitemia (HDP; >10,000 parasites/ $\mu$ L) and circulating IL-1 $\beta$  was investigated in children with acute malaria ( $n=566$ ) residing in a holoendemic *P. falciparum* transmission area. Hematological and parasitological profiles were determined in all study participants. IL-1 $\beta$  -31C/T genotyping was carried out by PCR and Alu restriction enzyme digestion, while -511A/G genotypes were determined using a Taqman 5-allelic discrimination assay. Circulating IL-1 $\beta$  concentrations were determined using the Cytokine 25-plex assay. Frequencies of -31C/-511A, -31C/-511G, -31T/-511A and -31T/-511G haplotypes were 87.2%, 29.1%, 2.1%, and 30.9%, respectively. Multivariate logistic regression analyses controlling for age, gender, sickle-cell trait, HIV-1, and bacteremia revealed that -31C/-511A was associated with increased risk of SMA (OR; 1.98, 95% CI, 1.55-2.27;  $P<0.05$ ) while -31T/-511A was non-significantly associated with protection against SMA (OR; 0.52, 95% CI, 0.18-1.16;  $P=0.11$ ). Consistent with these observations carriage of CA and TA haplotypes was associated with reduced ( $P<0.05$ ) circulating and elevated ( $P<0.05$ ) IL-1 $\beta$  production, respectively. Additionally, IL-1 $\beta$  levels were lower in SMA compared to non-SMA children. These results demonstrate that variation in the IL-1 $\beta$  promoter conditions susceptibility to SMA and functional changes in circulating IL-1 $\beta$  levels.

**1231****INHIBITION OF ANCYLOSTOMA CEYLANICUM MACROPHAGE MIGRATION INHIBITORY FACTOR (ACEMIF): POTENTIAL FOR PREVENTING HOOKWORM-ASSOCIATED IMMUNOMODULATION AND DISEASE PATHOGENESIS**

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Hookworms, parasitic nematodes that infect nearly one billion people worldwide, are a major cause of anemia and malnutrition. We hypothesize that hookworms actively manipulate the host immune response through the elaboration of specific molecules at the host-parasite interface designed to facilitate infection by larval stages and adult worm survival within the small intestine. Full-length cDNAs encoding two orthologs of the human cytokine, Macrophage Migration Inhibitory Factor (MIF) have been cloned from the hookworm *Ancylostoma ceylanicum*. Elucidation of the three dimensional crystal structure of recombinant AceMIF-2 (rAceMIF-2) revealed an overall structural homology with significant differences in the tautomerase sites of the human and hookworm

proteins. The relative bioactivities of human and hookworm MIF were compared using *in vitro* assays of tautomerase activity, monocyte migration, and binding to the MIF receptor, CD74. These data provide evidence that the hookworm-derived AceMIF molecules are bioactive and functional orthologs of human MIF. Vaccination of laboratory animals using purified rAceMIF-2 was associated with partial protection against anemia and growth delay following challenge infection, compared to adjuvant immunized controls. Selective *in vitro* inhibitors of rAceMIF activities were identified using high-throughput screening (HTS) of a small molecule library representing previously defined biologically active compounds. In summary, based on its unique immunological, structural, and functional characteristics, AceMIF is a viable target for novel drug and/or vaccine based strategies for selectively inhibiting these hookworm cytokine orthologs as a means of reducing parasite survival and disease pathogenesis *in vivo*.

**1232****PATENT FILARIAL INFECTION MODULATES MALARIA-SPECIFIC TYPE 1 CYTOKINE RESPONSES IN AN IL-10 DEPENDENT MANNER IN A FILARIA/MALARIA CO-INFECTED POPULATION**

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Human co-infection with malaria and filarial parasites is common in regions of Africa and particularly in Mali where both *Plasmodium falciparum* (Pf) and *Wuchereria bancrofti* (Wb) are transmitted by the same mosquito vector. As filarial infections can modulate responses to bystander antigens, we investigated the effect of filarial infections on malaria-specific immune responses. Blood samples were collected from individuals with Wb and/or *Mansonia perstans* (Mp) infections (Fil+;  $n=19$ ), as determined by a Wb Ag capture ELISA and/or circulating microfilariae (Mf), and those with no evidence of active filarial infections (Fil-;  $n=20$ ) from the same village. Whole blood samples were cultured *in vitro* with Pf-infected red blood cell lysate [MalAg] or *Brugia malayi* adult antigen (BmA) or medium alone for 24 hrs. The supernatants were assayed for IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17A, IP-10, TNF- $\alpha$  and IFN- $\gamma$  by Luminex™. Compared to the Fil- group, Fil+ individuals had significantly higher levels of IL-10 ( $p = 0.027$ ) and IL-17A ( $p = 0.037$ ) produced spontaneously. The Fil+ group also mounted a significantly lower IL-12p70 (GM 1.11 vs. 3.20 pg/ml,  $p=0.022$ ), IFN- $\gamma$  (4.77 vs. 17.32 pg/ml  $p = 0.06$ ) and IP10 (34.46 vs. 261.1 pg/ml  $p = 0.0023$ ) responses following MalAg stimulation but a significantly higher IL-10 response (7541 pg/ml vs. 3198 pg/ml,  $p = 0.022$ ) compared to the Fil- group. In contrast, BmA induced significantly higher levels of IL-2 and IL-4 in Fil- than in Fil+ individuals. To understand the role played by either IL-10 or TGF- $\beta$  in the regulation of Type 1 responses to MalAg in filarial infections, neutralizing antibodies to IL-10 and/or TGF- $\beta$  were utilized *in vitro*. Whereas, anti-TGF- $\beta$  had little effect on preventing the MalAg specific downregulation, anti-IL10 antibodies induced a significant reversal of IL-12p70, IFN- $\gamma$ , and IP10 ( $p<0.001$ ). Blocking both IL-10 and TGF- $\beta$  together did not augment the responses seen with IL-10 blockade alone. Taken together these data demonstrate that filarial infections clearly modulate the Pf-specific IL-12p70-IFN- $\gamma$  pathway known to play a key role in resistance to malarial parasites and do so in an IL10-dependent manner. Flow cytometric analysis is currently underway to determine if Pf-specific Type 1 response modulation extends to the level of CD4+ T cell frequencies of effector or regulatory cells.

**1233****CO-INFECTION WITH HELMINTHS AND MALARIA DURING PREGNANCY EFFECT SUSCEPTIBILITY TO FALCIPARUM MALARIA DURING CHILDHOOD**

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We have previously observed that a subset of offspring of malaria infected pregnant women fail to acquire fetal priming to malaria blood stage antigens *in utero*. These putatively tolerant children were more susceptible to malaria during childhood. Since co-infections with helminths and malaria are common in developing countries we hypothesize that helminth co-infection during pregnancy may down modulate fetal immune responses to blood-stage malaria antigens. To examine the impact of helminths (schistosomiasis, lymphatic filariasis and/or hookworm) on malaria susceptibility we undertook a prospective cohort study of 705 newborns in a malaria endemic region of Kenya in which children were examined every 6 months from birth to 4 years of age for *Plasmodium falciparum* infection and the presence of malaria antigen-specific T cell responses. Overall 26% of the pregnant women were co-infected with helminths and malaria, 16% with malaria and 34% with helminths alone. There was a 2-3 fold increase in risk of malaria infection in offspring of women with mixed helminth and malaria infection compared to offspring of women without either infection ( $p<0.01$ ) as measured by frequency of blood smear and PCR positivity at 12, 18 and 30 months of age. Similarly, offspring of women with mixed infection had significantly reduced hemoglobin levels at 12 months of age (geomean = 7.7g/dL,  $p=0.02$ ) compared to offspring of women with single infection. The increased susceptibility to malaria infection in offspring of women with mixed infection was associated with >2-fold reduced malaria-antigen-driven IFN- $\gamma$  production by peripheral blood mononuclear cells compared to offspring of women infected with malaria or helminths alone ( $p=0.01$ ). Thus, helminth co-infections during pregnancy may induce an immunomodulatory fetal response resulting in impaired fetal priming to malaria *in utero* that could enhance the risk for malaria infection during infancy. Treatment of women for helminth infections during pregnancy may have a beneficial effect on malaria susceptibility in childhood.

**1234****IDENTIFICATION AND CLONING OF BABOON TLF WHICH KILLS HUMAN INFECTIVE AFRICAN TRYPANOSOMES IN VIVO**

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African trypanosomiasis remains a scourge of public health and an obstacle to agricultural and economic development in sub-Saharan Africa. In humans the disease is caused by infection with *T. brucei rhodesiense* in east Africa and *T. brucei gambiense*, in west Africa. Cattle and other domestic animals are a major reservoir of *T. b. rhodesiense*. *T. brucei brucei* causes disease in cattle and other animals but cannot infect a subset of primates due to trypanosome lytic factors (TLFs) present in their serum. Human TLFs are high density lipoprotein complexes that contain Haptoglobin related protein (Hpr) and apolipoprotein L-I (apoL-I). Haptoglobin related protein forms part of a ligand (Hpr-Hb) that enhances the uptake of TLF. Apolipoprotein L-I is a pore forming protein that becomes active in acidic conditions and forms pores in the lysosome membrane. Human TLFs cannot kill *T. b. rhodesiense* due to the acquisition of SRA. Baboon sera and purified HDL can kill human infective *T. brucei*, confirming that baboon and human TLFs are different. To identify the trypanolytic component of baboon serum we purified TLF and analysed the protein components using Tandem mass-spectrometry. The peptide sequences obtained were used to clone two cDNAs encoding proteins with homology to human TLF components. Using a

hydrodynamic-based transgenic mouse model we show that expression of the cDNA clones confers TLF activity on mouse HDLs and protects mice from infection with both animal and human infective *T. brucei*. We propose that the production of baboon TLF transgenic cattle could be used to generate healthier livestock and reduce the transmission of human sleeping sickness in east Africa.

**1235****UNEXPECTED tRNA ENCODED WITHIN THE MITOCHONDRIAL 12S rRNA OF TRYpanosoma brucei**

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Typically mitochondria encode all of the transfer RNAs (tRNA) necessary for autonomous protein synthesis. However kinetoplastids, a protozoan parasite, have one of the smallest mitochondrial genomes and require unique modifications such as RNA editing to produce translatable mRNA. The kinetoplast maxicircle DNA (kDNA), which encodes the mitochondrial genome, is compact and contains little to no intergenic regions where most mitochondrial tRNAs are encoded. To date, there has been no evidence for tRNAs encoded within the mitochondrial kDNA, rather a full set of tRNAs are imported from the cytosolic pool of tRNAs. Analysis of the *T. brucei* nuclear genome with tRNAScan-SE verified this expectation and we identified genes for most of the tRNAs. However the tRNAPhe(AAA) was not detected. This was surprising since the UUU codon is the most abundant in mitochondrial mRNA due in part to uridine insertion during RNA editing. While third position wobble base pairing with the UUU codon would allow other phenalanine tRNAs to substitute for the tRNAPhe(AAA), there is one duplicated tRNAPhe(GAA) identified in the nuclear genome. We repeated the tRNAScan-SE analysis on maxicircle kDNA allowing for low covariance model values that are common for mitochondrial tRNAs. The most promising candidate for tRNA<sup>Phe(AAA)</sup> contained an unusually large intron and is encoded within the 12S rRNA. We have demonstrated the existence of this processed tRNA using 1D- and 2D- denaturing urea-polyacrylamide gels and northern blotting with probes specific for the tRNA. We can also demonstrate that these tRNAs retain their aminoacylation when isolated under acidic conditions and upon basic treatment aminoacylation is lost. Studies are underway to sequence and further characterize this unusual mitochondrial tRNA.

**1236****VALIDATION OF PLASMODIUM FALCIPARUM ISOLEUCYL tRNA SYNTHETASE AS A DRUG TARGET**

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Intraerythrocytic Plasmodium falciparum has minimal capability for amino acid synthesis. Amino acids are obtained via hemoglobin degradation and via uptake from the extracellular environment. Parasites are able to grow with isoleucine as the sole exogenous amino acid, relying on hemoglobin degradation for most of their needs (isoleucine is the only amino acid absent in human hemoglobin). Malaria parasites may be especially sensitive to perturbation of isoleucine uptake and/or utilization.

tRNA synthetases couple amino acids to their cognate tRNAs. Two isoleucyl tRNA synthetases (IRSs) are predicted in the *Plasmodium* genome, one cytoplasmic and one containing a putative apicoplast targeting signal. Mupirocin is a compound produced by *Pseudomonas fluorescens* and is used clinically for methicillin-resistant *Staph. aureus*. Mupirocin resembles the Ile-AMP transition state complex and has been shown to act as a competitive inhibitor of bacterial and archeal IRSs. We determined that mupirocin is a potent anti-malarial compound and kills parasites at nanomolar concentrations. Parasites are killed with "delayed-death" kinetics. Inhibition by mupirocin is attenuated by high isoleucine medium.

To show that mupirocin acts on the apicoplast-located IRS, we selected parasites resistant to mupirocin. Sequencing of cloned mupirocin-resistant parasites revealed a C to T change at nt 4034 of the predicted apicoplast IRS. This mutation results in an amino acid change of Pro1233 to Ser. Pro1233 is completely conserved in IRSs and is proximal to the active site.

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### PROBING CENTRAL CARBON METABOLISM IN PLASMODIUM FALCIPARUM

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Despite decades of study, central carbon metabolism in the Plasmodia remains poorly understood. Early microscopic and biochemical observations suggested that blood-stage Plasmodia possess a minimal mitochondrion with limited respiratory capacity that does not contribute to energy generation. However, recent whole-genome sequences and transcriptional profiling experiments have revealed that potential homologs to all the necessary tricarboxylic acid (TCA) cycle enzymes are both encoded by the plasmoidal genome and coordinately expressed during the trophozoite stage, strongly implying that some variant of the TCA cycle is active during asexual development. Several of these predicted enzymes have been confirmed biochemically or localized to the mitochondrion; however, the principal carbon source(s), directionality and ultimate role of the TCA cycle have not been established. We have used HPLC-MS/MS-based metabolomics and stable isotope-labeled nutrients to trace carbon flux through TCA cycle intermediates in *in vitro* cultures of *Plasmodium falciparum* (3D7 strain). Our data confirm the previously suggested disconnect between glycolysis and TCA metabolism and suggest an acyclic model in which glutamine and glutamate are the principal carbon sources and two discrete pathways act to generate energy, redox balance and biosynthetic intermediates. These results shed light on one of the most divergent examples of eukaryotic carbon metabolism and have implications for the effective design of therapeutic interventions.

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### RAPID MEMBRANE DISRUPTION BY A PERFORIN-LIKE PROTEIN FACILITATES PARASITE EXIT FROM THE HOST CELL

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The MACPF domain owes its name to the pore-forming proteins of the mammalian immune system where it is found in the final components of the complement cascade that form the membrane attack complex and in perforin released by cytolytic effector cells. Widespread genome sequencing in combination with recent crystallographic studies has revealed that the MACPF-fold is of ancient origin and expressed by many bacterial and protozoan pathogens. Within the Apicomplexa we found two or more MACPF family member proteins in most members of the phylum for which genome sequencing is available with a notable exception being Cryptosporidium. Our work on the Toxoplasma Perforin-Like Protein 1 (TgPLP1) revealed a 1151aa secretory protein containing an N-terminal propeptide, a well-conserved MACPF domain, and a predicted C-terminal beta sheet-rich domain. Antibodies raised against TgPLP1 allowed its localization to the micronemes of tachyzoites and confirmed its

secretion in response to calcium agonists. A TgPLP1-null strain displayed no noticeable *in vitro* growth defect but was severely attenuated *in vivo*. Closer examination revealed a defect in ionophore-induced egress from host cells despite an activation of gliding motility similar to wild-type levels. Additionally, the TgPLP1-null strain is severely impaired in its ability to permeabilize either the parasitophorous vacuolar membrane or the host plasma membrane following ionophore treatment of cytochalasin D-paralyzed vacuoles. Co-infection of host cells with wild-type and TgPLP1-null strains showed that egress of a WT vacuole was able to complement both the egress and permeabilization defects of the mutant vacuole. This marks the first time a secreted *Toxoplasma* protein has been shown to play a central role in egress.

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### A CALCIUM DEPENDENT PROTEIN KINASE MODULATES MICRONEME SECRETION IN TOXOPLASMA GONDII

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Apicomplexans rely on calcium as a second messenger to regulate motility, secretion, invasion and differentiation. Understanding calcium signal transduction is essential to elucidating the molecular mechanisms controlling these functions. Calcium dependant protein kinases (CDPKs) have been shown to respond to calcium, and genetic evidence in *Plasmodium* confirms their role in gametocyte exflagellation and ookinete motility. However, the basic pathways controlled by these kinases remain poorly understood, as does their role in other apicomplexans. Four canonical CDPKs conserved among apicomplexans are expressed in *Toxoplasma gondii* tachyzoites. Of these, TgCDPK1 and TgCDPK3 form a distinct phylogenetic lineage, sharing multiple characteristics. Both proteins are predicted to be N-terminal acylated and we show that these motifs are crucial for membrane localization of TgCDPK3. Additionally, both proteins are deposited in trails of gliding parasites, similarly to various components of the gliding machinery. To further examine the function of these CDPKs, we generated conditional inducible mutants employing the published tetracycline transactivator system. We successfully created a conditional TgCDPK1 mutant and have characterized its phenotype. When grown in the presence of anhydrotetracycline, mutant parasites grew normally and formed large stable vacuoles, suggesting an egress defect. When mechanically dissociated, the mutant parasites showed decreased adhesion and invasion into host cells, and reduced lytic ability in a monolayer growth assay. Reminiscent of mutations in certain microneme proteins, these phenotypes suggested a potential defect in secretion. Consistent with this hypothesis, we observed that TgCDPK1 mutants failed to secrete MIC2 in response to induction by ethanol, a potent agonist. These results suggest that TgCDPK1 may be activating microneme secretion, thus forming a critical signaling link downstream of calcium in this essential pathway.

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### THE ROLE OF TNF AND MYD88 IN THE INDUCTION OF B CEL PATHOLOGY FOLLOWING TRYPANOSOMA BRUCEI INFECTION

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Experimental *T. brucei* infections in C57BL/6 mice cause a severe defect in B lymphopoiesis in the bone marrow and show a depletion of splenic transitional T1 (AA4.1+, B220+, IgMhi, CD23-), T2 (AA4.1+, B220+, IgMhi, CD23+) and T3 (AA4.1+, B220+, IgMlo, CD23-) B lymphocytes in addition to the depletion of mature marginal zone (MZ) and follicular

(Fo) B cells. The decline in bone marrow B lymphopoiesis in infected mice appears to result from expulsion of B cell progenitor populations out of the bone marrow into the spleen as it was mirrored by an increase in common lymphoid progenitors (CLP; Lin-, AA4.1+, IL-7+), pre-pro-B (Lin-, IgM-, CD19-, AA4.1+, B220+), pro-B (Lin-, IgM-, CD19+, AA4.1+, B220+, CD43hi) and pre-B (Lin-, IgM-, CD19+, AA4.1+, B220+, CD43lo/-) in the spleen. In contrast, the loss of bone marrow immature B cells (Lin-, CD43lo/-, AA4.1+, CD19+, B220+, IgM+) and splenic transitional B cells appears to result from apoptosis. To define the mechanisms underlying *T. brucei* AnTat 1.1E induced B cell pathology, infections were established in TNF and MyD88 gene-deficient mice. Both the loss of developing B cell populations pre-pro-B, pro-B, pre-B and immature B from the bone marrow and the infiltration of these precursor populations in the spleen was significantly less pronounced in infected TNF-/ mice as compared to in the wild type mice and MyD88-/ mice. Interestingly, during infection the depletion of transitional B cells was not only rescued in TNF-/ mice but also appeared to a much lesser extent in MyD88-/ mice as compared to infected wild type mice. Finally, only in MyD88-/ mice reduced B cell pathology was observed with respect to the loss of FoB cells. In conclusion we show here that two important components of the innate immune system, TNF and MYD88, are involved in the induction B cell pathology during *T. brucei* infection and that at least 3 different processes, one TNF-dependent, one TNF-and MYD88-dependent and one MYD88-dependent, are responsible for respectively the loss of B lymphopoiesis from the bone marrow and the depletion of transitional B cells and FoB cells.

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### NEUTROPHILS ARE THE PREDOMINANT INITIAL HOST CELL FOR LEISHMANIA MAJOR AND ARE ESSENTIAL FOR THE ESTABLISHMENT OF SAND FLY TRANSMITTED INFECTION

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Infection with the obligate intracellular protozoan *Leishmania* is thought to be initiated by direct parasitization of macrophages, but the early events following transmission to the skin by vector sand flies have been difficult to examine directly. Employing 2-photon intra-vital microscopy and flow cytometry, we observed a rapid and sustained neutrophilic infiltrate directed towards localized bite sites and subsequent phagocytosis of *L. major*. Neutrophils ultimately defined the sand fly bite site by creating a neutrophil "plug" at the location of proboscis penetration in the skin. The formation of the neutrophil plug occurs with both infected and uninfected sand flies, suggesting that the wound response to sand fly bite is the primary driving factor in acute neutrophil recruitment. Recruited neutrophils, not macrophages, were the predominant host cell during the first 24 hours of infection following needle inoculation, and neutrophils contained viable parasites that could be propagated in culture. Following adoptive transfer of infected neutrophils into the ear dermis of naïve mice, neutrophil derived parasites established disease as efficiently as infectious parasites obtained from culture. Between 24-72 hours of infection, *L. major* parasites transitioned from neutrophils to their definitive host cell, the macrophage, a process that involved parasite release from infected neutrophils. Lastly, depletion of neutrophils prior to infected sand fly bite abrogated the ability of *L. major* to initiate productive infections and was associated with an increase in the production of the proinflammatory molecules, IL-1 alpha and IL-1 beta. These findings reveal the directed migration of large numbers of neutrophils to sites of *L. major* deposition by sand fly bite, identify neutrophils as critical to the infectious process and an essential cell in the parasite life cycle, and suggest *L. major* exploits

the early host response to sand fly bite in order to establish and promote disease.

## 1242

### DENDRITIC CELL IL-23 PRODUCTION IN RESPONSE TO SCHISTOSOME EGGS INDUCES TH17 CELLS IN A MOUSE STRAIN PRONE TO SEVERE IMMUNOPATHOLOGY

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Infection with schistosomes results in a CD4 T cell-mediated inflammatory reaction against parasite eggs that varies greatly in magnitude both in humans as well as in mice. In the murine disease, the severe form of immunopathology correlates with high levels of interleukin 17 (IL-17). We now report that live schistosome eggs stimulate dendritic cells from (high pathology) CBA mice to produce IL-6, TGF-&#946; and IL-23, whereas those from (low pathology) BL/6 mice only make TGF-&#946;. Moreover, egg stimulation of dendritic cells plus naïve CD4 T cells from CBA mice resulted in increased levels of IL-17 and the chemokines CXCL1, CXCL2 and CCL2, whereas similarly treated BL/6 cell co-cultures instead expressed higher IL-4, IL-10 and Foxp3. Neutralization of IL-23, but not of IL-6 or IL-21, profoundly inhibited egg-induced IL-17 production in the CBA co-cultures, and only the addition of exogenous IL-23 stimulated BL/6 cells to make IL-17. These findings identify IL-23 as a critical host factor that drives IL-17-production and suggest that a genetically programmed innate pro-inflammatory response against the parasite determines the development of Th17 cells and the outcome of immunopathology in schistosomiasis.

## 1243

### PERIPHERAL TREG INDUCTION CAN BE DIRECTLY MEDIATED BY HELMINTH-DERIVED PRODUCTS

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Epidemiological studies of human allergic disease have highlighted the discordance between their increasing prevalence in the western world, compared to lower incidence in the developing world, where endemicity of helminth parasites is high. In animal models, a direct link has been suggested between helminth infection and reduced allergic responses. For example, mice harbouring the chronic rodent gut nematode *H. polygyrus* show suppressed allergic airway inflammation, even if infection follows normal sensitization of the immune system. Moreover, transfer of CD4+CD25+ Tregs, which expand in the mesenteric lymph nodes following infection, to uninfected sensitized animals results in reduced bronchial inflammation upon allergen challenge. These results suggest that parasites have evolved mechanisms to exploit host regulatory networks thus gaining a long-term survival advantage. An intriguing question is whether *H. polygyrus* activates pre-existing "natural" Tregs, or induces regulatory activity in peripheral naïve T cells. We have addressed this by functionally analysing a set of proteins released by live parasites, termed excretory/ secretory products (ES). We found that *H. polygyrus* ES (HES) is able to mediate Treg induction, *in vitro*, as anti-CD3 stimulation of Foxp3 negative splenocyte cultures in the presence of HES leads to de novo Foxp3 expression. Foxp3 induction is not observed with anti-CD3 stimulation alone or with a range of other pathogen-derived products tested under the same conditions. Furthermore, Foxp3 induction is dependent upon signalling through the TGF-beta receptor as inhibition of this pathway with a specific inhibitor (SB431542) abrogates Foxp3 induction by HES. The Tregs induced by HES are able to suppress proliferation of effector T cells to a similar extent as TGF-beta generated Tregs. These data confirm that helminth derived products are able to

directly drive Treg expansion in the periphery, raising the possibility that they can mediate the airway allergy suppression observed in whole worm infection.

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