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Human babesiosis is a tick-borne malaria-like illness that generally resolves without complication after administration of atovaquone and azithromycin or clindamycin and quinine. Although failure of antibiotic therapy to clear Babesia microti parasitemia and associated symptoms in immunocompromised hosts has been reported, the pathogenesis, clinical course, and optimal treatment regimen remain uncertain. We used a case-control strategy to compare the immunologic status, clinical course, and treatment of 14 immunocompromised subjects who suffered morbidity or death following persistence of *Babesia microti* infection despite repeated courses of anti-babesial antibiotics with those of 46 case controls who cleared infection after a single course of standard anti-babesial therapy. All of the case subjects were immunosuppressed at the time of acute babesiosis compared to fewer than 10 percent of controls. Most subjects experiencing persistent babesiosis suffered from hematologic malignancies and were asplenic or had received immunosuppressive therapy shortly before initial babesial illness. The cases were more likely than control subjects to require hospital admission and to suffer hematologic, pulmonary, and renal complications from acute or relapsed babesiosis. Three case subjects died of complications related to their babesial infection. Resolution of persistent disease occurred in 11 patients after 3 to 10 courses of therapy and administration of a final antimicrobial regimen for at least 2 weeks after babesia were no longer seen on thin blood smear. In conclusion, immunocompromised patients infected by *B. microti* are at risk for the development of refractory babesiosis despite a course of standard anti-babesial therapy. In order to overcome persistent disease and achieve cure, such patients require an extended course of antimicrobial therapy, generally administered for at least 2 months, including 2 weeks of therapy beyond the time piroplasms are no longer detectable on blood smear.

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CLINICAL FEATURES OF THE HUMAN BARTONELLOSIS (ACUTE CARRION'S DISEASE) IN THE NORTHERN FOREST OF PERU

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Carrion's Disease is a reemerging disease in Peru with a broad clinical spectrum. Coinfections and superinfections are frequent, but this has been poorly investigated in endemic area. Our aim is to describe the clinical features of the acute Carrion's Disease (ACD) in the Northern forest of Peru as well as the coinfections. A cohort study was performed in the San Ignacio Health Center, Cajamarca department, located in the North forest of Peru, between July 2004 and June 2005. All patients with fever, without known source of infection and without previous antibiotic treatment, who turned to the San Ignacio Health Center, were enrolled. During the first consultation, a clinical-epidemiological survey was run and blood samples for culture, thin and thick smear, hematologic and biochemistry analysis, serological tests for infection by *Leptospira*, *Brucella*, *Salmonella typhi* and *paratiphy* were taken. People with positive blood culture to *Bartonella* were considered as ACD. From 476 enrolled 87 were cases of ACD. The median age of the cases was 14 years old (4 months - 82 years) and 52,3% were male; the main symptoms were general malaise 97,7%, headache 89%, arthralgias 78,2%, chills 75%, hyporexia 74%, myalgias 64,4%, abdominal pain 61%, dizziness 45,9%, cough 45,9%, conjunctival injection 35%, retroocular pain 26,4%, vomits 23% and diarrhea 21%. The main signs were hepatomegaly 19%, conjunctival pallor 17,2%, petequiae 11% and splenomegaly 8%. The main coinfections were leptospirosis 16,7% (8/48), rickettsiosis 4% (2/49) and *P. vivax* malaria 1,2%. The median of hemoglobin was 12,5 g/dl (5.8-16.1). Sensitivity of blood smear was 29,8%. Only 5.5% were inpatients and the fatality rate was 0%. In conclusion, unspecific symptoms are the most reported and retroocular pain is described for the first time in ACD. The conjunctival pallor was unusual and the main coinfections are leptospirosis, rickettsiosis and malaria. The blood smear has a low sensitivity for diagnostic of ACD.

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REDUCTION OF INFANT MORTALITY: LESSONS FROM CEARÁ STATE, NORTHEASTERN BRAZIL (1995-2002)

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Infant mortality (IM) remains a very important public health problem worldwide. Substantial reductions in IM were observed in the state of Ceará, northeastern Brazil, after the implementation of a state wide program. Ceará is one of most underprivileged states in the region in terms of natural resources and had a high infant mortality; 80/1,000 live births in 1994. The IM was associated with the lack of exclusive breast-feeding, insufficient prenatal care, inadequate water supplies, poor sanitation, and illiteracy of mothers. The state ministry of health implemented the following measures: 1) It increased the number of community health workers (CHW). Each CHW visited 100 families monthly, provided health and nutrition education, referred pregnant women for prenatal care and sick persons to the health unit, taught water chlorination and supplied chlorine, and collected health data. 2) It greatly expanded implementation of Family Health Teams of one nurse, one physician, and 10 CHWs for every 1,000 families. 3) A major breast feeding campaign was launched. It included a) training all health professionals on the importance and practice of breast feeding and b) using postal workers to deliver information to pregnant women encouraging prenatal care and breast-feeding. 4) Kangaroo Mother Care was initiated to provide supplemental care for underweight infants. 5) Human breast milk banks were created in maternity centers. 6) Prizes were awarded to child-friendly hospitals where 100% of babies delivered were breast-fed exclusively up to 6 months. From 1995-2002 IM rate in Ceara decreased from 80 to 25/1000, a 68% reduction; exclusive breast-feeding (up to 6 months) increased from 38% to 64%, an increment of 68%; and prenatal care increased from 68 to 90%, an increment of 32%. While incentivized breast feeding and other aspects of the program were directly responsible for decreased IM, successful implementation of the program was dependent on political will and cooperation among multiple agencies.

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IDENTIFICATION OF DEVELOPMENTALLY REGULATED GENES IN *ENTAMOEBA HISTOLYTICA*

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Entamoeba histolytica is a protozoan parasite and the second leading cause of parasitic death worldwide. There are two stages in the life cycle: a trophozoite that causes disease and a cyst form that transmits disease. Encystation is necessary for transmission of the parasite to new hosts, hence, blocking encystation would prevent spread of the disease. Unfortunately, research into the regulation of this developmental process has been severely hampered by the lack of an *in vitro* system of encystation in *E. histolytica*. We used *E. histolytica* clinical isolates, which contain cysts and a whole-genome microarray-based expression profiling to examine the transcriptomes of *E. histolytica* cysts and trophozoites.

We identified that ~15% of the 9,938 annotated amebic genes are developmentally regulated (672 cyst-specific genes and 767 trophozoite-specific genes). Among the cyst-specific genes included potential signal transducing genes such protein kinases and G-protein coupled receptors, which may play a role in the regulation of the developmental program. In order to identify the molecular signature that initiates the encystation program, we are functionally characterizing a Myb-domain containing gene, which was upregulated in cysts. We have confirmed that the homologue of this gene is upregulated in *Entamoeba invadens* cysts suggesting a potential conserved function in *Entamoeba* development. Furthermore, we have demonstrated that overexpression of the Myb-domain gene in *E. histolytica* trophozoites initiates a transcriptional profile consistent with encystation, including expression of cyst wall genes. Studies to identify the promoter motifs bound by the Myb protein are underway. This work will help to delineate the molecular basis of stage conversion in *Entamoeba histolytica* and lead to potential therapeutic measures against the cyst form of the parasite.

(ACMCIP Abstract)

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CYSTEINE PROTEASE ACTIVITY IN *SCHISTOSOMA MANSONI* RESISTANT AND SUSCEPTIBLE *BIOMPHALARIA GLABRATA* SNAILS

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A strong body of evidence exists for a role of hydrolytic enzymes in the defense of mollusks against invading parasites. It has been shown that the levels of these enzymes are elevated in *Biomphalaria glabrata* snails following exposure to pathogens such as parasitic helminths. The innate immune response of *B. glabrata* to *Schistosoma mansoni* is mediated both by the cellular (hemocytes) and plasma (hemolymph) components of the snail's internal defense system (IDS). In response to the invading parasite, the IDS is triggered within a short period post-exposure. This results in encapsulation of the parasite, where the action of hydrolytic enzymes released from the hemocytes and hemolymph may facilitate the death of the parasite. In the present study, SDS-page gelatin zymology was used to qualitatively determine proteolytic enzyme activity in resistant (BS-90 and LAC strains) and susceptible (NMRI strains) pre- and post- exposure to *S. mansoni*. Results indicated that protease activity was higher in parasite resistant compared to susceptible snails with most activity residing in the posterior, hepatopancreas, region. Enzyme activity corresponded to a complex high molecular weight smear (>220 to 66kDa) that was inhibited by the cysteine protease inhibitor E64. Because parasite exposure was found to affect the levels of cysteine protease activity in the snail, a cDNA library was made from the hepatopancreas from which several cysteine protease encoding transcripts were identified. One of these transcripts encoded the full-length cDNA for the cysteine protease, cathepsin B. Nucleotide and amino acid sequence analysis of the snail cathepsin B gene showed significant matches to schistosome and vertebrate (human and mouse) orthologues. In *B. glabrata*, Cathepsin B was found to occur as a single-copy gene. Genetic variation in the Cathepsin B locus in resistant and susceptible snails was investigated by Restriction Fragment Length Polymorphism (RFLP) analysis and results revealed the occurrence of polymorphisms between these snails. Real-time Reverse Transcriptase (RT)-PCR was used to determine the regulation of the Cathepsin B transcripts in resistant and susceptible snails pre-and post exposure to miracidia. Results consistently showed that up-regulation of Cathepsin B occurs shortly after parasite exposure of resistant but not susceptible snails.

(ACMCIP Abstract)

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IDENTIFICATION OF IMMEDIATE RESPONSE - GENES DOMINANTLY EXPRESSED IN *BIOMPHALARIA GLABRATA* SNAILS UPON EXPOSURE TO *SCHISTOSOMA MANSONI* INFECTION

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Intermediate snail hosts of parasitic infections are an integral part of the transmission of intractable chronic diseases such as schistosomiasis. An understanding of the snail host-parasite relationship at the molecular level is probably the best chance for the identification of novel tools that will help block parasite development in the snail. Non-self responses in *Biomphalaria glabrata* towards parasite infection depend on an innate defense system. This system is characterized by an immediate early response against miracidia that eliminates the parasite. In this study, we focused on the identification of immediate response-transcripts that may be involved in miracidia destruction before they develop into sporocysts. Suppression subtractive hybridization (SSH) was used to reveal the up-regulation of dominantly expressed transcripts in either resistant (LAC) or susceptible (NMRI) snails 5 hrs post exposure to *Schistosoma mansoni*. SSH cDNA libraries were also constructed from parasite- exposed juvenile resistant (BS-90) and susceptible (NMRI) snails. One thousand nine hundred and twenty Expressed Sequence Tags (ESTs) were generated from these libraries and clustered. We identified 41 dominantly expressed genes from parasite-exposed susceptible snails. These included transcripts encoding antioxidant, cell structure/signaling, immune related, metabolic/mitochondrial, transduction/translation and various enzymes. From the resistant snail-specific SSH libraries, ESTs generated included 25 genes that were up-regulated in these parasite-exposed snails. These included a similar repertoire of gene transcripts as those found in the susceptible parasite-exposed snails. Real time PCR was used to verify the specificity of transcription in resistant and susceptible snails at different times post-exposure. Immediate defense response genes were identified from the resistant snail. These included cytidine deaminase and 2 defense-related transcripts that were dramatically up-regulated shortly after exposure. Similarly, results showed that several receptor-encoding transcripts, including lectin-like receptor, low density lipoprotein receptor and receptor for protein kinase C, were significantly up-regulated in exposed susceptible but not in resistant snails. Differences in the relative expressions of the SSH transcripts identified in resistant and susceptible snails pre-and post exposure will be discussed.

(ACMCIP Abstract)

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ANTI-TRANSMISSION DNA VACCINE FOR SCHISTOSOMIASIS JAPONICA IN CHINA

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Despite intensive control efforts, schistosomiasis remains an endemic, zoonotic disease of major public health importance in China. In the marsh and lake regions of China, water buffalo account for approximately 75% of disease transmission. In addition to acting as the major reservoir,

infected water buffalo often experience poor growth and weight gain compared to non-infected animals. Thus, interventions which reduce schistosome infection in buffalo will be beneficial to buffalo health and aid in reducing disease prevalence in humans. In this regard, a mathematical model predicted that an anti-fecundity vaccine which reduces fecal egg output in water buffalo by 40-45% in conjunction with praziquantel treatment will significantly lead to reduction in transmission of schistosomiasis. In this study, we tested the ability of four schistosome-DNA vaccine constructs to reach these levels in water buffalo. The DNA vaccine constructs encode the glycolytic enzyme triose phosphate isomerase (SjCTPI) or the tetraspanin 23 kDa integral membrane protein (SjC23) or the same antigens fused to the N-terminus end of the bovine heat shock protein 70 (SjCTPI-Hsp70 and SjC23-Hsp70). We found that compared to buffalo vaccinated with the control plasmid DNA (pVAX), vaccination with SjCTPI-Hsp70 or SjCTPI plasmids reduced worm burdens by 51.2% and 41.5% respectively and importantly, fecal miracidial-hatching was reduced by 52.1% and 33.2% respectively. Vaccination with SjC23-Hsp70 and SjC23 plasmids reduced worm burdens by 50.9% and 45.5% respectively and fecal miracidial-hatching by 52.0% and 47.4%. Thus both the SjCTPI-Hsp70 and SjC23-Hsp70 plasmid DNA vaccines exceeded the level of protection predicted by the mathematical model to significantly reduce transmission of schistosomiasis in the lakes and marsh regions of China. These data support the use of either of these two vaccines in a field-based intervention to determine if vaccination of buffalo will reduce transmission of schistosomiasis in China.

(ACMCIP Abstract)

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A DNA VACCINE ENCODING A SAND FLY SALIVARY YELLOW RELATED PROTEIN (LJM11) CONFERS PROTECTION AGAINST CHALLENGE WITH *LEISHMANIA MAJOR* IN THE PRESENCE OF *LUTZOMYIA LONGIPALPIS* SALIVARY GLAND HOMOGENATE

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Sand fly salivary proteins are injected in the host skin during blood meal and they act helping the feeding process by counteracting the coagulation and inflammatory cascades. Moreover, immune responses to sand fly bites or salivary gland homogenate from *Phlebotomus papatasi* were previously shown to confer protection against *Leishmania major* infection. The correlates of protection were a delayed type hypersensitivity (DTH) response with a Th1 profile in the presence of interferon γ. In order to identify a salivary protein from *Lutzomyia longipalpis* that induces a DTH response and potentially protect against *Leishmania* infection, we cloned in the mammalian expression vector VR2001-TOPO, the 17 transcripts encoding the most abundant *Lu. longipalpis* salivary proteins. We found two molecules, LJM11 (44 kDa salivary protein) and LJL143 (33 kDa salivary protein), that induced a DTH response in C57BL/6 mice. Since, there is no model to test protection to visceral leishmaniasis in mice; we tested if vaccination with these two plasmids could protect mice against *L. major* in the presence of *Lu. longipalpis* saliva. Mice vaccinated intradermally, three times, in the ear with 5ug of the purified plasmid LJM11 were protected against the *L. major* infection. No apparent protection was observed with LJL143 or other antibody producing salivary proteins. Evaluation of the immunological mechanisms of protection with the expressed recombinant LJM11 protein is underway. This functional genomic approach based on DNA immunization and identification of plasmids producing a cellular immune response allowed us to narrow down the number of potential vaccines candidates and the identification of a protective molecule from the salivary gland of this sand fly.

(ACMCIP Abstract)

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MULTIPLY PARASITIZED ERYTHROCYTES ARE ASSOCIATED WITH INCREASED SEVERITY OF MALARIA

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The ability of *Plasmodium falciparum* to invade host erythrocytes efficiently contributes to the severity of disease caused by this, the most lethal of the human malaria species. Observations from the Queen Elizabeth Central Hospital Paediatric Research Ward in Blantyre, Malawi suggest malaria patients with high numbers of erythrocytes with multiple parasites (EMPs) are more severely ill. We examined pretreatment thin smears from children with cerebral malaria (n=56) and uncomplicated malaria (n=98). For each slide the number of ring stage parasites per erythrocyte was counted and the peripheral parasitemia was calculated. Normally distributed data were analyzed using t-tests for continuous variables and chi-squared tests for categorical variables. In children with cerebral malaria and in those with uncomplicated malaria, there was a positive correlation between numbers of EMPs and parasitemia (r^2=.48 vs.37, respectively). After controlling for parasitemia, the difference between the groups remained significant. The mean percentage of EMPs was higher for cerebral malaria patients than for uncomplicated patients (5.5 vs. 3.0, $p<.001$). More cerebral malaria patients had erythrocytes infected by three or more ring stage parasites than uncomplicated patients (39% vs. 12%, $p<.001$). The mean percentage of EMPs was higher for cerebral patients with a fatal outcome (28/56) than those who survived (28/56) (6.8 vs. 4.3, $p<.05$). In conclusion, our data suggest a strong relationship between the number of EMPs and the severity of malaria illness. The biological basis of this phenomenon remains to be elucidated but may involve degree of anemia, intensity of sequestration or a distinct parasite phenotype. *In vitro* data relating to these possibilities will be presented.

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IN UTERO SELECTION AT THE FLT1 LOCUS IN A MALARIA-ENDEMIC AREA

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Placental malaria (PM) is a major cause of neonatal mortality, but its effect on pregnancy loss is less clear. Soluble fms-like tyrosine kinase 1 (sFLT1) is secreted by fetal cells of the placenta into the maternal circulation during PM of first-time mothers, and may be related to preeclampsia. We hypothesize that fetal FLT1 genotype may differentially promote survival for the fetus of first versus later pregnancies. Maternal and infant samples were provided by Tanzanian women aged 18 to 45 years delivering at the Muheza Designated District Hospital. Genomic DNA was extracted from filter paper or from frozen blood pellets. Genotypes from 1159 individuals were obtained. The genotype frequency of infants differed by maternal parity, but maternal genotype did not. The difference between infant genotype frequency persisted after correcting for maternal genotype. Reported miscarriages were most common in first time mothers who were homozygous, corresponding with the likelihood of having a homozygous fetus. Homozygous infants born to PM-positive first-time mothers had the highest prevalence of low birth weight. In conclusion, the data suggest that FLT1 homozygous offspring are at a selective disadvantage in first-time mothers in malaria endemic areas. Maternal malaria may exert selective pressure *in utero* at the FLT1 locus through pregnancy loss. This is

the first identification of a malaria resistance gene that confers protection *in utero*.

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IP-10, APOPTOTIC AND ANGIOGENIC FACTORS ASSOCIATED WITH MORTALITY OUTCOMES IN CEREBRAL MALARIA PATIENTS IN INDIA

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We conducted a prospective study in Jabalpur, India to assess the burden of neurological outcomes associated with cerebral malaria was conducted. The goal of this study was to understand the immunopathological mechanisms involved in cerebral malaria and to identify peripheral biomarkers (cytokines, growth factors, ligands, etc.) implicated in malaria severity that may be utilized to predict prognoses of severe CM cases in India. The central hypothesis is that altered levels of interferon γ inducible protein (CXCL10), soluble tumor necrosis factor receptor 1 and 2 (sTNFR1, sTNFR2), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGFbb) in cerebrospinal fluid (CSF) and plasma of CM patients are predictive for neuronal injury, cognitive impairment and mortality associated with CM. We comparing different malaria groups (healthy controls [HC], mild malaria [MM], cerebral malaria survivors [CMS], and cerebral malaria non-survivors [NSCM]) and investigated the immunological profiles of various biological mediators (IL-1, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, Eotaxin, Fas-ligand [Fas-L], soluble Fas [sFas], FGF basic protein, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1 (MCAF), MIP-1α, MIP-1β, PDGF bb, RANTES, TNF-α, sTNFR 1, sTNFR 2, TGF-β, and VEGF) in order to understand the role of immune factors which influence progression to severe outcomes associated with CM. Our findings suggest potential roles for IP-10, apoptotic factors and angiogenic factors in the cerebral malaria associated outcomes in Indian patients. The potential use of these results in establishing a prediction rule for CM prognosis is discussed.

(ACMCIP Abstract)

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SUPPRESSION OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IN CHILDREN WITH SEVERE MALARIAL ANEMIA: ROLE OF MONOCYTE ACQUISITION OF HEMOZOIN

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Severe malarial anemia (SMA), caused by *Plasmodium falciparum* infections, is one of the leading causes of childhood mortality in sub-Saharan Africa. Although the molecular determinants of SMA are largely undefined, dysregulation in host-derived inflammatory mediators influences disease severity. Macrophage migration inhibitory factor (MIF) is an important regulator of innate inflammatory responses that has recently been shown to suppress erythropoiesis and promote pathogenesis of SMA in murine models. To examine the role of MIF in the development of childhood SMA, peripheral blood MIF production was examined in Kenyan children (aged <3 years, n=357) with *P. falciparum* malarial anemia. All children in the study were free from bacteremia and HIV-1. Since deposition of malarial pigment (hemozoin) contributes to suppression of erythropoiesis, the relationship between MIF concentrations and monocytic acquisition of Hz was also examined *in vivo* and *in vitro*. Circulating MIF concentrations declined with increasing severity of anemia and significantly correlated with peripheral blood leukocyte MIF transcripts. MIF concentrations in peripheral blood were not significantly associated with the reticulocyte production. Multivariate regression analyses, controlling for age, gender and parasitemia, further revealed that elevated levels of pigment-containing monocytes (PCM) was associated with SMA and decreased MIF production. In addition, PCM levels were a better predictor of hemoglobin and MIF concentrations than parasite density. Additional experiments in malaria-naïve individuals demonstrated that hemozoin caused both increased and decreased MIF production in cultured peripheral blood mononuclear cells (PBMC) in a donor-specific manner, independent of apoptosis. However, PBMC MIF production in children with acute malaria progressively declined with increasing anemia severity. Results presented here demonstrate that acquisition of hemozoin by monocytes is associated with suppression of peripheral blood MIF production and enhanced severity of anemia in childhood malaria.

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IMPACT OF ARTMISININ-BASED COMBINATION THERAPY ON MALARIA TRANSMISSION IN MALI

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Most African countries have now changed their first line treatments from monotherapies to Artemisinin-based combination therapies (ACTs). ACTs are known to decrease the rate of gametocyte carriage and gametocyte density in a treated population. However, the impact of ACT treatment on gametocyte infectivity and malaria transmission is still debatable. During a randomized controlled Phase IV trial in Bougoula-Hameau, Mali, we compared the infectivity of post-AS/AQ, AR-L and AS/SP to *Anopheles gambiae*. Patient with uncomplicated malaria were randomised to one of the three treatment arms and followed for 28 days. Gametocyte carriage was assessed by microscopy before and after treatment. Whenever gametocytes were found, starved mosquitoes were direct-fed and kept in laboratory for 8 days. The presence of oocysts was determined and the number estimated by dissection on day 8 post feeding. Before any treatment 12% (n= 728) of mosquitoes were oocyst positive at day 8. After treatment we found that 34% (n= 224), 28% (n=288) and 8% (n=602) of mosquitoes were oocyst positive at day 8 in the AS/AQ, AR-L and AS/SP arms, respectively. AS/AQ and AR-L significantly increased gametocyte infectivity ($p < 0.0001$) while AS/SP significantly decreased gametocyte infectivity ($p = 0.01$). This data show that the impact of ACT treatment on malaria transmission and spread of resistance may vary from one ACT to the other.

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BLOOD GROUP O PROTECTS AGAINST SEVERE *PLASMODIUM FALCIPARUM* MALARIA

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Malaria has been a major selective force on the human population, and several erythrocyte polymorphisms have evolved that confer resistance to severe malaria. *Plasmodium falciparum* rosetting, a parasite virulence phenotype associated with severe malaria, is reduced in blood group O erythrocytes compared to groups A, B and AB, but the contribution of the ABO blood group system to protection against severe malaria has received little attention. We hypothesised that blood group O may confer resistance to severe malaria via the mechanism of reduced rosetting. In a case-control study of 670 Malian children, we found that blood group O was present in only 22% of severe malaria cases compared to 40-45% of healthy controls and uncomplicated malaria cases. Blood group O was associated with a 66% reduction in the odds of developing severe malaria compared to the non-O blood groups (odds ratio (OR) 0.34, 95% confidence interval (CI) 0.21-0.54, P<0.0005). In the same sample set, *P. falciparum* rosetting was reduced in parasite isolates from blood group O children compared to the non-O blood groups (P= 0.003, Kruskal Wallis test). A second study of 144 Kenyan children also showed that group O was associated with reduced rosetting (P= 0.0001) and protection against severe malaria (OR 0.37; 95% CI 0.17-0.80, P<0.05). This work highlights the importance of *P. falciparum* rosetting as a pathogenic factor in severe malaria, and suggests that the selective pressure imposed by malaria may contribute to the variable global distribution of ABO blood group types.

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α+-THALASSAEMIA PROVIDES A HAEMATOLOGICAL ADVANTAGE AGAINST MALARIA

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The heritable haemoglobinopathy α+-thalassaemia is caused by the reduced synthesis of α-globin chains that constitute normal adult haemoglobin. Individuals homozygous for α+-thalassaemia have an increased number of microcytic erythrocytes that contain less haemoglobin than those of normal genotype. α+-Thalassaemia homozygosity confers considerable protection against severe malaria, as well as severe non-malaria. We investigated whether a haematological advantage may explain these observations. A number of haematological parameters as well as acute phase protein levels were determined in a cohort of Papua New Guinean children who had previously demonstrated the protective effect of α+-thalassaemia. Here we show that the erythrocyte profile associated with α+-thalassaemia would be a haematological benefit during the significant erythrocyte loss that occurs in acute malaria. This may contribute substantially to the protection of α+-thalassaemia homozygous children against severe malaria anaemia.

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MODELING THE DISTRIBUTION OF THE HOST-SEEKING NYMPHAL *IXODES SCAPULARIS* TICKS IN THE USA USING CLIMATE AND LANDSCAPE PREDICTORS

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Human Lyme disease risk in the eastern United States is dependent on the abundance of nymphal *Ixodes scapularis* ticks infected with *Borrelia burgdorferi*, since nymphs represent the only important stage for transmission to humans. While many studies have estimated local and regional *I. scapularis* density, there has never been a large-scale study using a common, standardized methodology. To develop a nationwide spatial Lyme disease risk model, we designed a four-year survey of *I. scapularis* covering its known geographic range. Here we report results for tick density, for the first three years. The density of host-seeking *I. scapularis* nymphs was measured by drag sampling of closed-canopy deciduous forest habitats in 304 sites spaced among 95 two-degree quadrants covering the USA, east of the 100th meridian. Thirty of those sites were resampled in subsequent years. Within each site, one thousand meters were sampled along five transects, 3-6 times during the summers of 2004-2006. We used a logistic regression model to predict the probability of finding a nymph in a site based on climate, landscape and altitude. Climate variables included monthly maximum and minimum temperature, precipitation and vapor pressure deficit. We also derived the magnitude and phase of the annual temperature cycle, and the magnitude of vapor pressure deficit, using Fourier transformation. Landscape variables included the proportion of forest in an 8 km area surrounding the sampling site. The variables in the best fitting model were altitude and the magnitude and phase (quadratic) of the annual minimum temperature cycle. Model sensitivity and specificity were 71.1% and 72.5%, respectively. The model delineates two areas with high probability of host-seeking nymphal *I. scapularis* presence in the Northeast and Upper Midwest and correctly predicts the absence of nymphs in most of the Southern sites. Negative sites that where predicted positive could represent areas of future *I. scapularis* expansion.

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IMMUNITY TO SALIVA AT THE TICK-HOST INTERFACE: IDENTIFICATION OF *IXODES SCAPULARIS* SALIVARY PROTEINS ELICITING A CELLULAR IMMUNE RESPONSE

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Tick saliva contains pharmacologically active ingredients that enable and enhance blood feeding. In susceptible hosts, such as guinea pigs, exposure to saliva confers resistance to ticks. Tick resistance may have a protective effect against transmission of tick borne diseases, such as Lyme disease, yet the immunological mechanisms involved in tick resistance have not been thoroughly elucidated. *Ixodes scapularis* saliva consists of at least 25 different families of proteins containing over 470 individual proteins. Using a reverse antigen screening approach, whereby guinea pigs were sensitized to *I. scapularis* and then challenged intradermally with 72 individual salivary specific DNA plasmids, we identified 9 salivary gland proteins that induced a statically significant cellular recall skin response based on redness, induration and histological analysis. This reverse antigen screening approach, which identifies molecules that produce a delayed skin response, a surrogate of cellular immunity, was validated using the corresponding recombinant proteins produced in mammalian cells. As observed during normal tick feeding, basophils and eosinophils were the major cellular infiltrate at the inoculation site. Six of the nine skin response inducing DNA plasmids belong to a protein family characterized by cysteine framework and a lysine rich basic tail and shares homology with a known tick salivary anti-coagulant. In addition to cellular recruitment, this group of proteins elicits an antibody response in tick sensitized guinea pigs as measured by western blot. When vaccinated with a combination of the six basic tail DNA plasmids, guinea pigs showed higher levels of tick resistance than control animals. With this approach we have identified tick salivary proteins that produce a strong cellular immune response in the

skin of animals. Furthermore, the immune response generated by these tick salivary proteins appears to play an important role in resistance to tick feeding.

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METHODOLOGICAL CONSIDERATIONS IN DESCRIBING THE POPULATION DYNAMICS OF DEER TICKS ON WHITE-FOOTED MICE

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Studying the population dynamics of deer ticks (*Ixodes dammini*) is facilitated by counting subadults infesting white-footed mice (*Peromyscus leucopus*), which serve as unbiased sampling devices. Typical summary statistics for infestation include the mean number of larvae or nymphs infesting the average mouse. Because macroparasites are usually overdispersed, such a parametric summary statistic as the mean and standard deviation is poorly representative. We sought to develop alternative methods for representing the population dynamics of deer ticks on white-footed mice in space and time. Mice were trapped over 8 transmission seasons (1994-2001) on two geographically separated 0.4 ha grids on Martha's Vineyard, Massachusetts, where Lyme disease incidence is among the greatest reported for the U.S. Demographic information and counts of identified tick larvae and nymphs were recorded for each individual animal (N=1133). More than half of all mice were uninfested during the typical nymphal or larval activity months. The mode for nymphs infesting each mouse was 2 or fewer but 2.5% of mice served as host for more than 10, that is, a small number of mice may function similar to "super-spreaders" in that they are heavily parasitized while the majority of mice carry a low to moderate tick burden. Total expected productivity of a trapping grid during a transmission season, estimated by the area under the curve of a scatterplot of individual infestations by epidemiological week was more predictive of the subsequent year's infestations than was measuring the association between mean infestations of larvae of one year and that of nymphs during the next. Alternative methods that describe the intensity as well as heterogeneity of tick parasitism may provide us with more realistic parameters to use for predictive models of Lyme disease transmission.

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IDENTIFICATION OF A NATURAL FOCUS OF TULAREMIA TRANSMISSION USING GIS MAPPING OF INFECTED *DERMACENTOR VARIABILIS*

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During the last 7 years, Martha's Vineyard, Massachusetts has experienced a prolonged epizootic of tularemia due to *Francisella tularensis tularensis* (Ftt). Although the mode of perpetuation remains undescribed, our previous work on the island has implicated dog ticks, *Dermacentor variabilis* (Dv), as a critical element. Using variable number tandem repeat (VNTR) analysis, we have shown that Ftt on MV is highly diverse, indicative of long-standing transmission on the island. It may be that such diversity is maintained by enzootic transmission of Ftt in small isolated natural foci of transmission. To examine this hypothesis, we sought to map the location of ticks testing positive for Ftt to determine whether such ticks cluster together in putative foci. Using a handheld GPS unit, we mapped 86 waypoints along 3 transects approximately 3400m total in length in a field site on MV that has sustained a high prevalence of infection since 2002. From 2004-2006 questing ticks were collected at each waypoint by flagging. Ticks were tested for evidence of Ftt in pools of 6 by PCR targeting the FopA gene. Ticks from positive pools were then retested individually. VNTR analysis was done on individual ticks using 4 loci. We identified an area along the transects, approximately 180m long, where PCR positive ticks appeared to cluster. Of 5106 ticks collected during this study; 27% (1383) of these were collected from this section. However, a disproportionately large percentage of ticks testing positive for Ftt, 54% (110 of 204 total), derived from this small area. VNTR analysis showed that most, 83.8% (171), of the positive ticks harbored one of 2 dominant haplotypes. These two types were distributed throughout our field site. However, of the 33 uncommon haplotypes, 81.8% clustered together in this one section. We conclude that this 180m transect represents a natural focus and is likely to be a source of genetic diversity for Ftt.

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EHRLICHIOSES IN CAMEROON

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Ehrlichia are tick-transmitted obligately intracellular gram negative bacteria of medical and veterinary importance. Two species, *Ehrlichia chaffeensis* and *E. ewingii* cause human monocytotropic ehrlichiosis (HME) and ehrlichiosis ewingii, respectively, two emerging and life-threatening human zoonoses. They are transmitted primarily by the Lone Star tick, *Amblyomma americanum,* in the United States. *E. canis* is the globally distributed cause of canine monocytic ehrlichiosis (CME) and is transmitted by the brown dog tick, *Rhipicephalus sanguineus. E. canis* has also been isolated from a human while *E. chaffeensis* and *E. ewingii*, also cause serious disease in canines. *E. ruminantium* causes cowdriosis, an economically important disease of cattle in Africa. Reports describing the prevalence of *E. ruminantium* in Cameroon are available; however, the presence of other ehrlichial agents is only being investigated. Recent molecular and serological evidence suggest that *E. chaffeensis* is also found in areas where *A. americanum* is not indigenous suggesting that ehrlichial agents could be maintained and transmitted by different reservoirs and tick vectors respectively. A study was designed to determine the prevalence of *Ehrlichia* spp. (*E. canis, E. chaffeensis* and *E. ewingii*) in human, dogs and tick populations in Cameroon. Results indicate that in addition to *E. canis* and *E. ruminantium, E. chaffeensis* and *E. ewingii,* agents of important emerging zoonosis circulate in Cameroon. *R. sanguineus* was identified as a probable vector of *E. canis, E. chaffeensis* or *E. ewingii* with a possibility of simultaneous infections This study also identifies *E. chaffeensis* as a prevalent but unrecognized cause of undifferentiated febrile illness in Cameroonian patients. These findings offer conclusive evidence that multiple *Ehrlichia* species are present in Cameroon and identify *R. sanguineus* ticks as a primary vector of *Ehrlichia* species with the potential to transmit these previously unrecognized zoonotic agents to humans Cameroon.

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RISK OF SPOTTED FEVER GROUP RICKETTSIA INFECTION TO U.S. MILITARY PERSONNEL

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This report describes an investigation to characterize the risk of spotted fever group rickettsia (SFGR) infection to U.S. military personnel by evaluating: 1) 10,000 sera from military members for the presence of antibodies to SFGR by a *Rickettsia rickettsii* antigen adsorbed ELISA and 2) 1,399 ticks removed from individuals presenting to medical clinics on military bases throughout the country for the presence of rickettsiae by genus- and species-specific real-time PCR assays. It was ascertained that 6.0% (597/10,000) of military personnel sera tested were positive for SFGR-specific antibodies. This SFGR seroprevalence is similar to that reported from a previous study performed on pre- and post-deployment sera collected from 865 military personnel during Operation Desert Storm

(9.8%) and on sera collected from U.S. civilian populations (4-10%). In the second part of the study it was determined that of 808 *Dermacentor variabilis* and 220 *Amblyomma americanum* ticks evaluated none were identified as having *Rickettsia rickettsii* the causative agent of Rocky Mountain spotted fever (RMSF). However, 47 of the 808 (5.8%) *D. variabilis* were infected with rickettsiae and 17 (2.1%) of these were *R. montanensis*, and one (0.1%) was *R. felis*. The other 29 (3.6%) rickettsiae were not identified further to the species level. Of 44 pools containing 5 *A. americanum* individuals/pool 35 pools (87.5%) were positive for *R. amblyommii* and no other SFGR. Moreover, 246 (66%) of 371 additional *A. americanum* ticks tested were positive for *R. amblyommii*. The high prevalence of antibody to SFGR among U.S. military personnel and the high prevalence of SFGR infected ticks recovered from humans detected in this study suggests that U.S. military personnel are at risk of infection with SFGR but the risk of infection solely due to *R. rickettsii* appears to be low. This conclusion is especially important in light of the recent reports of the previously thought to be non-pathogen *R. parkeri* causing SFGR disease in military personnel.

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HUMAN ANTIBODY-REACTIVE EPITOPES ON THE CONSERVED 47 KDA ANTIGEN OF *ORIENTIA TSUTSUGAMUSHI* AND THEIR SIMILARITY TO EPITOPES ON HUMAN SERINE PROTEASE

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Scrub typhus is an acute, febrile disease caused by infection with *Orientia tsutsugamushi*. One of its immunodominant antigens is a conserved 47 kDa protein, a homologue of heat shock protein HtrA. To identify the human antibody-reactive epitopes on this antigen, a series of overlapping decapeptides encompassing the whole Karp strain protein were synthesized by solid phase pin technology. A modified ELISA was used to measure the immunoreactivity of sera from scrub typhus patients to each of the peptides. All of the five patient sera tested reacted with peptides located near the N-terminus and from amino acid 377 to the C-terminus. Two peptide clusters and three peptide clusters were identified near the N-terminus and C-terminus, respectively. The reactivity of each serum toward the central part of the protein was highly patient specific. This central part (aa 85-235) exhibited a high degree of sequence homology with human serine protease11. Collectively, 10 peptide epitopes were identified in this region. Previously we constructed a DNA vaccine plasmid expressing the conserved 47 kDa antigen of Karp strain (pKarp47) which provided 70-100% homologous protection, 80% protection against three antigenically unrelated strains, only partial protection against three other strains and no protection against another ten strains in a mouse challenge model. Although immunized mouse sera did not react with the recombinant human protease11, the identification of peptide epitopes with sequence homology to human protein has raised concern about possible autoimmune responses if this antigen were used as a vaccine candidate. Elimination of the central part of the 47 KD antigen to avoid introducing potential cross-reactive epitopes may enhance the safety of the vaccine candidate.

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ARTEMISININ DERIVATIVES ACCUMULATE WITHIN DIGESTIVE VACUOLE-ASSOCIATED NEUTRAL LIPID BODIES IN *PLASMODIUM FALCIPARUM*

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Artemisinin (ART) is one of the most valuable antimalarials currently available. The activity of ART against Plasmodium is due to cleavage of the endoperoxide bridge by ferrous heme iron predominantly located in the parasite digestive vacuole (DV). This reaction initiates the formation of cytotoxic ART intermediates that may alkylate heme and proteins. To provide further insight into the targets of ART, we used microscopic imaging to study the cellular distribution of novel fluorescent ART derivatives in living malaria parasites. Exposure of *P. falciparum*-infected erythrocytes to the trioxane derivative (12C) resulted in rapid accumulation of fluorescence within neutral lipid bodies (NLBs) associated with the DV exterior. Pre-treatment of cultures with ART demonstrated a 75% decrease in total accumulation of 12C signal within NLBs. In contrast, pre-treatment with deoxydihydroartemisinin (DeoxyART), an inactive derivative lacking the endoperoxide, had little effect on 12C localization. Additionally, application of a fluorescent deoxy-dimer derivative (DeoxyASR) failed to produce NLB-associated fluorescence, confirming necessity of the endoperoxide pharmocophore for the observed drug accumulation. TLC analysis of parasite lipid extracts following exposure to ART and ART derivatives supported peroxidation, as opposed to alkylation, of lipids. This was confirmed in parasites through microscopic evaluation using an oxidation sensitive lipid probe. In the parasite DV, NLs associate with heme and promote hemozoin formation. We propose that ART activated by heme iron within this NL environment may increase heme-catalyzed peroxidation of these vital cellular components. This process could be essential to the antimalarial capability of ART and its derivatives.

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A LARGE RETROPOSON FAMILY IS INVOLVED IN THE REGULATION OF GENE EXPRESSION IN THE PROTOZOAN

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Leishmania are unicellular parasites that exist in two developmental stages: free-living promastigotes in the alimentary tract of a sandfly vector and intracellular amastigotes residing in phagolysosomes of mammalian macrophages. These developmental stages display distinct morphologic and metabolic characteristics, consistent with a highly regulated level of differential gene expression, which is central to the parasite's intracellular survival. In *Leishmania* stage-regulated gene expression is often mediated by sequences within 3'-untranslated regions (3'UTRs), since these parasites have lost the ability to regulate transcription initiation. Using in-silico screening and bioinformatic analyses, we have recently identified two new families of widespread extinct retroposons (more than 2000 in the *Leishmania* genome), LmSIDER1 (**S**hort **I**nterspersed **DE**generated **R**etroelements) and LmSIDER2, that are predominantly located within 3'-untranslated regions of *Leishmania* mRNAs. We investigated the regulatory potential of these elements, using microarray analyses, reporter gene assays and polysome profiling studies and found that members of the LmSIDER1 family are associated with stage-specific translational regulation by enhancing binding of SIDER1-containing mRNAs to highly translating polyribosomes. Interestingly, members of the LmSIDER2 retroposons are also involved in the regulation of gene expression, however, they act on the level of mRNA stability and promote destabilization of SIDER2-bearing mRNAs. Microarray analysis combined to actinomycin D assays indicate that several SIDER2-containing *Leishmania* transcripts are low abundant and short lived, suggesting a common mechanism to regulate multiple genes in a coordinated manner. The considerable expansion of SIDERs within 3'UTRs and their role in regulating gene expression support that *Leishmania* have recycled and probably expanded these elements to fulfill critical regulatory functions. We are currently investigating the mode of action of these widespread retroposons and the underlying molecular mechanisms that govern developmental gene regulation in this important human pathogen.

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THE TOXOPLASMA GONDII ORTHOLOGUE OF TIC20 (*TGTIC20*) IS ESSENTIAL FOR PROTEIN IMPORT, APICOPLAST BIOGENESIS AND PARASITE SURVIVAL

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Apicomplexan parasites contain a plastid organelle called the apicoplast. The apicoplast is a validated drug target that likely functions in several essential metabolic pathways. The majority of apicoplast proteins are nuclear-encoded, and must be directed to the apicoplast following translation. Apicoplast-targeted proteins typically have an N-terminal signal peptide that likely directs co-translational import into the ER. A second N-terminal domain known as a transit peptide then directs targeting of apicoplast proteins from the ER into the apicoplast stroma. Although the features of apicoplast transit peptides have been studied in some detail, little is known about the molecular mechanisms of protein targeting to the apicoplast, in particular how proteins cross the four delineating membranes of the organelle. We have mined the *T. gondii* genome for proteins known to be involved in protein import into plant plastids. Here we describe a *T. gondii* orthologue of the plant Tic20 protein, which forms part of the translocon of the inner chloroplast membrane (Tic) complex in plants. We demonstrate that *Tg*Tic20 is a membrane-spanning protein of approximately 20 kDa that localises to the inner apicoplast membrane. We have generated an inducible *Tg*Tic20 knock-out strain. We show that *Tg*Tic20 is essential for parasite growth, and that disruption of this gene leads to defects in protein import into this organelle, and subsequent defects in apicoplast biogenesis. Our results suggest that *Tg*Tic20 likely functions in protein import into the apicoplast. In the absence of apicoplast protein import, most apicoplast functions will be disabled, and we are now in the process of further characterising this apicoplast protein import mutant to gain an understanding into the functions of this organelle.

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ELONGATION FACTOR 1A MEDIATES THE SPECIFICITY OF MITOCHONDRIAL TRNA IMPORT IN *T. BRUCEI*

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Mitochondrial tRNA import is widespread in eukaryotes. Yet, the mechanism that determines its specificity is unknown. The T-stem nucleotide pair 51:63 is the main localization determinant in tRNAs of *Trypanosoma brucei*. In the cytosol-specific initiator $tRNA^{Met}$ this nucleotide pair is identical to the main anti-determinant that prevents interaction with cytosolic elongation factor (eEF1a). Here we show that ablation of cytosolic eEF1a, but not of initiation factor 2, inhibits mitochondrial import of newly synthesized tRNAs well before translation or growth is affected. $tRNA^{Sec}$ is the only other cytosol-specific tRNA in *T. brucei*. It has its own elongation factor and does not bind eEF1a. However, a mutant of the $tRNA^{Sec}$ expected to bind to eEF1a is imported into mitochondria. This import requires eEF1a and aminoacylation of the tRNA. Thus, for a tRNA to be imported into the mitochondrion of *T. brucei* it needs to bind eEF1a and it is this interaction that mediates the import specificity.

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A ROLE FOR IRF-7 IN REGULATING THE INTRACELLULAR FATE OF *LEISHMANIA DONOVANI*

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Leishmania donovani infects a broad range of host cells, including monocytes, dendritic cells and, a variety of tissue- and microenvironment-specific macrophages and stromal cells. In spite of evident host cell heterogeneity, little is known about the outcome of infection of specific groups or the underlying mechanisms involved. To begin to address this question, we performed comparative genome-wide expression analysis and identified differential activation of the type I IFN cascade in cell lines that differed in their capacity to support *L. donovani* infection. The transcription factor IRF-7 is critically involved in the regulation of Type 1 interferon gene expression and triggering of IFNα/β production following challenge with many pathogens. As such, IRF-7 acts as a key regulator of innate immunity and subsequent adaptive responses. To test the hypothesis that regulation of IRF-7 may underlie the cell-specific responses to *L. donovani*, and confirm our initial analysis, quantitative RT-PCR analysis was performed. RAW264 macrophages, which maintain intracellular amastigotes over an extended period of time, accumulated minimal *Irf-7* mRNA over a 12-48h period post infection (p.i.). In contrast, amastigote burden was significantly reduced in the stromal macrophage cell line (14M1.4) and this cell mounted a robust and infection dose-dependent *Irf-7* response, detected from 12h p.i., Comparative analysis of intracellular amastigote division by CFSE dilution indicated that the response of 14M1.4 cells was likely to be leishmanicidal rather than leishmanistatic. Confocal microscopy revealed that IRF-7 protein is localised in the cytoplasm of uninfected 14M1.4 cells and co-localises with the adaptor protein MyD88 in punctuate vesicular organelles. By 48h p.i., MyD88 and IRF-7 dissociate, as IRF-7 is translocated to the nucleus and also appears to be recruited to amastigote-containing phagosomes. In addition to identifying a potential basis for host cell permissiveness to *L. donovani* infection, these findings suggest the possibility of MyD88-independent, IRF-7- dependent signal transduction from the *Leishmania* phagosome. Further studies to test this possibility are underway.

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PRESENCE OF AN IL-7Rα^{HI} MEMORY CD8+ T CELL POPULATION DURING PERSISTENT *TRYPANOSOMA CRUZI* INFECTION

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Trypanosoma cruzi establishes a persistent infection in mice despite a potent and highly focused *T. cruzi*-specific CD8+ T cell response. Chronic intracellular infections are known to significantly impact the phenotype and function of host memory CD8+ T cell responses and these changes are thus of interest in *T. cruzi* infection. The IL-7Rα chain is one of the early memory markers modulated by persistent antigen. CD8+ T cells responding to persistent pathogens generally fail to express IL-7Rα while those targeting cleared pathogens upregulate IL-7Rα. We examined IL-7Rα expression on parasite-specific CD8+ T cells during chronic *T. cruzi* infection in mice using MHC class I tetramers to identify CD8+ T cells specific for immunodominant and subdominant parasite epitopes. While the majority of splenic *T. cruzi*-specific CD8+ T cells fail to express IL-7Rα, between 15 and 30% expressed this receptor during the chronic phase of infection. These IL-7Rα^{hi} antigen-specific CD8+ T cells were also capable of producing IFN-α following peptide restimulation. Additionally, purified IL-

7Rα^{hi} CD8+ T cells from chronically infected mice were better maintained following transfer into naïve mice than their IL-7Rα^{lo} counterparts, suggesting that a stable central memory CD8+ T cell population capable of antigen-independent survival may be present in an environment where antigen persists. This population of central memory T cells likely comprise a subset of parasite-specific cells which have not recently encountered antigen and thus can preserve long-term T cell memory in situations where antigen is cleared - such as after successful drug treatment. Indeed, we have shown that a *T. cruzi*-specific CD8+ T central memory population emerges in mice cured with the drug benznidazole. These results suggest that long-term T cell memory can be maintained even in the face of antigen persistence during *T. cruzi* infection. Retention of such a memory population could have implications for long-term protection in individuals successfully treated with benznidazole.

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CHARACTERIZATION OF THE APIAP2 DNA-BINDING PROTEINS IN *PLASMODIUM FALCIPARUM*

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The mechanisms underlying transcriptional regulation in apicomplexan parasites remain poorly understood. In addition, genome sequence data has revealed a dearth of specific transcription factors in *Apicomplexa*. Conserved regulatory sequence motifs that are sufficient for gene expression in *Plasmodium* have been poorly characterized and their cognate DNA-binding proteins remain unknown. We have initiated the characterization of the role of a predicted family of putative transcriptional regulators – the Apicomplexan AP2 proteins (ApiAP2) in *Plasmodium* development. ApiAP2 proteins contain AP2 domains homologous to the second largest family of transcriptional regulators in plants. Plant AP2 proteins play key roles in development and response to environmental stress. ApiAP2 proteins in *P. falciparum* show stage-specific gene expression patterns spanning the 48-hour intra-erythrocytic development cycle (IDC). We hypothesize that the ApiAP2 proteins may be the master regulators responsible for the coordination of gene expression throughout the IDC. Using a high-density protein-binding DNA microarray, we have assayed the DNA binding properties of AP2 domains from several ApiAP2 proteins. Our results demonstrate that isolated AP2 domains from ApiAP2 proteins in *Plasmodium* bind unique and highly specific DNA sequences found only in *Apicomplexa*. Furthermore, we have computationally examined the 5' upstream region of all plasmodium genes, and find that sequence motifs bound by our ApiAP2 proteins are significantly enriched in genes sharing similar stage-specific gene expression. These genes likely represent potential downstream targets of our ApiAP2 proteins. This study provides the first example of *Plasmodium* proteins that specifically bind DNA and lays the foundation for exploring the role of ApiAP2 proteins during development. AP2 proteins may prove to be ideal anti-malarial targets, as they have no counterparts in mammalian systems.

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POLYADENYLATION STABILIZES TRANSLATIONALLY-COMPETENT MRNAS IN TRYPANOSOME MITOCHONDRIA

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Polyadenylation is a ubiquitous mechanism regulating mRNA stability, although the functions of the poly(A) tail are phylogenetically divergent and may vary between cellular organelles. In human mitochondria poly(A) tails stabilize mRNAs, whereas in plant mitochondria and chloroplasts polyadenylation serves as a degradation signal. We have identified the trypanosomal mitochondrial poly(A) polymerase, termed kPAP1, as a protein homologous to terminal uridylyl transferases (TUTases). kPAP1 is a developmentally regulated gene that is essential for mitochondrial function. The inhibition of kPAP1 expression results in loss of both the short (~20 nts) and long (~120-250 nts) poly(A) tails, followed by the rapid decay of non-edited and edited mRNAs. The stability of pre-edited transcripts, however, is unaffected by the lack of poly(A) tails. The uridine insertion/deletion editing directed by a single guide RNA is sufficient to switch the function of the pre-existing poly(A) tail from a neutral to a stabilizing signal. In the mitochondrial extract, kPAP1 exists as part of high-molecular weight complexes that interact with the 20S editosome and RNA Editing TUTase 1 (RET1). The recombinant kPAP1 and the affinity-purified kPAP1 complex are capable of adding only short A-tails to synthetic RNA substrates, but not the long poly(A) tails reported *in vivo*. We further demonstrate direct involvement of RET1 into mRNA processing via contribution to the synthesis of a unique 3' end structure. In our model, kPAP1 synthesizes a short poly(A) tail thereby creating a platform for the recruitment of RET1 and possibly other factors. A long (A/U) heteropolymer of ~ 100 nucleotides in length is then added to the short A-tail of fully edited mRNAs by the concerted action of RET1 and kPAP1.

1075

A CONSERVED BASIC GROOVE ON ALDOLASE MEDIATES MIC2 CYTOPLASMIC TAIL AND F-ACTIN BINDING

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Apicomplexan parasites rely on actin-based motility to drive host cell invasion. To power motility, actin filaments must be coupled to surface adhesions in the thrombospondin-related anonymous protein (TRAP) family. This crucial linkage is provided by the glycolytic enzyme fructose-1,6-bisphosphate aldolase. Prior investigations have demonstrated aldolase forms a critical bridge between actin filaments and the short, acidic, cytoplasmic tail of the adhesin. By virtue of its key role in glycolysis, aldolase is a highly conserved enzyme at the levels of sequence, structure, and function. Based on published crystal structures, we developed a molecular homology model of *Toxoplasma gondii* aldolase and identified a large, basic, surface-exposed groove along each monomer of the tetrameric enzyme. Guided by this model, we selectively mutated several charged residues to alanine. The basic residues identified in the current study are conserved in the *Plasmodium falciparum* aldolase and, based on co-crystallization studies, have been implicated to participate in the association with the C-terminus of TRAP. Homology modeling supports a similar interaction between aldolase and the MIC2 C-terminus. We tested this model using biochemical analysis, investigating three critical functions of aldolase: substrate catalysis, binding to the C-terminal tail of MIC2 and interaction with F-actin. Our studies reveal the aldolase binding surface for the MIC2 tail overlaps with the enzyme active site, yet adhesin binding and substrate catalysis can be separated as two distinct sub-domains on the surface of aldolase. In contrast, the interactions between aldolase and F-actin and the MIC2 tail appear to be completely overlapping. These findings identify specific mutations that will allow dissection of the role of aldolase in bridging to TRAP-adhesins *in vivo*.

1076

IDENTIFICATION OF A GPI-ANCHORED *THEILERIA SURFACE* PROTEIN POTENTIALLY INVOLVED IN CYTOKINESIS

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The apicomplexan protozoal parasites *Theileria parva* and *Theileria annulata* cause diseases in cattle known as East Coast Fever and tropical Theileriosis. Sporozoites invade leukocytes where they develop into a syncytial schizont, which resides freely in the cytosol and activates anti-apoptotic pathways and continuous host cell proliferation. Parasite and host cell cytokinesis are synchronized and the parasite appears to use the mitotic spindle apparatus to ensure its distribution over the two daughter cells. Recently, we have identified a parasite gene encoding a 34 kDa GPI-anchored protein (gp34) that may be involved in this process. Antibodies raised against gp34 label the schizont surface. When expressed in HeLa cells, gp34 localizes to the plasma membrane. Ectopical expression of soluble forms of gp34, revealed cell cycle-dependent co-localization with M phase structures (spindle poles, spindle microtubules and midbody) and also provoke host cell multinucleation in *Theileria*-infected macrophages. Induction of multipolar spindles in *Theileria*-transformed cells confirmed a spindle pole association of the parasite. We used GST-gp34 fusion proteins in pull-down experiments to screen for proteins known to be associated with M phase structures. Among the interacting proteins are γ-tubulin, Plk1 as well as important components of the chromosomal passenger complex and the central spindlin complex. We could demonstrate that gp34 functions as an in vitro substrate for Plk1 and GST-gp34 was found to bind in vitro translated forms of Plk1. These findings are strengthened by the observation that endogenous and ectopically expressed Plk1 localize to the parasite surface in a cell-cycle dependent manner. Taken together, these findings point towards a potential role for *Theileria* gp34 in the host-parasite interactions during host cell mitosis and cytokinesis.

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