TWO-DAYS MELOquine-ARTesunate COMBINATION THERAPY FOR UNCOMPLICATED MULTIDRUG RESISTANT FALCIPARUM MALARIA AMONG GOLD MINERS IN VENEZUELA

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In the last decade, Plasmodium falciparum has shown significant clinical failures to chloroquine, falciparum and quinine in Southern Venezuela. The Venezuelan Ministry of Health has decided in 2004 to switch to artesunin based combination therapy with mefloquine plus artesunate for 3 days. However, the main goal is the supervision of all doses of treatment (DOT). Since 30 to 40% of the malaria cases occur in mining areas, a two-days mefloquine-artesunate treatment was evaluated as an alternative for indigenous and illegal gold miners. The efficacy of the treatment was evaluated following the WHO in vivo protocol. Patients were followed up on days 1, 2, 3, 7, 14, 21 and 28. parasite clearance time and fever clearance time as well clinical and parasitological responses were evaluated. During 2005, a total of 51 patients were enrolled into the trial. Most patients presented an adequate clinical response and there was one clinical failure on day 28. All patients became afebrile and cleared parasitaemia by day 2. Mefloquine-artesunate 2 days represents an alternative treatment for special groups such as gold miners or indigenous people.

EMERGING PATHOGENS, INCREASED SOCIAL INTERCONNECTIVITY AND DEMOGRAPHIC COLLAPSE IN BAJA CALIFORNIA (1697-1830)

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When the Spanish friars established the first town in the peninsula of Baja California in October 25, 1697, the population of central Mexico was already decimated, as a result of the devastating epidemics, famines and wars of the sixteen and seventeen centuries that took 90% of the population. However, the population of Baja California remained intact at that time, with a robust size of 60,000 inhabitants. Yet, with the formal initiation of colonization and religious conversion of the peninsula in 1697, a rapid process of depopulation began. This process started 178 years after the arrival of the Europeans to Mexico. At this time, the coast of Sinola and Sonora, just one or two days across the Gulf of California, was densely populated and provided regular visitors to the coast of Baja California in the form of explorers, pirates, smugglers and soldiers. Why did the local populations resist the introduction of measles and smallpox until that time? This delay can be explained by how the native population was distributed throughout the region. The inhabitants of the peninsula were organized in small nomadic groups, fighting constantly with their neighbors for resources. This isolation worked as a barrier for transmission of human-to-human diseases. One of the first objectives of the Spanish priests was to build a major road associated to a chain of Missions along the territory and to concentrate the native nomadic groups in permanent settlements around the Missions. This proved fatal for the native population that was annihilated by a rapid succession of epidemics of measles and smallpox. Because the population size was not enough to sustain a permanent circulation among the settlers of the peninsula they were re introduced periodically from the continent, causing great mortality each time. After 133 years, the whole native population was extinct.

The introduction of new human airborne pathogens simultaneous to an increased intercommunication among members of a previously isolated population resulted in a demographic catastrophe. Although remote in time, these events constitute an interesting model for today's world: The emergence of airborne infectious diseases, and increased intercommunication by means of trade and travel. Are we repeating history?

VENTRICULO-PERITONEAL SHUNTING IN THE MANAGEMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS

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Over 80% of cryptococcosis cases occur in HIV-infected patients, and cryptococcal meningitis (CM) is a frequent AIDS-defining illness. Fever, malaise, headache, and various neurologic symptoms are common, and mortality is high. Treatment requires antifungals and frequent lumbar puncture (LP) to manage increased intracranial pressure (ICP). The neurosurgical literature encourages the use of ventriculo-peritoneal (VP) shunt placement in managing ICP in HIV-infected patients with CM, but surgeons are often reluctant to do so. We report successful use of VP shunting in an HIV-infected patient with CM who had failed repeated LPs. Case Report: A 32-year-old Honduran female presented with a 3-week history of fever, headache and diplopia. Neurological exam revealed nuchal rigidity, left 6th nerve palsy and no papilledema. Cerebrospinal fluid (CSF) analysis was consistent with CM, showing elevated opening pressure and high titer of cryptococcal antigen. The CD4 count was 27cells/mm. Liposomal amphotericin (LA) was started with minimal improvement. Repeated LPs provided relief of headache and diplopia after each tap. She completed therapy with LA and was discharged on highly active antiretroviral therapy (HAART). Two months later, she returned complaining of headache and diplopia. Repeated LP again showed elevated CSF pressure, and temporary relief was reported after each. Imaging studies of the brain failed to show classical features of increased ICP such as periventricular diffusion of CSF or hydrocephalus. A right parietal VP shunt was placed with complete resolution of symptoms. She was discharged on maintenance fluconazole and HAART and is currently doing well. In conclusion, neurosurgeons often are reluctant to implant a VP shunt in patients with HIV-infection and CM. Reasons cited include: poor prognosis and life expectancy, risk of infection, shunt obstruction, and theoretical peritoneal seeding with infected CSF. Ventricular size is often normal despite increased ICP and even after VP shunting no decompression occurs. It is postulated that cryptococca cause a "frozen" brain by coating it with polysaccharide, making the ventricles stiff and not readily able to change in size. A recent review in the neurosurgery literature encourages the use of VP shunting in managing ICP in HIV-infected patients with CM, and recommends it as a safe and reasonable treatment modality. Our case lends support to this approach.

EFFECTS OF GLUTAMINE ALONE AND IN COMBINATION WITH ZINC AND RETINOL ON INTESTINAL BARRIER FUNCTION, DIARRHEAL DISEASES MORBIDITY AND GROWTH IN UNDERNOURISHED CHILDREN IN THE NORTHEAST OF BRAZIL

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The objective of this study was to study the impact of glutamine, zinc, and retinol alone or in combination on intestinal barrier function, diarrhea and growth in undernourished children in an urban Brazilian community. After parental consent children aged 2 months - 8 years with a height-for-age z-score less than the median (-0.06) were enrolled and
randomized to receive zinc (40 mg twice weekly for 12 months), retinol (100,000-200,000 IU for children < or > 1 yo every 4 months), both or neither (ie placebo) for 12 months. 324 children were enrolled. After 1 mo, half of each group (all having been randomized into 8 blocks) were given glutamine (Q, 17.2 g/day) or placebo (glycine 8.3 g/day) for 2 weeks and retested for absorptive function, diarrhoea-morbidity, lactulose and mannitol excretion ratios (UM), weight and height were measured. Patients were initially similar in age, sex, nutritional status (except for HAZ in one group) and UMI. Q or zinc alone significantly improved (decreased) the serum L excretion up to four and 1.5 months, respectively. Zinc plus retinol improved L excretion at long-term follow-up 4 months. Q alone significantly reduced the excretion of M during the follow-up period and retinol alone reduced the percent M excretion at 4 months. Zinc alone and other combinations of micronutrients did not change the percent M excretion. Q plus retinol significantly improved (decreased) lactulose:mannitol ratio throughout the whole follow-up periods. Glutamine plus zinc also improved UMI at long-term follow-up 4 months. Placebo controls, glutamine, zinc alone, Q plus zinc or Q plus retinol had reduced HAZ z-scores during follow-up periods. However, Q plus retinol increased significantly HAZ z-score at 1.5 and 4 months periods of time. Q, zinc, retinol alone or Q plus zinc had a significantly improvement on WHZ z-scores during the follow-up periods. The proportion of diarrhea reduced significantly only in the Q combined with zinc and retinol. In conclusion, Q, zinc, Q plus retinol or zinc plus retinol supplements repaired intestinal barrier function in undernourished children. Q, zinc or retinol alone or Q plus zinc reduced wasting in undernourished children. Q plus retinol, but not other micronutrients alone or in combination, reduced stunting. The combination of Q, zinc, and retinol decreased diarrheal diseases morbidity in undernourished children in the northeast of Brazil.

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POTENTIAL USE OF MULTIPLE FREQUENCY BIOIMPEDANCE FOR DIAGNOSIS AND MONITORING OF LYMPHATIC FILARIASIS LYMPHEDEMA

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An important innovation in general lymphedema research and treatment was the development of multiple frequency bioimpedance (MFBI) technology that can simply and non-invasively yet accurately measure the volume of intra and extracellular fluid in a limb. A study of patients undergoing surgery for breast cancer showed that MFBI could detect lymphoedema up to ten months before it could be clinically diagnosed or detected by morphometric measurements. The instrument is portable, robust and able to be used in remote locations, making it ideal for diagnosing and monitoring lymphatic filariasis-associated lymphedema and trials need to be undertaken to assess its usefulness in this setting.

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BURDEN OF MALARIA IMPORTED CASES IN VENEZUELA DURING 2004-2005

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Venezuela is a malaria endemic country with different circulation of Plasmodium spp. in different States. Identification of imported malaria cases has from endemic areas within Venezuela and from contiguous countries has implications for malaria control programs and travel recommendations. Through the national malaria program, Ministry of Health, all cases of malaria were analyzed to assess the importance of imported malaria cases. The study period was from January, 2004 to December, 2005. All cases were microscopically confirmed at least twice by a quality control system. During the study period, a total 92,331 cases of malaria were recorded (45,655 in 2004 and 45,676 in 2005). From this total, 91,572 (99.1%) cases were autochthonous and 759 (0.9%) imported. Cases came from Colombia (55.2%), Guyana (26.9%), Brazil (4.1%), Nigeria (0.5%), Mozambique (0.1%), Haiti (0.1%) and South Africa (0.1%). A mean of 7 imported cases per week was observed during the period. Distribution of autochthonous malaria cases through the period was as expected but with the imported cases occurred a mean of 2.11%, with 5 important peaks that were observed during weeks 5th, 23rd and 46th of 2004 (these weeks accounted 20.63% of the year’s imported cases) and 23rd and 37th of 2005 (these weeks accounted 9.5% of the year’s imported cases). In 2004, cases from Colombia, Brazil and Guyana were only reported, but in 2005, cases from other Caribbean and African countries were notified. Strengthened surveillance such as the carried out by this study is helpful to assess the potential introduction of other Plasmodium species or resistant strains that may subsequently spread through transmission of local mosquitoes from countries where malaria is endemic as occurs with all the border countries with Venezuela.

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SEROSURVEY AGAINST RICKETTSIA RICKETTSII AMONG HEALTHY INDIVIDUALS OF IN VILLETA, COLOMBIA

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Rocky Mountain spotted fever has re-emerged in Colombia near the same locality where it was first recognized in 1937. We have reported three fatal cases and confirmed that Rickettsia rickettsii was the etiologic agent. This is the first study based on a probability-sample in Colombia. To determine the prevalence of positive antibody titters against R. rickettsii in rural inhabitants of Villeta, Cundinamarca - Colombia and its relationship with some demographic and epidemiological characteristics. Sera from 392 randomly recruited healthy adults, a representative sample of the rural population of Villeta were analyzed by indirect immunofluorescent antibody assay to detect IgG against R. rickettsii as antigen. A cutoff titer of 1:64 was used. A structured questionnaire and informed consent was filled and signed by all people involved. The seropositivity rate for R. rickettsii was 40.56% (159 of 392 samples, CI 95%: 35.6-45.6). We did not find any association between a positive test and gender, age, occupation, education level, building material of the house, ownership or free circulation of domestic animals in and out of the house, time of permanence in the area, or number of people living in the house. Seropositivity was less frequent among those who referred previous contact with the nymph (OR:0.53, IC95%:0.32-0.89) or larvae stages (OR:0.39, IC95%:0.20-1.66). The prevalence of positive antibody titters against spotted fever group rickettsiae in this Colombian locality is high; however, it remains to be established whether R. rickettsii or other less pathogenic rickettsiae explain these findings. The lack of association between a positive test and several demographic and epidemiological characteristics could be a reflection of unique features of this area (including the high prevalence of infection). The lower frequency of positive tests among those who had contact with larvae and nymphs (as determined by their ability to identify them), suggests that learning to identify these immature forms is a preventive strategy that needs to be investigated.
NUTRIKINE DYSREGULATION: A NEW PARADIGM FOR THE IMMUNODEFICIENCY OF MALNUTRITION

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Childhood malnutrition is associated with growth stunting, increased susceptibility to infection, and increased mortality. Thus, there may be a underlying immunologic relationship between growth failure and immunodeficiency. Previously, we have described a murine model of human wasting malnutrition which leads to: (1) increased visceralization after infection with Leishmania donovani; and (2) defective macrophage pro-inflammatory cytokine response (decreased levels of tumor necrosis factor-α, IL-10, and NO and increased levels of IL-6 after IFN-γ/LPS stimulation); and (3) an increased ratio of immunosuppressive prostaglandins compared to inflammatory leukotrienes after LPS stimulation. There are particular molecular links between the nutritional status and immune function. We have coined a new term for these: nutrikes. Nutrikes include the adipokines (leptin, adiponectin, and resistin), insulin-like growth factor-1 (IGF-1), corticosterone, and gherelin. Relatedly higher levels of leptin, IGF-1, resistin, and gherelin observed in a well-fed host would be expected to increase the pro-inflammatory response, whereas high levels of adiponectin and corticosterone (observed in malnutrition) would be expected to decrease the inflammatory response. Nutrikie dysregulation (abnormal levels of the aforementioned nutrikes) in the malnourished host may lead to defective macrophage priming, and a decreased pro-inflammatory response. Weaning mice received a diet deficient in protein, calories, zinc, and iron for 6 weeks and caged to mice on a control diet. Serum levels of leptin, IGF-1, and resistin were all significantly lower in the malnourished mice compared to controls (53%, 71%, and 22%, respectively). In contrast, levels of the immunosuppressive nutrikes adiponectin and corticosterone were significantly higher (60% and 287%, respectively) in the malnourished mice. Levels of gherelin were comparable. Thus, lower serum levels of immunostimulatory nutrikes (leptin, IGF-1, and resistin), and higher serum levels of immunosuppressive nutrikes (adiponectin and corticosterone) may be a factor in the defective macrophage pro-inflammatory response in the malnourished host and be a factor in the increased susceptibility to infection in the malnourished host.

A MYCOBACTERIUM BOVIS BRAIN ABSCESSES IN A 34-YEAR OLD HISPANIC MALE WITH AIDS

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Infection with Mycobacterium bovis has been rare in developed countries since the implementation of control measures in cattle and routine pasteurization of dairy products. In developing countries, however, it remains a significant proportion of mycobacterial infections. Consumption of unpasteurized dairy products from infected cows is the primary route of transmission to humans. Case: A 34-year-old Hispanic male with AIDS (CD4 count 22/mm³, HIV viral load 22,000 copies/mL) presented with a one week history of worsening left posterior headache, imbalance, and nausea with vomiting. The patient had emigrated from Mexico three years ago. He was from an urban area, with no direct contact with livestock and with no specific recall of consumption of unpasteurized dairy products. Physical exam was significant for a wide-based ataxic gait, dysmetria, and dysdiadochokinesia. MRI showed a ring enhancing bi-lobed right cerebellar mass with associated edema and midline shift. PP and serologies for Toxoplasma, Coccidiodomycosis were negative. Urine histoplasma antigen and cryptococcal antigen tests were negative. A chest radiograph showed no acute disease. A SPECT scan of the brain did not suggest primary CNS lymphoma. Lumbar puncture was deferred due to the risk of herniation. Empiric therapy for toxoplasmosis was started. As no clinical improvement was seen, an MRI brain was repeated on hospital day 14 that showed interval enlargement of the cerebellar mass with new daughter lesions. Neurosurgery subsequently performed a craniectomy, with evacuation and drainage of the abscess. The smear of the pus was 4+ for AFB, promoting empiric rifampin/isoniazid/pyrazinamide/ethambutol therapy. Ultimately, M. bovis was identified in cultures from the abscess and sputum. The patient was subsequently treated with a rifampin/isoniazid/ethambutol/fluoroquinolone and trimethoprim. In conclusion, disseminated M. bovis infection in patients with AIDS has not been commonly reported. Although the primary route of infection is via consumption of unpasteurized dairy products, infection can also occur by inhalation of infected aerosols from cows or aerosols generated during the handling of infected carcasses. M. bovis infection should be considered in any patient with these identifiable risk factors and also in patients from developing countries in which M. bovis remains endemic, even without these obvious risk factors.

WHERE THERE ARE NO HEALTH POSTS: THE CHALLENGE OF COMMUNITY-DELIVERED INTRAVENOUS ANTIMONIAL THERAPY FOR LEISHMANIASIS IN NORTHEAST BRAZIL

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Leishmaniasis presents in various clinical forms in northeast Brazil, including cutaneous, mucosal and disseminated disease. In the Corte de Pedra endemic area of Bahia, the health post serves as the main referral center for the diagnosis and treatment of leishmaniasis and attends to 600 cases per year. The post is located in an isolated agricultural region and serves a population of approximately 500,000 people. Standard first line therapy is intravenous antimony (20mg/kg) delivered once a day for 20 or 30 days, depending on the clinical form of the disease. Due to long distances, poverty and limited access to transportation or feasible routes due to poor weather and road conditions, many individuals cannot reach their nearest health post daily and instead acquire intravenous treatment through volunteers in their remote community. The informal delivery of intravenous therapy by community volunteers poses challenges to the safe and reliable treatment of leishmaniasis. Our experience has shown that community volunteers do not have formal training and may not consistently provide clean, safe injections of antimony, which is not a benign medication and may have serious adverse effects, such as cardiac arrhythmias. Community delivery of therapy also raises questions about the storage of antimonials, supply delivery, and the safety of community volunteers and their families with regards to the safe needle use and disposal. We have begun community-based trainings of volunteers who deliver intravenous antimony in the Corte de Pedra area. Volunteers are recruited with particular emphasis in areas of high prevalence. Lectures are delivered by health post staff on the basics of leishmaniasis, intramuscular and intravenous treatment for children and adults, safe needle use and disposal, and basic treatment of potential side effects. We also track the adherence to and delivery of antimonial therapy prescribed at the health post. Lastly, we follow up with trainees after 3-6 months in the field to reevaluate their performance and acquisition of knowledge and practice techniques. Community volunteers provide an important service in areas where there are no health care professionals to deliver intravenous therapy, and their support and training are crucial to leishmaniasis treatment in this rural area.
DETECTION OF HUMAN FECAL MARKER, CRYPTOSPORIDIUM, AND GIARDIA USING IMMUNOMAGNETIC SEPARATION IN CONTAMINATED WATER SAMPLES

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Due to the increased prevalence of pathogens in contaminated water supplies, there is a pressing need for a rapid yet sensitive assay that detects human fecal contamination. We examined human-specific fecal markers as an indicator of water contamination and developed a method for sequentially detecting human fecal markers, Cryptosporidium and Giardia in water. Isolation of human fecal markers, Cryptosporidium and Giardia from 8 ml water samples was done using immunomagnetic separation, in which paramagnetic particles were coated with analyte-specific antibodies. Commercially available anti-Cryptosporidium and anti-Giardia antibodies were used to detect these pathogens, while a procedure developed in collaboration with Techlab, Inc. was used to detect human fecal markers. Human fecal markers were separated initially, followed by Cryptosporidium and Giardia. The sensitivities of the individual assays were compared to the sequential detection using a quantitative LISA for local markers and quantitative PCR for Cryptosporidium and Giardia. Detection levels for human fecal markers, Cryptosporidium and Giardia were as low as 0.1 ppb (which approximates standards for fecal coliform levels), 10 oocysts/mL and 100 cysts/mL respectively. Preliminary testing done on drinking water samples taken from households in Fortaleza, Brazil revealed a number of highly contaminated samples (based on coliform counts) and showed that several were positive for human fecal markers and that one was positive for Cryptosporidium. In conclusion, antibody-coated immunomagnetic beads rapidly detect human fecal contamination in water samples less than 10 mL. Cryptosporidium and Giardia can also be detected with this method. Using the assays together to rapidly detect human fecal contamination and transmissible enteric pathogens can provide an early warning of water supply contamination helping to limit disease transmission.

CONTROL OF CLONORCHIASIS REQUIRES REPEATED CHEMOTHERAPIES

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The present study was undertaken to determine the best strategy of clonorchiasis control by repeated mass chemotherapies in China. Seven different control strategies were implemented, and all subjected residents (mass treatment) or egg positive cases (selective treatment) were treated with praziquantel repeated every 6 months, once or two years. The recommended dosage of praziquantel, 25 mg/kg, 3 times a day, was used. The egg positive rates in 3 heavy endemic villages were 44.8-70.0%. All subjected villages showed 72.8% to 92.0% reduction of egg positive rates after 2, 3 or 6 repeated chemotherapies. Mass treatments in 2001 and 2003 reduced the egg positive rate from 68.8% to 18.7% and 4 annual mass treatments reduced the rate from 44.8% (2001) to 8.7% (2004). Selective annual treatments reduced the rates from 50.8% (2001) to 13.8% (2004) or from 70.0% (2001) to 11.6% (2004), and two treatments in a year reduced the rate from 57.6% (2001) to 4.6% (2004). In moderate endemic areas, the egg rates were 22.6% and 28.3% in 2001, but 1.7% and 1.1% after 2 or 3 selective treatments. The repeated treatments reduced EPG counts significantly. Among treated residents, one showed drug eruption, and was treated at a hospital. The present results confirm clonorchisiasis is widely prevalent and heavily endemic along the rivers in Heilongjiang Province, China. The reduction of clonorchisiasis by one mass chemotherapy was about 50% of pre-treatment positive rates. The more treated showed the better reduction but mass or selective treatments showed no differences in endemic areas. The chemotherapy of reservoirs hosts showed little impacts on the control effect. Mass or selective chemotherapy is effective to control clonorchiasis in heavy endemic areas when the chemotherapy is repeated while one selective treatment is effective enough in moderate endemic areas.

PERSISTENCE OF BACTEREMIA BY BARTONELLA BACILLIFORMIS POST TREATMENT WITH CLORANFENICOL. ANCASH - PERU

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Bartonella bacilliformis (Bb) is an obligate intracellular bacterium that initially invades the endothelial cells and then the red blood cells, its target cells, in which it reproduces finally destroying them. Chloramphenicol was the antibiotic of choice for the treatment of the acute phase of bartonellosis (Carrion's disease) in Peru. However, there is not scientific evidence of its efficacy. The aim of this study was to determine the persistence of bacteria by Bb after the treatment with chloramphenicol. A cohort study was performed in the Hospital of Caraz, Ancash department, located in the northeastern highlands of Peru, between July and December 2003. All patients who had a positive culture for Bb were enrolled. A clinical-epidemiological questionnaire was completed and blood samples were obtained for blood culture, thin smear, hematological and biochemical tests. All patients who after finishing treatment with chloramphenicol PO for 14 days had a positive culture or positive PCR for Bb, or presented typical eruptive lesions of Carrion's disease were considered as persistence of Bb. A total of 66 patients were enrolled. Of them, 53% (35/66) were male, the median of age and the time of disease were 11 years (range 3 months-72 years) and 6.5 days (range 1-90 days) respectively. The samples for culture were taken between 36 and 408 days after the beginning of treatment with chloramphenicol. The half of patients (50%) persisted with the presence of Bb in blood, of whom 10.6% (7/66) had a positive culture for Bb. 28.8% (19/66) presented eruptive lesions, 7.6% (5/66) had a positive culture and eruptive lesions, and 3% (2/66) had a positive PCR. All thin smears taken after treatment were negative. The lethality rate was 0%. In conclusion, chloramphenicol, the antibiotic of choice for the acute phase of Bartonellosis in Peru, produces clinical amelioration among cases, however it is not efficacious to eliminate Bb from blood. Patients may keep the viability of Bb in blood for months, increasing the risk of transmission in endemic areas.

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MAGNITUDE OF THE FIRST OUTBREAK OF DENGUE FEVER IN THE DISTRICT OF COMAS, LIMA-PERU

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Dengue fever (DF) is currently one of the most important vector-borne disease in Peru. Outbreaks from some departments of the northern coast and jungle of Peru have been reported. Native cases of DF in the city of Lima, capital of Peru, have never been reported. Our aim of this study was to determine the magnitude of the first outbreak of dengue in Lima city and identify the dengue serotype involved. We made an analytical cross-sectional study in the district of Comas, located in northern Lima, department of Lima, between April and May 2005. We selected a random sample of the population, collected blood samples in filter papers to determine antibodies IgM and IgG against the dengue virus and applied a clinical-epidemiologic questionnaire. In turn, a 5 cc blood sample was obtained for viral isolation from febrile patients visiting a Ministry of Health facility in the involved area. All specimens were tested in the National Institutes of Health of Peru. A total of 295 voluntary subjects were enrolled. Of them, 36.7% was male and the median of age was 32 years old (range 1-72 years). The 27.5% (21.0-34.0%) had IgM antibodies and the 26.5% (20.3-32.9%) IgG antibodies against dengue virus. All subjects that had IgG also had IgM antibodies. The estimated population in the study area was 42800 (CI 95%: 37140-48460) inhabitants and the number of DF cases estimated was 11780 (7810-16485); nevertheless only 845 cases had been reported in the surveillance system. From subjects with positive IgM only did the 29.5% fulfilled the dengue case definition and of them only did the 48% looked for medical attention. The serotype identified was dengue D3. In conclusion, the magnitude of this outbreak by dengue virus D3 was greater than did we expect. Less than 10% of infected people were reported by the surveillance system. This finding may be explained by the high frequency of subclinical or asymptomatic cases among infected people and the lack of expertise from the health personnel to identify this condition.

ROLE OF HSP70 IN DENGUE VIRUS REPLICATIVE CYCLE

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Dengue virus is the causative agent of dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Dengue virosomes are enveloped and icosahedral containing a single stranded positive polarity RNA as genome. The unique open reading frame encodes three structural, C, prM and E, and seven nonstructural proteins. E protein is the most exposed protein and interacts with the receptor on the surface of the host cell. This first contact is a very important step in the establishment of dengue infection. Several authors have reported the interaction of dengue virus with different molecules such as heparan sulfate, DC-SIGN, a laminin receptor and some heat shock proteins such as GRF78, HSP70 and HSP90. Our group, using an affinity chromatography technique, found that HSP70 and HSP90 from neuroblastoma and monocyte cell lines bind to the E protein from dengue virus. Infection inhibition assays using antibodies against both proteins supported that HSP70 and HSP90 are part of the receptor complex in human monocytes and neuroblastoma cell lines. In an attempt to demonstrate firstly the function of HSP70 in dengue virus entry we used the interferent RNA technology to knock down the expression of this molecule. The RNA-mediated knock down expression of HSP70 caused an important reduction in viral yield. Studying the ability of dengue virus to bind and enter into the silenced cells will allow us to propose a role of HSP70 in dengue virus replicative cycle and in viral entry.
VECTOR COMPETENCE FOR DENGUE 2 VIRUS IN WILD COLLECTIONS OF Aedes aegypti FROM VENEZUELA

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Aedes aegypti from 8 collections in Venezuela were orally challenged with dengue 2 virus JAM1409 (DEN-2 JAM1409). In contrast with Aedes aegypti populations from Mexico, United States and Colombia, the vector competence of Venezuelan collections ranged from 77-95%. The presence or absence of a midgut infection barrier (MB) and a midgut escape barrier (MEB) was determined in each collection. The percentage of mosquitoes exhibiting an MB ranged from 2-15%, and those exhibiting an MEB ranged from 2-18% in the collections.

CLINICAL AND MOLECULAR CHARACTERIZATION OF DENGUE PATIENTS COHORT IN RECIFE, BRAZIL FOR EPITOPE MAPPING AND IMMUNO PATHOGENESIS STUDIES

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Aiming to enable more efficient development of dengue vaccines and identification of dengue disease diagnostic markers, we intent to characterize the human immune elicited by dengue virus infection, focusing on the comparison between the dengue and dengue hemorrhagic fever syndromes. Our study cohort is composed of dengue fever and dengue hemorrhagic fever (DF/ DHF) volunteers from the city of Recife-PE, Brazil that are enrolled among patients with clinical diagnoses of DF. Volunteers are examined on the first and fourth days of symptoms, with additional evaluations if necessary. Each case is collected 4 to 5 blood samples, from day 1 to day 30 from the start of the symptoms and one additional sample six months later. The clinical characterization includes clinical history and examination, hemogram, platelet count, liver enzymes, HLA typing, MB2 and IL-18 SNPs analyzes, dengue serology (IgM and IgG), virus isolation and dengue quantitative PCR diagnosis and serum cytokine data (IL-2, IL-4, IL-10, TNF-α and IFN-γ). The study data is integrated in a customized database that includes complete clinical data, research results and the respective inventories of cryopreserved samples of PBMCs, plasma and serum. Our cohort studied enrolled 471 patients. The first serum samples from all patients were submitted to RT-PCR, virus isolation in C6/36 cell line, ELISA-IgM and ELISA-IgG. Serology (IgM and IgG) was performed in all additional serum samples collected, a total of 1512. Among these 471 patients, 239 (50.7%) are female and 232 (49.3%) male; age ranging from 5 to 84 years old, being 103 cases <15 years and 366 ≥15 years old. From a total of 230 (48.3%) laboratory positive cases, 89 (38.2%) were classified as primary and 89 (38.7%) secondary dengue infections. Among these 230 cases, 18 (7.8%) were classified as DHF, according to the WHO criteria and in contrast to what would be expected, 9 (50%) of our DHF cases were primary dengue infections. 62% of the RT-PCR samples collected before 5 days were positive, whereas only 38% of the samples collected after day 5 were positive. DENV-3 was the predominant serotype isolated and this fact has facilitated many of the studies. These samples and clinical data are being used for the development and validation of the dengue molecular diagnostics, epitope mapping of immune responses, dengue immunopathology, and the ex vivo evaluation of the immunogenicity of candidate vaccines.

INACTIVATION OF DENGUE VIRUS USING HIGH HYDROSTATIC PRESSURE (HHP)

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Although several approaches are being investigated, no licensed vaccine to prevent dengue currently exists. In this study we investigated the use of High Hydrostatic Pressure (HHP) to inactivate a live dengue-2 virus. One advantage of HHP is that complete virus inactivation can be achieved under controlled conditions without exposure of the virus to radiation or chemicals such as formalin. DENV-2 was propagated in Vero cells and the culture supernatants were concentrated approximately 50 to 100-fold. The concentrated virus was sealed in polyethylene pipettes and subjected to different conditions of varying pressure, temperature, and time of inactivation. Following treatment, residual virus infectivity was determined by direct plaque assay and by a 14-day amplification assay on Vero cells. Viral antigenicity was measured by ELISA using monoclonal and polyclonal antisera. As a measure of functional activity, investigators determined the ability of the treated virus to hemagglutinate goose red blood cells. Of the 31 high-pressure conditions tested, 19 conditions resulted in complete virus inactivation from initial infectivity titers as high as 7 log10 PFU/ml. There appeared to be a small loss in antigenicity of the HHP-inactivated virus compared with untreated and formalin-inactivated virus; the loss was difficult to quantify. Both HPP- and formalin-inactivated virus also lost the ability to hemagglutinate goose red blood cells. Immunogenicity of HPP-inactivated virus will be tested in a murine model and compared with formalin-inactivated and vaccines.

CHARACTERIZATION OF MICROVESICLES FROM IMMUNE CELLS INFECTED WITH DENGUE VIRUS

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Microvesicles were produced in large amounts after dengue virus infection of human immune cells. We characterized these particles by sucrose gradient ultracentrifugation and transmission electron microscopy. They are electron-dense particles with 70-90 nm in diameter. By Mass Spectrometry (MS), Flowcytometry and PAGE the microvesicles showed quantifiable differences between infected and infected cells. Microvesicles were labeled with fluorescent probes for lipids (PKH-26, Sigma) and for RNA (SYTO9 RNAselct, Invitrogen). Lipid probes were used to monitor microvesicle transfer to HUV-ECs. RNA labeling of microvesicles was resistant to RNase A treatment, and positive for dengue virus by qRT-PCR, suggesting virus particles were protected inside the microvesicle. All microvesicles derived from PBMCs, B, monocytes, myeloid derived dendritic cells were positive for CD45 by Flowcytometry, and for each microvesicle type the cell surface markers present in the progenitor cell were also found in the corresponding microvesicle (CD14, CD19, CD56, CD11a, PECAM-1 for Monocytes, B, NK, dendritic cells and CD4+ and HUV-ECs, respectively). All the microvesicles tested increased in net number after 48 hours of in vitro dengue infection at MOI of 1 but did not after IFN α treatment. Proteomics using MS quantitative analysis (iTRAQ) determined the proteins that were differentially present.
in microvesicles of human B cells infected with dengue virus. Membrane, cytoplasmic and nuclear proteins were identified. Of those, plasma membrane proteins were confirmed by Flowcetometry. The implications of microvesicle formation and fusion during dengue infections could explain the pathological conditions of the disease, including activation of coagulation and immune modulation. Plasma and sera from acutely infected dengue patients with fever had 20-fold increased CD14 and CD19 positive microvesicles obtained with the procedure used for in vitro studies. Microvesicles from Monocytes and B cell resulted from the infection in vivo. Implications of microvesicle formation in dengue infection are discussed.

(ACMOP Abstract)

PRIMARY HUMAN MYOBLAST ARE TARGETS FOR DENGUE VIRUS INFECTION

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One of the main features of dengue fever is myalgia, involving the muscles of back and limbs. Therefore, the disease has been described as “Break Bone Disease.” It is paucity of literature regarding the involvement of muscle during dengue viral infection. This study demonstrates dengue virus infection of primary human myoblasts. FACS analysis detected dengue virus in primary myoblasts (0.3-6%) infected for 48 hours at MOI of 1. In addition, electron microscopic studies show presence of virus in D-V infected primary myoblasts. These observations were supported by the TaqMan RT-PCR analysis of D2V. Increased gene expression levels of six innate response genes (Nmda-5, CXCL6, IRF7, IDO, TRAIL and Lgal3Ep) and up-regulated surface expression of MHC-I suggests myoblasts response upon infection. Previous studies have demonstrated that skeletal muscles in mice can be infected with adapted dengue virus leading to ultrasturctural changes in skeletal muscles and biochemical changes physiologically. Also, recent human study has observed myocytosis in dengue patients during acute viral infection. Our study indicates that these observed changes in skeletal muscles might be an outcome of dengue virus infection of myoblasts.

(ACMOP Abstract)

TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) REDUCES DENGUE VIRAL LOAD IN HUMAN INFECTED CELLS

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TNF-related apoptosis inducing ligand (TRAIL) was identified from Affymetrix gene expression analysis of in vitro infections and from PBMCs of dengue patients as a potential link between the IFN type I, II and TNF-α response by comparing different cell permisive to dengue virus (HIV/ECs, Monocytes and B cells). A significant increase in dengue viral progeny was observed in anti-TRAIL Ab treated monocytes, B cells and HIV/ECs and conversely a decrease in viral progeny was seen in recombinant TRAIL treated monocytes. Type I and Type II interferons regulate dengue virus progeny. We found that IFN-α mediated antiviral effects was partly dependent on TRAIL function. Thus, this study identifies TRAIL as a novel antiviral molecule able to limit dengue virus spread.

(ACMOP Abstract)

QUASI SPECIES OF DENGUE RNA: MOLECULAR DISSECTION OF DENGUE VIRUS INFECTIONS IN MONOCYTE-DERIVED HUMAN DENDRITIC CELLS

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Dengue is a single-stranded, enveloped RNA virus that infects human dendritic cells (DCs) primarily at the immature stage of their differentiation. In this study, the growth kinetics of dengue 3 virus in human DCs was assessed. We have shown that dengue 3 virus infects human DCs as a DC-SIGN, C-type lectin dependent process via dengue specific real-time RT-PCR quantification. The majority of dengue infections in dendritic cells were blocked by anti-DC-SIGN antibodies, but not by antibodies to other molecules on these cells. In contrast to real-time RT-PCR, agarose-gel based electrophoresis of dengue cDNA identifies genotypes within allelotypes (based on size polymorphisms) derived from dengue infected DCs at 48, 96 and 144 hours post-infection. The presence of various NS5 deletions (500-600s bps) in addition to the intact non-deletion NS sequences was identified from DCs 48 and 96 hours post dengue infection. However, no detectable NS5 deletion was detected 144 hours post dengue infection. Similarly, it was also found that blocking of dengue 3 infections by anti-DC-SIGN antibodies eliminated the heterogeneous dengue NS5 RNA species, i.e., RNA population with NS5 deletions. The observed quasi dengue RNA species is both time and DC-SIGN dependent. The rise and fall of the quasi dengue RNA species in DCs coincided with DC maturation. For example, the peak cytokine production was observed at 144 hours post infection. The presence of quasi dengue RNA species might reflect a strategy that dengue virus uses to minimize host responses.

(ACMOP Abstract)

MULTIPLEX DENGUE TQAUMAN ASSAY (DEN1-4) FOR THE DIAGNOSIS OF ACUTE DENGUE SECONDARY INFECTIONS

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Dengue is an arthropod-borne flavivirus composed of single stranded RNA of approximately 11kb and is subdivided into four distinct serotypes (DEN1-4). Dengue is hyperepidemic in Puerto Rico therefore most dengue infections are secondary infections. The Centers for Disease Control and Prevention surveillance system has determined the circulating dengue viruses in Puerto Rico for the last 30 years. The present study was a retrospective study of secondary dengue infections in which we randomly identified 100 candidates from each serotype that were confirmed using standard tissue culture isolation and immunofluorescent assay (IFA) for virus identification from the last ten years. These samples were used to validate the results of a multiplex TaqMan assay for all four dengue serotypes. In addition, we measured the antibody response of these sera using a MAC-RUSA at these early time points. These data determined that circulating viral titers during the first 5 days post onset of symptoms (DPO) were elevated throughout the entire 5 days determined by the multiplex TaqMan assay. In addition, the minimum cycle threshold value determined from the TaqMan assay for virus detection could not be related to the qualitative IFA results. Because virus isolation results were based on a qualitative determination of the amount of viral antigen present in serum infected cells, the results did not correlate. These results demonstrated the limitations of viral isolation during secondary infection because of the presence of cross protective neutralizing antibodies that may inhibit the infection of the cells used to amplify the virus. We concluded that the multiplex TaqMan assay was a valid assay for dengue serotype diagnosis in acute serum specimens and was more sensitive than virus isolation assay for secondary infections. However both assays should
be used for confirmation of a diagnosis of dengue due to the potential of contamination of the TaqMan assay because of its sensitivity and the decreased sensitivity of the virus isolation assay following secondary infections. In addition, because TaqMan assay is quantitative and relatively rapid test compared to virus isolation, viral load can be measured in the serum and could be used as an early and predictive measure of disease severity.

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SEROEPIDEMIOLOGY OF HOSPITALIZED DENGUE PATIENTS IN THE PHILIPPINES

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Dengue is a major health concern in the Philippines. Although dengue has been a notifiable disease in the Philippines since 1950s, there remains a scarcity of dengue data due to the limited diagnostic capabilities. We determined the seroepidemiologic profile of patients admitted with dengue-like illness to San Lazaro Hospital, a tertiary infectious disease referral hospital in Manila. Sero-type determination was conducted using RT-PCR and nested PCR. Patients were characterized as having acute dengue virus infection (primary or secondary) based on the IgM/IgG ELISA. Between September 1, 2005 to January 17, 2006, a total of 275 patients were screened and 104 patients were enrolled in the study; 90 (87%) were confirmed to have dengue. There were 6 (7%) primary and 84 (93%) secondary infections. Forty (38%) were female and 64 (62%) were male. The mean age was 18 years, with 50% of cases between 12-20 years, only 3% were < 7 years and 1% > 35 years. There was no significant difference in the clinical manifestations and laboratory parameters between the pediatric and adult age groups. Most study participants were diagnosed with DHF gr II (49%) and DHF gr I (25%). Fever was the most common presenting manifestation (100%) followed by loss of appetite, headache, and muscle pains. Hemorrhagic tendencies were seen in 86 (95%) patients. DEN-3 was the predominant serotype, though all 4 serotypes were identified during the study period. There was equal distribution between pediatric and adult groups; this may reflect changing epidemiology of dengue in terms of age affected. Similar observations have been noted in Latin America and Southeast Asia since the 1980’s. Studies have shown not only an increase in dengue incidence rates among young adults but also a shift in peak dengue mortality. This apparent shift suggests later exposure to dengue viruses. This epidemiological change may have important implications for health service planning and dengue control strategies. However, further study is warranted to determine if there is a real shift in modal age or whether such trend is the result of improved detection and diagnosis of dengue among the adult population.

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ACTIVATION OF COAGULATION AND FIBRINOLYSIS IN DENGUE VIRUS INFECTION: RELATION TO THE BLEEDING SYMPTOMS

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Dengue virus infection may result in a wide range of clinical manifestations, from mild to severe with hemorrhage and vascular leakage. Blood mononuclear cells are targets of dengue virus and diminished platelets count is a consistent finding. There are abnormalities in the coagulation and fibrinolytic processes that contribute to the pathogenesis and severity of the disease. We focused our study in the activation of coagulation process. A cohort of 113 patients with dengue (diagnosed by RT-PCR and serology) and 38 non-dengue viral infection was studied. Mean age was 25 +/- 16 (SD). Abnormal bleeding were petechiae, mucous bleeding, positive tourniquet test or vaginal bleeding and they were present in 77% of cases, 4 cases with severe leaking. The following tests were done for all cases at the febrile period until 72 h of apyrexia and at convalescence: platelet count, plasma thrombin time (TT), PT, aPTT, fibrinogen (Fbg), D-dimer (DD), factor II fragment 1-2 (F1-2), von Willebrand Factor (vWF), thrombin-antithrombin complex (TAT) and soluble Tissue Factor (TF). For 70% of all dengue cases TT was higher with increased vWF and DD. In febrile phase there is a significant rise in TAT, at defervescent Fbg and platelets reached their minimal values. This characteristic behavior was more marked in the group with hemorrhagic manifestations. The TAT results suggests that activation of coagulation starts at the febrile (viral replication phase) followed by a fibrinolysis response (increased DD and a negative correlation of Fbg with DD). The hemorrhagic symptoms are a sign of severity, prolonged TT and TAT are precursory signs of outcome and prolonged TT can be used for differential diagnosis in dengue infections.

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YUCATAN MINIATURE SWINE DEVELOP DENGUE VIREMIA AND ARE A POTENTIAL MODEL FOR DENGUE IMMUNOPATHOLOGY

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Evaluation of dengue vaccines has been limited to date by availability of adequate animal models, in particular for dengue disease and protective immunogenicity. Due to their physiological and immunological similarities to humans, we chose Yucatan Minipigs as a novel species in which to study dengue virus infection and resultant immune responses. We now expand upon our successful development of the first intermediate animal model for dengue viremia and report immunopathologic features of the swine model. We previously established a dengue viremia model using subcutaneous (SC) inoculation of swine. In the current work we expand the earlier results by evaluating intravenous (IV) inoculation as well as re-challenge of previously infected animals. Swine were inoculated with 1x10^7 PFU of virus by central IV and to evaluate the impact of administration route. Presence of viremia was tested using cell culture isolation and RT-PCR. In contrast to SC inoculation, no animals inoculated IV developed detectable viremia, though all animals developed antibody responses as determined by EUSA and neutralization assays. In subsequent experiments, previously inoculated animals (both SC and IV primary inoculation) were re-challenged by SC inoculation approximately 6 months after initial infection to assess the degree of immunologic protection. Unexpectedly, 5 of 6 animals previously infected SC, and 4 of 4 animals previously inoculated IV, developed a pronounced petechial rash upon SC rechallenge with virus. Cutaneous manifestations developed between day 3 and 4 post-inoculation and resolved by day 10. Histopathologic examination of skin biopsies revealed intradermal inflammation. Dengue immunoreactive immune complexes were detected in the sera of all animals that developed rash, but none of contemporaneously inoculated primary infection controls. In conclusion, Yucatan Miniature swine are susceptible to experimental dengue viremia and develop immune responses following dengue infection. Induction of viremia is dependent upon route of administration, suggesting the importance of initial target cells in initiating productive infection. Rechallenge of previously sensitized animals results in a cutaneous disease phenotype resembling skin lesions in human dengue infection, and the cutaneous findings are associated with dengue-containing immune complexes.

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DEN GUE-2 AND YELLOW FEVER VIRUSES INDUCE GENE EXPRESSION CHANGES IN THE MIDGUT OF THE DISEASE VECTOR, A EDES AEGYPTI

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Dengue and yellow fever viruses are two flaviviruses that are extensively studied due to their ability to cause human disease. These viruses seem to be mainly studied in the mammalian system with little effort going towards the understanding of how these viruses act within the vector. By studying these viruses within the vector itself, we can dissect pathways that these viruses utilize within the host for entry, replication, and release. We are using Aedes aegypti, the vector that is largely responsible for transmission of these viruses, to further understand the pathways involved in viral infection. Using microarrays previously constructed in our laboratory, we compared both Dengue-2 (Jamaica 1403 strain) and yellow fever (Akits strain) infected midguts with noninfected at 4, 7, and 14 days post infect. There were a few genes that showed a dramatic increase following viral infection, including a Tshin binding protein and a synaptic vesicle protein. These genes are suggested to be involved in immunity and viral release, respectively. We are currently conducting studies on viral entry and release using specific inhibitors. These viruses are able to use host machinery for entry, replication, and release. By knowing what pathways are exploited by the virus, we may be able to block viral spread at the site of the vector.

DEVELOPMENT OF A DEN GUE ELISA-BASED MICRO NEUTRALIZATION ASSAY

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Dengue virus infections occur in most of the tropical and subtropical areas of the world, and dengue is considered as one of the most important infectious diseases. Various serological techniques have been used for the detection and quantification of neutralizing antibodies to dengue. The plaque reduction neutralization test (PRNT) is the gold standard to measure dengue-neutralizing antibodies. However this test is labor intensive, time-consuming, and carried out in 6 or 24-well plates, which limits its usage for epidemiological and immunological trials especially in the context of vaccine development and large-scale evaluation studies. To address the above shortcomings, we have developed an ELISA-based micro neutralization assay for the detection and quantification of dengue neutralizing antibodies. The principle of this assay is similar to PRNT. However, the new assay is carried out in 96-well plates with optical density measurements and does not require viral plaque counting. This assay uses dengue virus serotype specific primary and peroxidase-labeled secondary antibodies with a chromogenic soluble substrate for viral antigen detection in the infected cell monolayer. We have optimized the ELISA based microneutralization assay for the four serotypes of dengue virus and demonstrated that this assay is sensitive and reproducible. A concordance study was conducted to evaluate the agreement between ELISA-based and PRNT methods with a panel of human serum samples. Our results indicate an acceptable relationship between the neutralizing antibody titers obtained by ELISA-based micro neutralization assay and standard PRNT.

HIDDEN TRANSMISSION OF DENGUE VIRUS IN KAOHSIUNG SCHOOL CHILDREN DURING 2004-2005 AFTER THE 2001- 2003 LARGE-SCALE EPIDEMIC OF DENGUE/DENGUE HEMORRHAGIC FEVER IN TAIWAN

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The severest epidemic of dengue fever (DF) and dengue hemorrhagic fever (DHF) occurred in Kaohsiung, Taiwan in 2002 [5387 DF, 242 DHF, 13 dengue shock syndrome (DSS), and 21 deaths]. It is interesting to investigate whether the dengue virus (DENV) might circulate silently among school children after this large-scale epidemic. This study was to measure the seroprevalence and seroconversion rates and the magnitude of silent transmission in primary school students and to investigate whether fever alert in communities might be feasible to detect the trends of DENV transmission in cities in advance. A prospective cohort was used to recruit grade 2-3 school children located in high or low 2002 epidemic areas and also considering population density of Kaohsiung. Serum samples from 8-to-11 year-old children were collected on June 29th, 2004 and Jan 17th 2005 for both pre-epidemic and post-dengue epidemic time points, respectively. The results found the overall seroprevalence rates of DENV infection before the 2004 epidemic season (1342 children:11 schools) and after the 2004 epidemic season (612 children 4 schools) were 4.55% (61/1342) and 5.56 % (34/612), respectively. Among the 174 paired serum samples, four children from three different schools were seroconverted as anti-dengue IgG (+). Thus, the overall seroconversion rate was 2.30% (4/174). Interestingly, the geographical distributions of seroprevalence of DENV infection among these children’s schools were parallel to the incidence rate of the dengue cases in 2001-2002. Besides, we used the five serological assays, including the three different ELISA assays at Taiwan-Centers for Disease and Prevention for capture dengue and Japanese encephalitis IgM and IgG and serotypes by NS1 indirect IgG plus plaque reduction neutralization test (PRNT) and western blot to confirm the results at the National Taiwan University. In addition, mostly ELISA positive samples were primary DENV-2 infection. The paired-serum samples show the consistency between PRNT and NS1 IgG ELISA serotyping. Most importantly, the five symptomatic DENV infected children showed different symptoms: fever, abdominal pain, cough, running nose, fatigue, and nausea. Risk factor analysis found that the family history of DENV infection was statistically significant with the children acquired the DENV infection (p=0.0371). In conclusion, our seroconversion rate (2.30%) was much lower than those reported from Thailand in 1999-2000 and Singapore in 1996-97.

DISTRIBUTION AND TISSUE TROPISMS OF THREE PHENOTYPICALLY DISTINCT YELLOW FEVER VIRUS STRAINS IN A EDES AEGYPTI

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Arbovirus dissemination from the midgut of a vector mosquito is a critical step in facilitating virus transmission to a susceptible host. We have previously characterized the genetic determinants of yellow fever virus (YFV) dissemination from the Aedes aegypti mosquito midgut using two genetically and phenotypically distinct strains of yellow fever virus (YFV) the wild-type, disseminating YFV AK1 strain and the attenuated, midgut-restricted YFV 17D vaccine strain. In our current study we examined the

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tissue tropisms of three YF viruses in *Ae. aegypti*: Asibi, 17D, and a chimeric virus containing the Asibi M and E structural protein genes and 17D non-structural genes (17D/Asibi M-E). Mosquitoes were infected via intrathoracic inoculation or artificial bloodmeal and were sampled days 7 and 14 post-inoculation or days 3, 7, 10, 14, and 21 post-oral infection for paraffin embedding and serial sectioning. YFV antigen was visualized in the mosquito tissues by immunohistochemical staining. In orally infected Asibi and 17D/Asibi M-E mosquitoes, virus antigen was observed in the posterior and anterior midgut, cardia, salivary glands, fat body, and nervous tissues. As expected, 17D infection was limited to cells of the posterior midgut following oral infection. Differences in virus distribution in the posterior midgut between disseminating and non-disseminating viruses have been observed, and we have identified tissues important in virus amplification and dissemination. These observations will be discussed in the context of the hypothesized progression of events which occur during productive infection of *Ae. aegypti* with YFV Asibi.

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A MULTIPLEXED REAL-TIME QUANTITATIVE RT-PCR ASSAY FOR ARTHROPOD-BORNE FLAVIVIRUSES

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Arboviruses in the genus Flaviivirus are major causes of human disease worldwide, and therefore it is essential to be able to rapidly and accurately test for them. Classical methods of detection using serologic or immunologic techniques are typically insensitive or require biological containment facilities in order to manipulate infectious cultures. Molecular detection assays based on broadly reactive (degenerate) primers also tend to have low sensitivity for many flaviviruses. In this study, we designed an assay to test for many flaviviruses using species specific primers in a single reaction. This real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay combines flavivirus ssp-specific Taqman primers and probes (some previously described) into a single tube subjected to standardized thermocycling conditions. The multiplex assay contains specific detection for St. Louis encephalitis, West Nile, Dengue 1, 2, 3, 4, and 5, and consensus sequences for Tick-borne encephalitis complex groups: Russian Spring-Summer encephalitis and Central European encephalitis viruses. Viral RNA was extracted from infected cell-culture derived stock viruses, field samples, or was synthesized as subgenomic target RNA molecules. All flavivirus species and sample types were detected by the multiplex assay. A sensitivity analysis of the assay suggested that the multiplex was no less able to detect low virus titer samples than the single pathogen assay. This technique allows for a collection of specific assays to be used to screen for the presence of many flaviviruses of interest while saving labor and reagents, and without sacrificing sensitivity. The results demonstrated that these viruses can be screened for specifically using the rapid and sensitive method of real time qRT-PCR.

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HETEROLOGOUS INTERACTIONS OF WEST NILE VIRUS AND ST. LOUIS ENCEPHALITIS VIRUS: EFFECT ON REPLICATION AND VIRAL GROWTH KINETICS

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West Nile virus (WNV) has moved rapidly through the US since its introduction in 1999. Following the arrival of WNV to Florida in 2001, increased findings of WNV in the state surveillance system have coincided with a decrease in reports of Saint Louis encephalitis virus (SLEV). WNV is a flavivirus closely related to SLEV, which is endemic to Florida, and may be utilizing the same hosts and vectors as SLEV for transmission. These viruses may be interacting in a competitive manner for resources in this transmission system, and this interaction could partially explain the reduction in detected SLEV activity. To explore this possibility, we evaluated the effect of simultaneous and sequential infection on WNV and SLEV growth kinetics in vitro. When SLEV was introduced prior to WNV, SLEV infection was enhanced compared to when SLEV was introduced at the same time or after WNV inoculation. The same pattern was apparent for WNV, although WNV growth rates and titers were higher than those of SLEV when introduced simultaneously with equal titers of SLEV. We also tested asymmetric and equal multiplicities of infection (MOIs), monitoring viral titer over time. When introduced at asymmetric MOIs, the virus added at a lower MOI was inhibited by more than 10-fold versus control infections, with growth of the virus delayed over time. Results from in vivo co-infection studies will also be presented.

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FURTHER CHARACTERIZATION OF A WEST NILE VIRUS SMALL PLAQUE VARIANT ISOLATED IN NEW YORK, 2000

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A small plaque variant (SP) of West Nile virus (WNV) was selected from an isolate (WT) made from the kidney of a dead crow in New York in 2000. We previously reported growth of SP and WT viruses in vitro, in DF-1 and C6/36 cells maintained at different temperatures, and in vivo in mosquitoes, chicks and mice. SP had lower rates of replication in Vero and DF-1 than WT, but grew equally well as WT in C6/36. In Culex pipiens, WNV SP had lower infection and dissemination rates following peroral infection and a higher ID50 after intrathoracic inoculation. The mean virus titers of WNV SP in mosquito bodies were significantly lower than WT. Chicks demonstrated delayed peak viremia and lower viral titers in blood following infection with WNV SP compared to WT. Current studies are investigating whether the differences previously observed in viral growth between SP and WT in vitro were due to temperature of incubation or cell line. Further studies in mosquitoes indicate decreased rate of viral replication and lower peak titers after three days post-inoculation. Adult house sparrows, natural hosts of WNV, were inoculated subcutaneously with 10^4 pfu SP or WT. The viremic response of the SP infected birds was variable, and the virus appeared to revert to WT plaque morphology. The average viremia titer of SP infected birds was lower than WT during the first 3 days PI, but then approached the WT titers. Peak viremia for SP occurred at days 3-4, for WT day 2. C3H mice were inoculated SC in the footpad with 10^4 and 10^6 PFU WNV SP or WT. WNV SP had significantly lower morbidity and no mortality, and a lower viremic profile compared to WT. Virus was recovered with consistent high virus load from brains and footpads of all WT mice at the time of death in one experiment and on day 7 in a second experiment. No virus was recovered from brains of SP infected mice on day 7 pi demonstrating that SP has lower neuroinvasiveness compared to WT. Further studies are underway to clarify pathogenesis in sparrows and mice.

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POST HURRICANE JEANNE MOSQUITO BORNE INFECTIOUS DISEASE SURVEILLANCE AND HUMAN WEST NILE VIRUS INFECTION IN HAITI IN 2004

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We established laboratory-based fever surveillance in the three clinics providing health care in Gonäive, Haiti following hurricane Jeanne in 2004. Patients who were febrile (temperature ≥ 38.5°C Centigrade) at the time of presentation were asked to provide blood for a serum sample and thick and thin malaria smears. The treating physician completed a brief medical history and physical on each of these patients and indicated the discharge diagnosis and treatment. All patients were asked to return in two weeks for a convalescent blood sample. Malaria smears were stained and read

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using standard methods. To diagnose dengue, we used an IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) to detect anti-dengue IgM antibodies in all serum specimens. All the serum specimens were also tested for the presence of anti-dengue IgG antibodies to determine previous exposure to dengue. In paired samples, a full titration of four-fold dilutions of serum specimen was used for the IgG ELISA and the end-point titration of these samples was used to assess seroconversion. To determine the presence of dengue viral RNA in acute serum samples (≤ 5 days post-onset of symptoms) we used both a nested polymerase chain reaction (PCR) and TaqMan assay. Because of the cross reactivity of flavivirus antibody tests, we utilized a plaque reduction neutralization test (PRNT190) to determine the specificity of the antibody response to the infecting virus. The serum was tested against West Nile virus (WNV), St. Louis Encephalitis (SLE) and dengue 1-4 viruses. In conclusion, of the 116 acutely febrile patients tested, three were diagnosed with malaria, two with acute dengue, and two with acute WNV infection. This is the first report of human cases of WNV in Haiti.

PERSISTENCE OF WEST NILE VIRUS IN EXPERIMENTALLY INOCULATED ANIMALS

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Most RNA viruses cause a transient infection and are subsequently cleared from the host. Recently we found persistence of West Nile virus (WNV) RNA in house sparrows and pigeons six months after experimental inoculation. Our long-term research goal is to understand the mechanism of WNV persistence. Six-week-old, female C57BL6 mice were inoculated subcutaneously (SC) with 10^4 PFU WNV, and eight to nine WNV-inoculated mice and one mock-inoculated mouse were sacrificed at monthly intervals. Brain, spinal cord, spleen, skin, lymph nodes, kidney and heart were harvested for virus isolation and WNV RT-PCR. Virus isolation was performed by co-culturing tissue homogenates on vero cells, followed by blind passages on vero cells. At one month post-inoculation (p.i.), all eight WNV-inoculated mice had infectious virus in at least one of seven tissues. Infectious virus was found most frequently in the skin. WNV RNA was found more frequently than infectious virus, and all eight mice were positive for WNV RNA in the brain, spinal cord and skin. At 2 and 3 months p.i., no infectious virus was detected, but WNV RNA persisted in at least one tissue for all eight mice at 2 months and 8 of 9 mice at 3 months. WNV RNA was detected most frequently in the brain, spinal cord and skin. A similar study was conducted in one-day-old chickens inoculated SC with 10^4 PFU WNV. The seven surviving chickens and one mock-inoculated chicken were sacrificed at one month. In contrast to the mice, no infectious virus was detected in any of the tissues from the chickens. WNV RNA, however, was detected in at least one tissue for all seven WNV-inoculated birds and was detected most frequently in the heart, brain and spinal cord. All tissues from mock-inoculated birds and mice were negative for infectious virus and WNV RNA. Sequence analysis and further studies to examine viral persistence in mice for up to one year post-inoculation are in progress. In summary, WNV can persist in diverse vertebrate hosts, and these results have potential implications in organ transplantation and overwintering of virus.

EFFICACY, DURATION, AND ONSET OF IMMUNITY OF A WEST NILE VIRUS CHIMERA VACCINE

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The purpose of this research was to demonstrate the efficacy, duration and onset of protection of a chimera vaccine using a model of induced West Nile Virus (WNV) clinical disease in horses. Horses were inoculated one time with a 1 ml dose of vaccine virus that represented a 1X serial release dose for short term efficacy, duration and onset of immunity. Horses were monitored after vaccination daily for injection site reactions, general health, including temperature, pulse and respiration rate, and neurological condition. Efficacy and duration of the vaccine was demonstrated by challenge of vaccinated and control horses with virulent WNV using a model that induces signs of neurological disease that are consistent with those observed in horses infected with WNV under natural field conditions. Data from the efficacy studies with horses vaccinated one time with a 1 ml dose of vaccine showed a 95% protection against severe neurological disease, including encephalitis, compared to controls and a 100% protection against viremia compared to controls. Data from the duration of immunity studies with horses vaccinated one time with a 1 ml dose of vaccine showed a 95% protection against severe neurological disease, including encephalitis, compared to controls and a 100% protection against viremia compared to controls. Additional efficacy studies using the same challenge model demonstrated onset of immunity as rapid as 10 days post vaccination. A live West Nile Virus Chimeric vaccine has been developed that provides protection of horses against severe neurological disease and viremia as demonstrated using a model of induced West Nile Virus clinical disease.

CHANGES IN GENE EXPRESSION PROFILES IN BRUGIA MALAYI L3 INDUCED BY CULTURE AND RADIATION

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Brugia malayi (Bm) L3 are developmentally arrested in mosquitoes; they resume development in mammalian hosts. Irradiated L3 fail to develop normally in mammals and have a special ability to induce protective immunity. The purpose of this study was to examine changes in gene expression that occur when mosquito-derived BmL3 (L3i) are cultured for 2 days (L3c) under conditions that mimic the mammalian environment and permit molting (37°C in MEM/10%FCS) and also to examine how gene expression in L3c compares to that in L3i that are cultured after irradiation (L3ir). The Version 2 B. malayi oligonucleotide slide array contains 17,300 elements that are derived from fialarial ESTs and predicted ORFs that cover ~85% of total Bm genes. Arrays were co-hybridized with labeled L3 cDNAs (either L3i with L3c or L3ir with L3c). A gene was considered to have upregulated expression relative to the comparator if it showed a ≥ 2-fold hybridization signal with P < 0.01. 353 genes were upregulated in L3i relative to L3c. These included putative immune evasion genes (such as Bm-Cpi-1, All-2, Bm-Mol-1, Serpin, Bm-Mif-1) and putative parasitism genes (ES22/24, SOD and TPX). 165 genes were upregulated in L3ir relative to L3c. Some 40% of these genes/clusters were also upregulated in L3i. The overlap genes include many novel genes with unknown functions. Most of the putative immune evasion genes were downregulated in L3ir, but genes for DNA repair and genes encoding the highly immunogenic Ovegi and Juv-p120 were upregulated. This suggests that the immunogenicity of L3ir may be related in part to their failure to down-regulate certain genes that are highly expressed in L3i. 244 genes were involved in transcription, translation, and growth (e.g., ribosomal and RNA
bining proteins and structural proteins such as actin and tubulin). These preliminary results suggest that expression profiling may shed light on the biological basis of irradiated L3 vaccines and on critical adaptations that occur when filarial L3 enter mammalian hosts.

(ACMCP Abstract)

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### VACCINATION WITH ONCHOCERCA VOLVULUS GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (OVPDH) USING THE MOUSE/LITOMOSOIDES SIGMODONTIS MODEL

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Recent studies identified Ov-GAPDH as a possible vaccine candidate molecule against human onchocerciasis. In this study the immunogenicity and protective potential of vaccination with Ov-GAPDH was tested in BALB/c mice infected with the rodent filaria Litomosoides sigmodontis, the mouse model of human onchocerciasis. BALB/c were vaccinated with either Ov-GAPDH DNAiP alone or in combination with the recombinant protein. Challenge of immunized and infection of naive mice with L. sigmodontis was performed 20 days post boost with 40 infective larvae by artificial infection (s.c. injection) or by natural infection via mites. Both types of vaccination led to protection in a subgroup of immunized mice affecting to a significantly higher degree female worms than males. The course of infection was associated with distinct ratios of Ov-specific IgG subclasses pre and post challenge. Apart from protection, enhanced susceptibility as well as pathology or combinations of both occurred. Pathology was observed in artificially infected mice irrespective of immunization. Recent studies in mouse models of autoimmune disease and inflammation emphasize the relevance of the relative proportion of antigen-specific IgG subclass responses with respect to Fc receptor-mediated effector mechanisms. Our results suggest that the same principle applies to a filarial infection, whereby the pattern of the antigen-specific IgG subclass response is relevant for the determination of protective immune responses, pathology or both.

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### TISSUE MIGRATION OF BRUGIA PAHANGI THIRD STAGE INFECTIVE LARVAE: AN IN VITRO MODEL

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Mosquito borne third stage infective larval (L3) of filarial nematodes emerge from the mosquito labella into a drop of haemolymph during mosquito feeding. They reportedly migrate into the host through the vector produced wound and then through various connective tissues of the skin to a lymph or blood vessel. Earlier published studies have demonstrated the capacity of Brugia to penetrate a variety of tissue types rapidly. Nothing is known regarding the mechanisms associated with this process. An in vitro model was developed attempting to quantify this early pre-migratory phase in order to define the parasite molecules involved in this process. Skin from gerbils (Meriones unguiculatus) was cut into 1cm circles and inserted into the top of a blind well chamber and sealed. Different procedures were used to prepare the surface of the skin. These included: clipping, puncturing, shaving, tape stripping and combinations of each. RPMI 1640 without sera was used to fill the bottom of the chambers and 200µl were placed in 100µl of media on the top of the chamber. Chambers were incubated for 1, 2 and 3 hrs at 37°C in 5% CO2. Larvae were counted in the upper and lower chambers after incubation. The total number that penetrated the skin was calculated. Large numbers (>80%) of L3 rapidly (1hr) migrated through the punctured skin and this system was discounted. L3 also migrated into and through the skin prepared using all other methods. Depending on the initial preparation this resulted in 69.9-29.7% entry and 17.2-14.4% complete migration of L3 through the skin. Histologic examination of the skin showed larvae in all tissues. It appears that larvae penetrate minimally disrupted epidermis and in some cases hair follicles. Initial attempts to alter this migration with anti-L3 excretory-secretory antibodies were not successful.

(ACMCP Abstract)

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### MAJOR SPERM PROTEIN: A POTENTIAL TARGET FOR ONCHOCERCIASIS VACCINATION AND DETECTION

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While a great deal of effort has been devoted to regional programs aimed at reducing the risk of onchocerciasis, significant obstacles remain which must be overcome prior to successful eradication. A key hurdle is the improvement of current diagnostic methods to monitor infection and possible recurrence. Great strides in detection technologies have been made using various assays including skin biopsies, detection of parasite DNA, and antibody-based strategies for specific parasite proteins. However, none of the current methods are capable of detecting sexually mature parasites. Major sperm protein (MSP) is the major component of nematode sperm and is critical for sperm motility. Because MSP represents one of the unique features of nematode reproduction, we propose that it is a potential target for diagnosis of nematode infection. In addition, while it is accepted that MSP polymerization is important for nematode motility, it is debated how this protein polymerizes. Evidence exists indicating that MSP dimer could be a single unit of MSP polymer, suggesting that antibody-mediated disruption of MSP polymerization could result in inhibition of nematode reproduction. We will present our results on the bacterial expression of O. volvulus MSP2 as well as the biological properties of this protein. Furthermore, our efforts aimed at isolating human monoclonal antibodies specific for MSP2 will also be discussed.

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### ANTI-WSP IGG1 ANTIBODY RESPONSE PREDOMINATES IN CHRONIC LYMPHATIC FILARIAIS

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Wolbachia have been believed to play an important role in the pathogenesis of filariasis. In order to evaluate their roles in the adaptive immune responses, we performed an ELISA to monitor IgG production (IgG1 and IgG4) against recombinant Wolbachia surface protein (WSP) in patients with lymphatic filariasis. The effect of diethylcarbamazine (DEC) treatment of the disease on the antibody levels was also studied. Seventy-five serum samples were collected from the endemic areas of the western region of Thailand. The antibody responses against WSP appeared to be IgG1 subclass, but not IgG4. The levels and seroprevalence of anti-WSP IgG4 antibodies were not significantly elevated among filarial groups, while the levels and seroprevalence of anti-WSP IgG1 antibodies were significantly elevated in individuals with chronic filarial symptoms. In contrast to filarial-specific IgG4 response which predominated in asymptomatic microfilaria-positive individuals, IgG1 antibody response to WSP was not found to increase in either asymptomatic microfilaria-positive and antigen-positive nor microfilaria-negative and antigen-positive individuals. DEC treatment had no effect on the levels of anti-WSP IgG1 and IgG4 antibodies in microfilaremic patients with adverse reactions. In
conclusion, the presence of anti-WSP IgG1 antibodies was found to be associated to chronic filarial, but not to parasitological status in patients. The specific antibody response may play a role in the pathogenesis of lymphatic filariasis, but not in the adverse reactions.

(ACMCP Abstract)

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**TEMPERATURE-INDUCED DIFFERENTIAL GENE EXPRESSION PATTERNS IN THIRD STAGE BRUGIA MALAYI LARVAE**

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Little is known about the changes in gene expression that occur as infective filarial larvae transition from the vector to the human host. To examine one aspect of this transition, 1,500 Brugia malayi L3 were cultured at room temperature in serum-free medium or at 37°C in the presence of human serum for 4 hours to simulate the mosquito vector and human host, respectively. Following RNA isolation, cDNA was amplified and labeled with Cy3 or Cy5 for hybridization to a slide array spotted with 65-mer oligos corresponding to 3569 clusters from the Brugia malayi EST database. Three technical replicates were performed, including one dye swap. Expression was detectable for 6,016 genes, of which 325 gave concordant values among all 3 replicates. 114/325 (35%) genes were upregulated by at least two-fold in L3 cultured at RT, and 33/325 (10%) were upregulated by at least two-fold in L3 cultured at 37°C in the presence of serum. Although a majority of the differentially regulated genes showed no homology to known genes, upregulation of genes involved in protein synthesis, including ribosomal proteins, was more common in the L3s cultured at room temperature (10 of 114 vs 1 of 33 upregulated genes, respectively). Confirmation of the array results by quantitative RT-qPCR and further characterization of the differentially regulated genes is currently underway. Identification of genes involved in the transition of infective L3s from the insect vector to the human host could lead to the development of novel strategies to prevent filarial infection.

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**CRYOPRESERVATION OF A. CANINUM, A. CELYANICUM AND N. AMERICANUS INFECTIVE LARVAE AT -196°C**

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Hookworm Necator americanus, Angiostrongylus ceylanicum and A. caninum strains are maintained in our laboratories for research on human hookworm vaccine. To prevent an accidental interruption in their maintenance or to store infective L3 larvae as a backup for a strain not needed temporarily (without associated effort and expense of continuous maintenance on laboratory animals), we studied the cryopreservation of their (ensheathed) L3 larvae. Several cryoprotectant agents and two cryopreservation protocols with different cooling speeds were studied. In all experiments, the cryovials with larval suspension complemented with cryoprotectant agents were first equilibrated for one hour at room temperature then cooled rapidly by direct immersion in liquid nitrogen at -196°C. Alternatively, prior to storage at -196°C larvae were cooled slowly at -20°C overnight in mechanic deep-freezer. The larval survival was assessed by L3 motility test under stereo-microscope. The slow cooling protocol gave higher survival rates for infective larvae of all three hookworm strains. Overall, A. ceylanicum survival rates were higher than those of N. americanus. Cryopreserved A. ceylanicum larvae preserved the infective power for golden hamster. The L3 quality prior to freezing was of cardinal importance for larval survival regardless of the cooling protocol or cryoprotectant agent.

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**UNCARIASIS IN PANAMA**

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The objective of this study was to describe the epidemiology of geohelminthiasis in Alto y Baía, Veraguas, Panama. Previous voluntary acceptance of participation and informed consent, all subjects 2 years old and older were included in the survey. The study consisted in a questionnaire regarding various predisposing factors and visual evaluation of the house. Laboratory analysis included: complete blood count, cellular immunophenotypes and feces analyses (Kato-Katz and concentration). Alto y Baía is a rural community of 292 inhabitants allocated in 88 houses. A total of 228 (78%) subjects, 48% males and 52% females accepted to participate in the study. The mean age for the study group was 29 ± 1.65 years; 42% were younger than 15 years old. The majority of the subjects were housewives, farmers or students. Mean school attendance was 2.7 ± 0.20 years and the family income was less than US$100 per year. About toilet facility, 82.8% of the subjects defecated in latrines, but 47% referred open-field defecation. It was observed that 84% of the subjects claimed to wear shoes, but only outside the house. A total of 188 subjects (84%) were positive for the presence of intestinal parasites in the fecal exam. Helminths were observed in 72 subjects (32%), protozoa in 38 (17%), and mixed infections were detected in 78 (35%) of the participants. The most frequent helminth infection observed were caused by: Necator americanus [52% (117)] and Ascaris lumbricoides [33% (73)]. In all age groups evaluated, hookworm infection showed a prevalence of 40-61%. With respect to intestinal parasite infection, latrine use was a protector factor [0.41 (CI95% 0.17-0.91)] and open-field defecation a risk factor [2.21 (CI95% 1.04-4.74)]. In the group of subjects 12 years old and younger, mean hemoglobin concentration was 12.7g/L±0.13, in 10.5% (4/38) of the subjects the levels were lower than 12g/L, and all the positive subjects for intestinal parasites showed eosinophils percentages higher than 5%. In conclusion, our study confirmed a high prevalence of intestinal parasite infections in the study area that correlated with eosinophilia. Lower Hb concentrations were observed more frequently in man than in women. Our preliminary findings support future studies for the control of soil-transmitted helminthiasis, based on regular anthelmintic treatment, health education and improved sanitation standards, until a vaccine is approved.

(ACMCP Abstract)

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IMMUNODIAGNOSIS OF STRONGYLOIDIASIS: SCREEN WITH ELISA AND CONFIRM WITH IMMUNOBLOT USING A RECOMBINANT ANTIGEN

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The diagnosis of strongyloidiasis remains difficult and the consequences of a missed diagnosis can be serious. The clinical features of infection can be subtle and non-specific. Both standard ova and parasite examinations and more specialized clinical laboratory concentration (Agar plate, Baermann extraction, Harada Mori culture, charcoal culture) are a biohazard and are insensitive compared to strongyloides serology. The sensitivity of the ELISA using crude antigen is high, however the specificity is low due to antibody cross-reactivity to antigens present in other parasites, such as filarial parasites, Ascasis lumbricoides, hookworm and Schistosoma sp. We compared three S. stercoralis antigens (one recombinant and 2 crude) with both ELISA and immunoblot. Sera from four clinical subsets were tested (S. stercoralis larva-positive patients, HTLV-1 S. stercoralis positive patients, eosinophilic esophagitis cases and travelers/minmigrants judged at risk for Strongyloides but negative for Strongyloides larvae). With the ELISA all three antigens demonstrated 100% sensitivity in the strongyloides larvae-positive, with or without HTLV1 positivity. The specificity for the crude batch 1, crude batch 2 and recombinant antigens was 67.75 and 85% respectively. The immunoblot using the recombinant antigen showed a specificity of 98%. Further measures of specificity with various helminthes are in progress. This recombinant antigen has the benefit of reproducibility. The ELISA with the recombinant antigen is sensitive and the Western blot appears specific.

(ACMOB Abstract)

DISSEMINATED HISTOPLASMOsis IN AIDS PATIENTS IN GUATEMALA: PRELIMINARY RESULTS FROM A SYMPTOMATIC PATIENT COHORT

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Systemic fungal infections represent a diagnostic challenge to the growing immunocompromised patient population. In many countries where histoplasmosis is endemic, the burden of this disease in AIDS patients is unknown. As a part of a histoplasmosis diagnostic assay evaluation, we recruited symptomatic AIDS patients in Guatemala City to assess for the presence of histoplasmosis. We present the preliminary descriptive results of the clinical characteristics and epidemiology of histoplasmosis affecting this population. The prospective study was initiated at Clinica Familiar Luis Angel Garcia (CLIFAC), where HIV-infected patients receive care in Guatemala City, in January 2005. Patients with symptoms of fever plus one of the following: weight loss, skin lesions, diarrhea, pancytopenia, hepatomegaly (>- spleenomegaly), or radiographic evidence of histoplasmosis were asked to participate (suspect histoplasmosis case). We performed routine diagnostic work-up which included blood and bone marrow cultures by regular methods, routine histopathology examination and histoplasmosis serology testing. We classified patients into confirmed, probable, and possible histoplasmosis case definitions based on results of these tests. Other diagnoses were infectious, non-infectious or unknown etiology. A total of 87 patients suspect histoplasmosis cases were identified; 52 were enrolled to date (67%). Their ages range from 18 to 54 years; 18 (34.6%) were female. The most common presenting symptom was fever (70%); 51% had pulmonary complaints, 56% had gastrointestinal complaints, and 41% had AIDS. Ten (19.2%) met confirmed case criteria, by culture. Serology results were available from 15; 5 met probable and 2 met possible histoplasmosis case definitions. Eleven (21, 2%) deaths were recorded in this cohort. In conclusion, in histoplasmosis endemic areas, among HIV-infected patients with systemic illness, the diagnosis of histoplasmosis is not infrequent and mostly achieved by culture. In settings where resources are limited, a rapid and reliable histoplasmosis diagnostic test is needed to decrease morbidity and mortality in this population due to delay in diagnosis and treatment.

IMPACT OF THERAPEUTIC REGIMEN FAILURE IN THE RESISTANCE TO ANTIRETROVIRAL DRUGS IN NORTHEAST BRAZIL

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Highly potent antiretroviral therapy is necessary to avoid viral replication in HIV patients, but also allows resistance mutations to appear. 41L, 67N, 70R, 210W, 215Y/F, 219E/Q, 44D and 118I are mutations defined as nucleoside analogues mutations (NAMs) because they compromise all Nucleoside Reverse Transcriptase Inhibitors (NRTI). 184V is important as it is associated with high level resistance to lamivudine. 103N is the mutation that occurs more frequently in Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) causing cross resistance to this class. 33RF, 82AF/FT, 84V and 90M are called protease inhibitor resistance-associated mutations (PRAM) because they cause cross resistance among Protease Inhibitors (PI). The objective of this study was to evaluate the development of resistance mutations and susceptibility profile to antiretrovirals in HIV-1 patients failing therapy. We evaluated 101 results of genotyping test from patients with therapeutic failure to 2- or 3-drug regimens with NRTI, NNRTI or PI. We utilized the Stanford Database for defined susceptibility profile. The samples were divided in three groups of failure: first (I), second (S) and multiafailure (three or more fails) (MF) to antiretroviral regimens and correlated the groups with the profile of resistance and main mutations. Increased resistance mutations 82AF/FT, IS4V, L90M, M41L, K70R, L210W, T215Y/F and K239Q/E were observed in MF (p<0.05). High resistance detected to zidovudine, didanosine, estavudine and abacavir in MF (p<0.05). There was not observed increase resistance to tenofovir (p=0.28) and lamivudine (p=0.075) in MF. There was predominance of high resistance to lamivudine and mutation M184V (p=0.24) in all patient groups. Predominance of high resistance to NNRTIs was also observed in all groups. In conclusion, accumulation of resistance mutations due to therapeutic failure will affect future options to HIV patients’ treatment in Brazil since it allowed significant increase in resistance to actual antiretroviral drugs in use.

VALUE OF THE NEOPTERIN CONCENTRATION AND THE NPT/CD4 RATIO AS PROGNOSTIC MARKERS OF AIDS IN HIV INFECTED PATIENTS

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Neopterin (NPT, pteridine derivative of guanosine triphosphate) is released into circulation from Interferon y (IFN-y) activated macrophages. Three categories of subrogate markers are used for prognostic evaluation in HIV infection: CD4 T-cell counts, viral load and markers of immune activation (cytokines, IL-2, NPT). In the current study we evaluated the
prognostic value of concentrations of plasma neopterin in the progression of the HIV infection and its correlation with others markers. We have evaluated CD4 T-cell counts, HIV viral load and neopterin levels in 108 blood samples including 88 HIV+ patients (with and without AIDS) and 20 HIV negative subjects. CD4 T-cell counts, HIV viral load and neopterin levels were evaluated using flow cytometry (Epics XL Coulter), the Amplicor HIV Monitor Test 1.5 (Roche) and by competitive ELISA (IBL), respectively. Mean neopterin concentration was 5.7 nmol/L in the control group, however, higher levels (45.8 nmol/L) were present in patients with AIDS without treatment. Strong direct correlation between CD4/CD8 and CD4/CD4+TP1 ratios was shown (r = 0.973) in patients with AIDS under HAART. The observed CD4/CD8 TP1 ratios were 182.2 (IC: 149.1-215.4), 57.9 (IC: 39.3-77.6) and 5.2 (IC: 0.6-9.8) in controls, patients with low viral loads (<100,000 copies/mL) and high viral loads (>100,000 copies/mL), respectively. Statistically significant differences (p<0.0001) in neopterin concentrations were observed in AIDS patients with respect to treatment. Strong direct correlation (r = 0.814, Spearman rank) between neopterin level and viral load was found in patients without AIDS, not receiving HAART; however, an inverse correlation was observed between neopterin levels and CD4 T-cell counts (0.485) in the same group of patients. Our results demonstrated the possible benefit of measuring plasma neopterin and the CD4/CD8 TP1 ratio as additive prognostic markers of progression in patients with AIDS.

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HIGH LEVEL OF POLYMORPHISM IN THE GAG REGION OF THE B-SUBTYPE HIV-1 STRAINS FROM NORTHERN VENEZUELA

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HIV-1 strains circulating in northeastern Venezuela and their relationship to the epidemiological characteristics of the patients. A total of 21 HIV-1 positive patients were chosen at random from three health care facilities in Sucre state. Viral RNA and proviral DNA were extracted using a commercial kit and the env and gag regions were amplified by nested PCR or RT-PCR, using the primers and protocols outlined by National Institutes of Health/AIDS. Molecular typing was carried out using the heteroduplex mobility assay (HVMA) and RFLP using Pst I restriction digestion. The studied patients were 12 males and 9 females, 71.4% were younger than 34 years age and 20% had homosexual behavior. Most of the individuals showed a low educational level, poor knowledge about the infection and declared that never used condoms. The epidemiological survey determines that all patients were infected through sexual intercourse, and they were infected in Caracas (15%), Margarita Island (10%), Bolivar (10%), Delta Amacuro (5%), Anzoategui (20%) and Sucre (40%) states. We found that all HIV-1 strains were of type B for the env region, while 85% were of type B, two were undetermined and one sample did not amplify for the gag region. The undetermined strains and the one that did not amplify were amplified for the RT gene and sequenced, showing homologies of 95.0, 93.6 and 86.2% to the reference type B strain. The RFLP analysis showed that no "Brazilian" type B strains were present in the sample. Polymorphism was seen in 5% of the type B strains for the env region but 45% for the gag region. Statistical analysis associated the type-B polymorphic strains to patients infected in Anzoategui and Sucre states. Simple and complex quasispecies were seen in 50 and 15% of the samples for the env and gag region, respectively. The clinical characteristics of the patients such as time of diagnosis, type of treatment, CD4 counts and viral load were not associated with the molecular characteristics of the strains.

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Molecular Characterization of Shigella and Plasmodium falciparum Co-occurrence in HIV-1 Seropositive Nigerian Children


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Shigellosis and falciparum malaria are clinically distinct causes of morbidity and mortality in Nigerian children with or without HIV seropositivity. There is currently paucity of scientific data to justify their co-occurrence as opportunistic infections and mediate evolution of new control and management strategies in Nigerian children with immunity compromised by HIV viremia. This study genotypes shigellosis and Plasmodium falciparum for plasmodium and msp1 diversity in children with HIV-1 seropositivity. Serogroup diversity and antigen of the shigella isolates were also determined. A total of 27 consenting HIV-1 seropositive Nigerian children aged 5 - 14 years (mean age = 6.4yr) presenting with diarrhea and fever (axillary temperature > 37.5°C) were enrolled into the study. Stool and blood samples of each child were cultured on conventional media with thick blood films analyzed for P. falciparum parasitaemia. Plasmodium were extracted from shigella isolates and characterized by alkaline lysis and electrophoresis respectively. While nested PCR was employed for msp-1 genotyping of P. falciparum. Of the 27 blood samples analyzed, 2 (7.4%) and 15 (55.6%) were positive for S. sonnei shigellosis and P. falciparum parasitaemia (GMFD = 13700 = 58500 parasites/µL) respectively. Stool cultures revealed mixed (3/27, 11.1%) and single shigella (8/27, 29.6%) (P < 0.05) constituted polymicrobial infections with S. flexneri exhibiting serogroup dominance (57.1% vs. 14.3 - 28.6%; P < 0.05) and isolates including E. coli and Salmonella serving as co-pathogens. Although all the isolates analyzed exhibited quinolone and quinolone sensitivity, the Shigella isolates were multidrug resistant with blood strains of S. sonnei exhibiting reduced susceptibility to ceftazidime (MIC = 10.0±6.0 µg/mL < 0.05) and ofloxacin (MIC = 0.2±0.06 vs. 0.08±0.01 µg/mL, P < 0.05) compared to strains recovered from stool cultures. The shigella isolates generally co-harbor low molecular weight plasmodiums of size 1.7, 2.0, 2.6 and 4.0kb but still display distinct extrachromosomal DNAs of 10.2kb in S. flexneri and 8.0kb in blood S. sonnei strains. The P. falciparum isolates exhibited msp-1 diversity with block 2 RO33 family predominating and multiplicity of infection range of 1 – 5 was observed. Eighty-five percent of the parasites genotyped had the pfcrfT767 chloroquine resistance allele (P < 0.05). In conclusion, infections due to shigella and P. falciparum exhibit complexity and diversity of occurrence at molecular level in Nigerian children with HIV-1 seropositivity. The parasite pfcrfT767 dominance and display of serogroup discordance by blood and stool shigella flora strongly raise the possibility of chloroquine failure and antibacterial therapy with third generation cephalosporins being compromised in co-infected patients.

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Cross Reference Between Cellulose Acetate Electrophoresis (CAE) and Ploymerase Chain Reaction (PCR) in Leishmania Diagnosis

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Effective diagnostic techniques for Leishmaniasis are very important and critical in starting treatment of an infected patient. Leishmaniasis is a potentially life threatening and mutilating disease and various species are lethal if not treated on time and appropriately. PCR is becoming the ultimate tool in Leishmania diagnosis due to its rapidity in providing
results, but is still lacking the full effectiveness of speciating all species of the Leishmania parasites. CAE is a technique that is been in place for a very long time, and it requires live parasites and a large number of promastigotes to work, but it remains the most effective technique in performing speciation of Leishmania parasites. Both techniques accommodate the diagnostic needs if used together, but until PCR is fully developed, Cellulose Acetate Electrophoresis remains the leading technique for Leishmania classification and speciation of all known species of Leishmania parasites. In doing this project, my intention is to examine how far CAE and PCR can extend its similarity common grounds in Leishmania identification and speciation.

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BLOOD AGAR SUBSTITUTE IN GROWING LEISHMANIA

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Leishmania diagnosis is essential to determine the best course of treatment and forecast its effectiveness in the infected patients. Until new and more effective techniques are found, culturing the parasites is a key step in treating this disease. Currently there are many ways to grow and expand Leishmania parasites, but the technique most commonly used is growing the parasites using Novy-MacNeal-Nicolle (NNN, blood agar). This blood agar medium for culture is used to grow and expand all Leishmania species and is more effective when parasites are very sensitive and difficult to expand in other culturing media. NNN is also very difficult to use as a standard media. NNN is very expensive and time consuming in its preparation. It is also easily contaminated because of the rich nutrients that compose the medium, which allow other unwanted microorganisms to grow and interfere with identification of Leishmania parasites. A control temperature environment is also needed to keep this culture medium active.

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DIAGNOSIS OF HUMAN BABESIOSIS USING SELDI PROTEINCHIP TECHNOLOGY

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Babesiosis, an emerging zoonotic disease transmitted through the bite of ixodes ticks and through blood transfusion, is caused by several species of hemoprotozoan parasites belonging to the genus Babesia. Prevalence of human babesiosis is worldwide but most cases have been reported in North America and Europe. The spectrum of disease manifestation is broad, ranging from silent infection to fulminant, malaria-like illness that may result in severe hemolysis, pulmonary, renal, or liver impairment, and occasionally in death. Laboratory diagnosis of babesiosis includes blood smears, indirect fluorescent antibody test (IFA) and PCR. These tests require highly trained personnel and are often expensive. Blood smears may be negative when patients first present to their physicians and it may be difficult to differentiate Babesia from Plasmodium. IFA requires babesia infected hamster erythrocytes as antigen that are difficult to procure and not suited to large scale testing. In addition, IFA can lead to misdiagnosis because of cross-reactivity with other parasites. To improve sensitivity, specificity, and provide better automated diagnosis of babesia, surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF-MS) serum protein profiling technology was used as a method to identify biomarkers specific to babesiosis infection. Serum samples were fractionated by pH using a Biomek 2000™ automated laboratory system and analysed by SELDI-MS (Ciphergen PBS LC) using metal affinity (IMAC30) weak cation exchange (CM10) and hydrophobic (HS50) protein-chip arrays. Analysis of sera from subjects with babesia infection alone (n=24), Babesia and Lyme disease co-infection (n=9), healthy controls (n=11), controls with flu-like illness (n=5), and other protozoan infections (n=10), led to the identification of a set of babesia specific polypeptide biomarkers in the range 2-150 kDa. Characterization of each of these babesiosis-specific markers by peptide mapping and tandem mass spectrometry is underway to determine potential biomarkers to be used to develop a faster, most cost-effective and accurate diagnostic test for this common, life-threatening parasitic infection.

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ANALYSIS OF BIOMARKERS ASSOCIATED WITH CHAGAS DISEASE USING ANTISERA TO NOVEL BIOMARKER EPITOPES

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Many parasites such as Trypanosoma cruzi, the causative agent of Chagas Disease (CD), can be transmitted by blood transfusion. Currently, there are no appropriate tests for screening blood products from Chagas infected donors. Surface-enhanced laser desorption ionization-time of flight mass spectrometry (SELDI-TOF MS) is a new strategy for the discovery of biomarkers for parasitic diseases. We have applied SELDI-TOF technology to identify biomarkers that could potentially be developed as improved diagnostic tests for parasite infections, such as Chagas disease, as well as to understand pathogenesis events associated with infection. Protein profiling of Chagas sera and endocardial control sera identified several biomarkers associated with infection, including fragments of human apolipoprotein A1 (ApoA1) and fibrinogen (Fbn). Both N terminal and C terminal fragments of ApoA1 (NtApoA1: 13.6 kDa, CtApoA1: 24.7 kDa, respectively) and a C terminal fragment of Fbn (CtFbn, 28.7 kDa) were identified. Polyclonal rabbit antiserum to the predicted novel peptide epitopes on these biomarkers were used to probe Western blots of sera from Chagas and uninfected control volunteers in order to confirm the identity of the truncation products in Chagas sera. The CtApoA1 sera detected the major truncation product near 24.7 kDa whereas the NtApoA1 sera detected a complex pattern of ApoA1 fragments. The CtFbn antiserum detected a novel fragment near 29 kDa. Further studies will be reported evaluating these specific antisera against sera from other parasitic infections to determine whether these ApoA1 and Fbn truncation products are specific for Chagas disease. This would validate their utility as target antigens in an ELISA-based diagnostic test for Chagas disease.

(ACMCP Abstract)

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EVIDENCE FOR GENOTYPE-BY-SEX INTERACTION IN THE GENETICS OF SEROPREVIRITY TO TRYPANOSOMA CRUZI IN A BABOON MODEL

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Baboons housed at Southwest Foundation for Biomedical Research in southern Texas are exposed naturally to the insect vector that transmits Trypanosoma cruzi, a kinetoplastid protozoan that causes Chagas disease. About 3% of the baboons in the colony are seropositive to T.cruzi. Infected baboons can develop Chagas disease and exhibit disease progression and pathology, including myocarditis and EKG abnormalities, similar to that seen in human cases. Previous work has suggested that variation in serostatus to T.cruzi in the baboon is significantly influenced

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by additive genetic effects, but these studies have assumed that the genes involved must act identically in males and females. In the present analysis we allowed for interaction between sex and genotype, such that different genes may be involved, and may have different effects, in males and females. In a sample of 1227 pedigreed baboons the heritabilities of senescence in males ($h^2=0.70, p=0.001$) and in females ($h^2=0.67, p=0.007$) were each significant, yet not significantly different. However, the genetic correlation between males and females was significantly different from zero ($r=0.71, p<0.001$) and negative. These results can be interpreted as indicating (i) that male and female baboons have in common a large proportion of the genes affecting their senescence at $T. cruzi$, and (ii) that these genes have different effects in the different sexes.

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**VIRULENCE AND IMMUNOLOGIC RESPONSE INDUCED BY A TYPE II A NORTH AMERICAN ISOLATE OF T. CRUZI AS COMPARED TO THE TYPE I BRAZIL STRAIN**

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Trypanosoma cruzi is widely distributed across North America, with Type IIa genotypes associated with placental reservoir hosts. While prevalence is high in sylvatic populations of raccoons (Procyon lotor), pathology is largely absent. We compared a Type IIa North American isolate from St. Catherines Island (SC), Georgia with the Type I Brazil strain (BS) to determine whether differences in virulence could be determined. We found that in vitro the rate of cell infection and percentage of cells becoming infected were statistically similar, but that the intracellular replication and emergence rates of the BS exceeded that of the SC isolate. When groups of BALB/c mice were infected with each isolate the BS strain induced consistently higher levels of morbidity and mortality, which was in agreement with the higher parasitaemias noted in these mice. Infection with the SC isolate induced protection against a challenge infection with the BS. Western blot analysis showed similar antigenic recognition profiles although minor differences could be noted. This demonstrates that strain genotype may translate into significant differences in pathology. Comparisons with Type I isolates from North America will also be discussed.

(ACMCI Abstract)
According to immunoblot, AbMSP51L is specific and will not recognize MSP2, whereas AbMSPX recognizes isoforms migrating with MSP52 and MSP51. These monoclonal antibodies are being used to study the intracellular trafficking of MSP isoforms.

(ACMOIP Abstract)

THE CHEMOKINE RECEPTOR CXCR3 IS NOT REQUIRED FOR HOST RESISTANCE TO MURINE VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) caused by L. chagasi and L. donovani is the most severe form of Leishmania infection and is fatal if left untreated. The CXC chemokine receptor 3 (CXCR3) is expressed on plasmacytoid DCs, NK cells and T cells and is involved in their trafficking to the site of inflammation. Three chemokines CXCL9 (Mig), CXCL10 (IP10) and CXCL11 (II-TAC) are the main ligands for CXCR3. Both CXCL9 and CXCL10 produced in high levels during L. major and L. donovani infections in man and mouse, and are believed to play a role in host immunity. We recently found that CXCR3 plays a critical role in mediating host resistance against cutaneous leishmaniasis (CL) caused by L. major by regulating trafficking of effector T cells to skin. Using an intravenous mouse model, we investigated the role of CXCR3 in the immune response to L. donovani. Here we report that C57BL/6 mice genetically lacking CXCR3 (CXCR3-/-) mount an efficient Th1 response following L. donovani infection, recruit significant number of CD4+ and CD8+ T cells to the liver and spleen and control parasite growth as efficiently as CXCR3+/- mice. These results demonstrate that although CXCR3 controls effector T cell migration to skin during L. major infection, it is not required for trafficking of T cells to the liver and spleen during L. donovani infection. Furthermore, they also indicate that CXCR3 is not required for induction of Th1 response and host resistance to L. donovani.

(ACMOIP Abstract)

IDENTIFICATION OF NOVEL PLASMODIUM KNOWLESI AND P. VIVAX MEROZOITE PROTEINS

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The erythrocytic stage of the malaria parasite (Plasmodium) lifecycle is responsible for the clinical symptoms associated with malaria. The parasite merozoite form, expressed in the erythrocytic stage, is responsible for invasion of RBCs and propagation of this stage of the life cycle. Numerous proteins are expressed in these merozoites and these proteins may mediate crucial functions during merozoite invasion. Many of the merozoite proteins have not been identified, nor have their functions been defined. We sought to identify novel merozoite proteins in P. knowlesi and P. vivax using a systematic approach to more readily identify specific proteins expressed at the merozoite’s apical end. P. knowlesi is a simian malaria parasite related to the human malaria P. vivax and has been used extensively as a model for merozoite invasion. Specific antibodies made against proteins expressed in P. vivax merozoites were used to identify homologous proteins in P. knowlesi. Identification of these proteins was accomplished using proteomics and screening of P. knowlesi genomic databases, followed by screening of the P. vivax genome database. Several proteins identified by these methods will be presented.

PLASMODIUM FALCIPARUM SYNTAXIN HOMOLOGUES

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Plasmodium falciparum, the causative agent of malaria, invades and occupies erythrocytes during its asexual lifecycle. Blood stage P. falciparum
parasites traffic proteins of parasitic origin to the surface of the infected erythrocyte that mediate cytoadherence and play a crucial role in the pathology of malaria. Mature red blood cells are terminally differentiated and do not possess the machinery necessary for protein synthesis and trafficking. How this parasitic exports protein beyond the boundaries of its own plasma membrane to the surface of the erythrocyte remains an enigma. Syntaxins are responsible for the specificity of vesicle trafficking and different syntaxins are present on each organelle of eukaryotic secretory pathways. Syntaxins are thus ideal for serving as organelle specific markers. Nothing is currently known about the P. falciparum syntaxins, and we aim to use them as tools to dissect the secretory system that is hypothesized to form in the erythrocyte cytoplasm. We have identified six putative P. falciparum syntaxins and have generated antibodies to three of them. Using biochemical and molecular techniques, we demonstrate that P. falciparum exports a subpopulation of several syntaxins into the cytoplasm of the erythrocyte. We also show that on a chimeric protein, P. falciparum protein that is exported to the Maurer's clefts, co-localizes with the exported syntaxins. Maurer's clefts appear to be an intermediate secretory compartment between the parasite and the erythrocyte membrane. Identification of syntaxins present in the Maurer's clefts is further evidence that transport to and from this secretory organelle involves vesicular intermediates.

(ACMCIP Abstract)

APICAL ORIENTED MEROZOITE PROTEINS WITH EGF-LIKE DOMAINS UPSTREAM FROM THE MSP-1 GENE ARE UNIQUELY EXPRESSED IN P. VIVAX AND P. KNOWLESI

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The release of Plasmodium merozoites into the bloodstream is responsible for the clinical symptoms seen in a malaria infection. Interventions to interrupt this step in the life cycle of the parasite require a thorough understanding of invasion mechanisms and molecular components that are involved. Here we present a unique gene encoding an apical pole protein, MAP-2 (Merozoite Apical Protein) in P. vivax and P. knowlesi. Using a gene fragment from the University of Florida EST database in conjunction with BLAST analyses, we identified two highly homologous contigs in the P. vivax and the P. knowlesi sequence databases. These features make these genes of particular interest: 1. The map-2 gene is part of a syntenic block that includes themsp-1 gene in P. vivax and P. knowlesi. 2. The map-2 gene are present in P. falciparum. 3. The C-terminal region of the putative MAP-2 contains two EGF-like motifs oriented head-to-tail. To characterize these proteins we produced a recombinant fragment representing the central region of PkMAP-2 in the pET system and the soluble form of the protein was utilized to produce rabbit polyclonal antisera. Data on the localization and the biochemical characteristics will be presented. By using RT-PCR we determined that the P. knowlesi map-2 transcript is synthesized as a full-length product during the schizogony stage and ongoing studies will determine the exact timing for the protein expression on the merozoite. We will discuss the molecular characteristics of the map-2 transcript in the context of the syntenic block containing the msp-1 gene in P. vivax and the simian malaria parasites.

(ACMCIP Abstract)

FUNCTIONAL CHARACTERIZATION OF REFOLDED DBL1D DOMAIN OF PLASMODIUM FALCIPARUM ERTHROCYTE MEMBRANE PROTEIN-1

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The ability of Plasmodium falciparum-infected erythrocytes to adhere with uninfected RBC (rosetting), other parasitized RBC (auto-agglutination), and to endothelial cell lining blood vessels (cytoadherence) is mediated by variant surface antigens, which are referred to as Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP-1) and are encoded by var genes. The extracellular regions of PfEMP-1 contain multiple conserved cytokine-rich domains that are referred to as Duffy-binding-like (DBL) domains. The DBL1D domain is a principle ligand identified on the parasitized RBC from children suffering from severe malaria often adhere to complement receptor 1 (CR1) on uninfected RBCs to form clumps of cells known as “rosettes”. Here we show that DBL1D domain from the PfEMP-1 protein containing nine disulfide bonds can be produced in E. coli and refolded into functional form. We expressed Pf DBL1D (NCI) in E. coli, refolded and purified the recombinant proteins from insoluble form. Refolded Pf DBL1D (NCI) exhibits functional binding to heparin in solution and to CR1 expressed on the CHO cell surface. Presently we are analyzing the

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immuno-functional responses of DBL1α as a potential vaccine candidate against malaria.

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**DISSEMINATED INTRAVASCULAR COAGULATION IN A RHESUS MACAQUE EXPERIMENTALLY INFECTED WITH PLASMODIUM COATNEY**

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Disseminated intravascular coagulation (DIC) is a common complication of sepsis. DIC is mediated by a massive intravascular activation of the coagulation and fibrinolysis cascades. Intravascular fibrin deposits lead to a severe disturbance of organ perfusion resulting in multiple organ failure. Subclinical coagulopathy is a common finding in malaria-infected individuals but DIC is very rare. Here, we describe the first case of DIC associated with Plasmodium coatney experimental infection in a rhesus monkey. This simian malaria parasite shares several features with *P. falciparum*. Thus, we have used it as a model of severe anemia. An Indian rhesus macaque was experimentally inoculated with 2 × 10^11 P. coatney-infected erythrocytes. The animal was closely monitored using daily clinical evaluation, temperature determination, parasite quantification, hemoglobin concentration, reticulocyte counts and platelet quantification. At day 10 after challenge, the animal developed anorexia and tachypnea and numerous petechiae were noted on the trunk and extremities. This clinical presentation was not correlated with hyper-parasitemia but with hypothermia and thrombocytopenia. Treatment with intravenous fluid support and complete blood transfusion was done. The animal also received artesunate by intramuscular route. Three days after therapy, the platelet counts returned to normal and parasitemia was abated. Nevertheless, both hands and the tail were gangrenous. In addition, ascral discoloration was observed. Laboratory tests were compatible with DIC and euthanasia was opted due to poor prognosis. A complete panel of clinical laboratory analyses and histopathology data will be discussed in the context of the relevance for developing novel experimental animal models of severe malaria.

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**IMMUNOELECTRONMICROSCOPY OF MALARIA MEROZOITE INVASION INTO RED BLOOD CELLS**

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Electron microscopy has contributed substantially to our understanding of malaria merozoite invasion into red blood cells since the seminal studies of the 1960s and 70s. In recent years immunoelectron microscopy has been used to establish the cellular locations of a number of invasion-related antigens within merozoites, but our knowledge of their behaviour during the invasion process has advanced little. The only effective model that in the past has been used for this purpose is Plasmodium knowlesi, a parasite that infects rhesus monkeys. In this study we use this approach to prepare specimens for both morphological and immuno-staining analysis by electron microscopy, and have re-examined this process in the light of molecular biological and proteomic advances. In this presentation we report the immuno-localization of a range of merozoite surface and secretory antigens during the invasion process, including Merozoite Surface Protein 1 (MSP-1) whose final processing we confirm takes place at the moving junction with the red blood cell.

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**CHARACTERIZATION OF P. FALCIPARUM MSP1-SPECIFIC MONOCLONAL ANTIBODIES WITH REGARDS TO REACTIVITY ON LIVE PARASITES, FINE SPECIFICITY, AND BIOLOGICAL FUNCTION**

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Merozoite surface protein 1 is a major surface protein expressed on merozoites and its function is essential to the *Plasmodium falciparum* parasite life cycle. It is synthesized as a 195 kDa precursor that undergoes several proteolytic processing events. The primary event yields a noncovalent complex attached to the merozoite surface through the C-terminal MSP1-42 Fragment. This fragment is cleaved secondarily to produce the MSP1-33 and C-terminal MSP1-19 Fragments with the later bound covalently to the surface of the invasive merozoite. Previously, MSP1-19-specific, conformation-dependent mAbs were developed by immunizing with parasite derived Ag and were shown to either interfere in vitro with erythrocyte invasion (mAbs 12.10, 12.8), or have blocking activity (mAbs 22, 7.5, 1E1) against the invasion inhibitory mAbs. These various mAbs have been used extensively to verify parasite-like structure on recombinantly expressed proteins thus serving as valuable tools for *P. falciparum* vaccine development. To test the question whether recombinant proteins could also induce mAbs with biologically relevant activities, we immunized mice with recombinant MSP1-42/52DA (GlasowSmikhline Biologicals) and characterized antibody specificity using MSP1 fragment-specific ELISAs on p42, p33, p19, EGF-domain 1 and EGF-domain 2, as well as by IFA, western blot, flow cytometry and functional assays (GIA and PIA). Correlating the fine specificities of these new mAbs with biological function will not only assist in localizing protective epitopes within the MSP1-42 molecule but also serve as useful tools for measuring correct structure.

(ACMOI Abstract)

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**A DATABASE MANAGEMENT SYSTEM (DBMS) FOR MOTHER OFFSPRING MALARIA STUDY (MOMS)**

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MOMS/DB is a DBMS that has been developed to store and track clinical and experimental data for a 5-year longitudinal cohort project to study severe malaria pathogenesis and protective immunity in mothers and infants. This study involves more than 2000 mother-infant pairs and is conducted in both Tanzania and the United States. Paper forms have been designed to collect clinical and experimental information (http://apps.sbsi.org/sgch/sbgch.htm) for this study. A barcode tracking system has been implemented to track all clinic and laboratory paper forms. The barcode system requires that preprinted barcode labels be placed on all forms before the data are entered into the database. An online data entry system (http://apps.sbsi.org/cohorts/) has been developed to help data entry persons to enter the information on the paper forms into the database which are hosted on a Microsoft SQL database server. The front end of this system was written in Java. A Linux server is dedicated as a web server. Double data entry technique, which is a way to verify the integrity of the data entry and protect data integrity from typographical or subject errors, has been applied in the system. In order to track all samples collected in
the study so we are able to link all experiment results to clinic information, a sample tracking system has been developed as well. Barcode labels are attached to all samples and boxes which are used to store samples. Experiment results, such as complete blood count and blood smear reading results, are captured through a Java standalone application and ultimately stored in a Microsoft SQL server database. MOMS/DB is compliant with the FDA guidelines of electronic records (21CFR11).

still limited. We tested the hypothesis that sulfalene/pyrimethamine/amodiaquine (SL/PQ/AQ) is as efficacious and safe as artemether/lumefantrine (Coartem® (AL)) in the treatment of uncomplicated P. falciparum malaria in multicenter study including Mali. We report here the results of this study carried from August to November 2005 in Sotuba and Kambia, in Mali. Treatment efficacy was assessed using 28 days WHO 2003 protocol. Safety was assessed clinically and biologically (hemogram and biochemistry). MSP1 and MSP2 and microsatellite CA1 were used to distinguish recrudescence from new infections. Of the total 450 required subjects for all the centers, 226 aged ≥ 2 years were enrolled in Mali, 113 in each treatment arm. SL/PQ/AQ was given once daily for 3 days. AEs were recorded twice daily for 3 days. The study was funded by Pfizer. Baseline characteristics of patients in the two treatment groups were comparable. The 28-day cure rate for SL/PQ/AQ was 90.7% (n=107) compared to 83.5% (n=109) for AL, p=0.1. After correction for re-infection, the 28-day cure rate was 98.1% for SL/PQ and 98.7% for AL; p=0.99. SL/PQ/AQ cleared fever faster than AL on day 1 (96.4% vs. 88.5% respectively, p = 0.074) while AL cleared faster the parasites on day 1 and day 2 compared to SL/PQ (54.5% and 97.3% vs 4.6% and 50.9% respectively, p<0.001). Gametocyte carriage rate was similar in both treatments arms. No serious adverse events or significant laboratory abnormalities occurred. Adverse events rate were similar in the two arms except for abdominal pain, vomiting, anorexia and fatigue which were higher in SL/PQ/AQ arm p<0.001. SL/PQ/AQ is as effective as AL for the treatment of P. falciparum malaria, with a faster fever clearance but frequent digestive symptoms.

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EFFECTS OF PYRIMETHAMINE-SULPHadoxINE, CHLORoQUINE PLUS CHLORoPHENIRAMINE AND AMODIAQUINE PLUS PYRIMETHAMINE-SULPHadoxINE ON GAMETOCYTES DURING AND AFTER TREATMENT OF ACUTE, UNCOMPPLICATED MALARIA IN CHILDREN

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The effects of pyrimethamine-sulphadoxine (PS), chloroquine plus chlorpheniramime, a H1 receptor antagonist that reverses chloroquine resistance in Plasmodium falciparum in vitro and in vivo (CQCP), and amodiaquine plus pyrimethamine-sulphadoxine (AQPS) on gametocyte production were evaluated in 157 children with acute, symptomatic, uncomplicated falciparum malaria who were treated with these drugs. PS was significantly less effective than CQCP or AQPS at clearing asexual parasitaemia or other symptoms of malaria. Gametocyte carriage on days 3, 7 and 14 were significantly higher in those treated with PS. The ratio of the density (per µl blood) of peripheral young gametocytes (PYG), that is, ≤2 stage III to peripheral mature gametocytes (PMG), that is, stage IV and V, an index of continuing generation of gametocytes, rose to 1 by day 7 of treatment in those treated with PS, but remained consistently below 1 in the other treatment groups. PYG-PMG density ratio increased significantly from day 0-14 in those treated with PS and CQCP (Z= 7.66, P = 0.00001 and Z = 4.22, P = 0.00001, respectively) but decreased significantly in those treated with AQPS (Z = 3.22, P = 0.001) and CQPS (Z = 3.02, P = 0.002). Both PS and AQPS produced less production of PYG, 18 of 29 vs. 13 of 20, Z = 0.40, P = 0.61) but PYG was present only in those with resistant response to CQCP. Combination of PS with amodiaquine (AQ), that is, (AQPS), resulted in less production of PYG, but in this setting, PYG was not indicative of response to AQPS. These data indicate that PS enhanced production or release of young gametocytes when used alone, but generated less young gametocytes when used in combination with AQ, PYG may be used as an indicator of response to CQCP but not PS or PQ-based combination drugs.

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THE EFFECTS OF ARTEMETHER-LUMEFANTRINE VERSUS AMODIAQUINE-SULFALENE-PYRIMETHAMINE ON THE HEPATOMEGALY ASSOCIATED WITH PLASMODIUM FALCIPARUM MALARIA IN CHILDREN

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An open randomized controlled study of artesether-lumefantrine (AL) and amodiaquine-sulfalene-pyrimethamine (ASP) for the treatment of uncomplicated Plasmodium falciparum malaria was carried out in 181 children. In 79 children, the hepatomegaly reduction ratios (HRR) and the speed of resolution of hepatomegaly, the hepatomegaly resolution rates (HRSR) were calculated and compared between the two treatment groups. HRR and HRSR were similar in the two treatment groups. HRSR was 71% and 62% in AL and ASP treated children, respectively by 14 days of commencing treatment. There was no significant correlation between HRR and parasite reduction ratio (PPR) in the same patient. In children in whom parasitaemia cleared and hepatomegaly resolved within 14 days, recurrence of parasitaemia was associated with re-occurrence of hepatomegaly, suggesting that, the propensity for recurrence of infection drives the malaria-attributable hepatomegaly in children from this endemic area. Combination therapy may provide additional beneficial effects on patho-physiological processes and change associated with falciparum malaria by rapid clearing of asexual parasitaemia and reducing the propensity for recurrence of infection.

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STAGE-SPECIFIC SURVIVAL OF PLASMODIUM FALCIPARUM TREATED WITH ANTI-MITOCHONDRIAL DRUGS

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The mitochondrion plays a critical role in the life and death decisions of many eukaryotic cells. Apoptotic pathways in metazoa and some unicellular organisms are initiated by the collapse of mitochondrial membrane potential. Atovaquone, an antimarial drug, has been shown to inhibit electron transport, leading to the collapse of the membrane potential in malaria parasites. We are exploring effects of mitochondrial physiology inhibition by atovaquone alone as well as in combination with its synergistic drug, proguanil, in malaria parasites. Our results suggest that, in P. falciparum, a collapsed mitochondrial membrane potential does not necessarily induce a cell death pathway. The effects of atovaquone and atovaquone/proguanil are highly dependent upon the erythrocytic stage of the parasites exposed to the drugs. We have found that ring-stage parasites are the most resilient to drug treatment and can survive for periods longer than 48 hours. During treatment, however, survival of the parasite seems to depend on its ability to exist in a 'static' phase, once treatment ceases, the parasites 're-enter' the erythrocytic development cycle. To further understand this static state, we have analyzed the global transcription profile of treated parasites to determine the mRNA stability and any transcriptional changes that may occur during drug treatment. These studies begin to provide information on the physiological state of the parasite at which mitochondrial DNA mutations could arise leading to drug resistance.

(ACMCIP Abstract)
BLACKWATER FEVER IN CHILDREN DURING CEREBRAL MALARIA: THREE OBSERVATIONS IN BAMAKO

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We carried out an exploratory study of cerebral malaria in the pediatric sector of National Hospital “Gabriel Touré” at Bamako. The study was conducted during transmission seasons of years 2003 and 2004. The aim goal of the study was to successfully find out children tolerance of quinine during cerebral malaria. The population study consist by children from 6 month to 14 years old with a drop thick positive (Plasmodium falciparum) and/or Optimal positive II, a “Score of Blantyre” less than 4. Repeated convulsions (at least 2 times per day followed by critical coma (for at least 15 minutes). Macroscopic haemoglobinuria after quinine administration. We observed 3 cases of macroscopic haemoglobinuria: 2,5% (3/119). Both cases had been treated by quinine. Haemoglobinuria was respectively observed 3 hours, 4 hours and 12 hours after treatment. About 373 cases, occurs complications as acute renal insufficiency with blood creatine level around 615,29 μmol and 719,10μmol. One of them died by respiratory distress which occurred 24 hours after admission in hospital. The treatment consists to immediately stop administration of quinine and replaced it by another in intra muscular: 3,2mg/kg the 1st day and 1,6mg/kg/per from 2nd to 5th day. Furosemide was administered to manage the complications: 2mg/kg/per. In most of the power country and specifically in Mali, people tend to get first self medication. Then, they are going to see a physician 3 to 5 days later in the case of medicine they were taken doesn’t make well they feeling. That can be cause of many complications as the one observed during this study. Actually, detecting haemoglobinuria seems to be very easy, but as proved by this study, it is essential for a best perceptive in the treatment and prevention of cerebral malaria if we know that patients always get hospital very late and used to take many kind of drugs as consigned by a pierce person (neighbors, parents etc...). Anyway, the first issue will be to stop immediately the medication by quinine.

DEVELOPMENT OF QUANTITATIVE REAL-TIME PCR AS A SENSITIVE AND EFFECTIVE APPROACH FOR DETECTING PLASMODIUM-INFECTED HC-04 CELL LINE

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Plasmodium spp., the causative agents of malaria, results in the illness and death of millions worldwide each year. The development of drug resistance has increased the urgency to develop new compounds against these parasites. Malaria prophylaxis targets blood and liver stage parasites. Development of drugs for blood stage parasites can be accomplished relatively easily compared to liver stage drug development because an in vitro system is available for rapid screening of compounds against blood stages. However, there has been no effective in vitro system available for evaluating compounds against the liver stage parasites of human malaria. A human hepatocyte cell line (HC-04) established in our laboratory can be optimized and used for in vitro screening of new compounds against liver stage parasites. This cell line maintains production of key proteins and enzymes which may be important for drug metabolism and parasite development. In addition, P. falciparum and P. vivax parasites can completely develop to mature merozoites in HC-04 cells that then invade red blood cells. To optimize this system, we need to develop a rapid assay that will allow us to follow parasite development in the liver cells without requiring microscopic examination. Quantitative PCR was considered for this purpose because of its rapidity and sensitivity. In this study, we developed universal genus specific primers for SYBR green probe and universal genus and species specific probes to 18s ribosomal RNA genes. The data indicated that the sporozoites required longer incubation time for cell lysis and the detection sensitivity was improved. There was no cross amplification of host and parasite when using primers for liver cell and Plasmodium spp. HC-04 DNA did not have a negative effect on the detection of parasites’ DNA although the DNA quantity of HC-04 was many times higher. The established protocol for this rapid quantification of parasites in the liver cells will enhance the development of in vitro system for drug sensitivity screening of new anti malaria compounds.

MOLECULAR DIAGNOSIS OF MIXED PLASMODIUM SPECIES AND SUB-CLINICAL MALARIA IN MINING REGIONS IN THE BOLIVAR STATE, VENEZUELA

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We compared a nested PCR assay and microscopic examinations of Giemsa-stained blood slides in the diagnosis of mixed and sub-clinical infections of malaria in mining regions in Venezuela. A first group of 158 volunteers seeking care at Malaria Center Dr. Francisco Vitanza in Tumeremo, Bolivar state with positive malaria diagnosis by microscopy was evaluated by a nested PCR assay. 39% (61 Plasmodium falciparum/Plasmodium vivax, 1 P. falciparum/P. vivax/P. malariae) of mixed infections were detected by the molecular diagnosis in contrast with 3% (4 P.falciparum/P.vivax) of mixed infections detected for the conventional microscopy. For each mixed infection identified by microscopy, the nested PCR detected 13 (Re=13:1). A second group of 91 asymptomatic miners from the mining village Villanovanas, Sifones District was studied for both methodologies. 34% (31 malaria cases) of the samples were detected positive to malaria by nested PCR, meanwhile the microscopic diagnosis detected only 7% (6 malaria cases). Also 4% (4 P.falciparum/P.vivax) of mixed infections were diagnosed by nested PCR, that were not identified by microscopic assay. For each malaria case detected in the asymptomatic miners by microscopy, the nested PCR detected 5 (Re=5:1). In our study, nested PCR showed a higher detection level than conventional microscopy in the diagnosis of mixed infections and sub-clinical malaria.
FIRST REPORT OF NATURAL PLASMODIUM KNOWLESI INFECTION IN WILD MACAQUES, THAILAND

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We recently reported the first naturally acquired Plasmodium knowlesi malaria in a patient who possibly acquired the infection in southern Thailand. Concurrently, a large outbreak of human cases infected with this simian malaria parasite were identified in Malaysian Borneo. It is of note that all human cases so far encountered have been confined to these regions including Malaysian Peninsula where natural reservoir hosts such as crab-eating macaque, Macaca fascicularis, have been populated in these regions. Therefore, we conducted an epidemiological surveillance of P. knowlesi among macaque monkeys in 3 localities along western and southern Thailand. We collected blood samples from 138 monkeys after temporarily caught under the safety guideline protocol. Thereafter, all monkeys were released back to their original habitats without any ensuing consequences. The macaque species were M. f. fascicularis, M. nemestrina, M. arcoides and certain hybrid or unidentifiable species. Each blood sample was searched for the presence of malaria parasites by the Giemsa-stained thick blood films. Results revealed that 36 samples (26.1%) contained ring stages of Plasmodium sp. while 4 monkeys (2.9%) were infected with Hepatocystis sp. Because ring stages of malaria parasites per se are not informative for species differentiation among simian malaria, we exploited the polymerase chain reaction method with P. knowlesi-specific primers targeting the 18S rRNA gene, followed by DNA sequencing for definite species identification. Of 36 ring stage-positive samples, P. knowlesi was detected in 9 M. nemestrina and none in other species of monkeys. We further examined the presence of other simian malaria potentially causing human infections by direct sequencing of mitochondrial genome. Of 7 samples analyzed, P. inui and P. cynomolgi were found in 2 and 5 isolates, respectively. The high prevalence of P. knowlesi and other simian malaria in macaque monkeys in Thailand emphasizes an important role of wild primate populations in malaria transmission to humans.

A SURVEY OF SYNTHETIC AND NATURAL PHYTOTOXIC COMPOUNDS AND PHOTOALENXS AS POTENTIAL ANTIMALARIALS

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Discovery of antimalarial drugs with new modes of action is a primary focus of malaria research because the malaria parasite has developed resistance against almost every chemotherapeutic regimen available. Malaria parasites are members of apicomplexa and have a plastid organelle called the apicoplast. There are several pathways and functions present in both the apicoplast and plant plastids which are fundamentally different to the analogous pathways and functions in humans that might be good targets for new antimalarial drugs. Apicoplast function is essential for survival and viability of Plasmodium, but all of the crucial apicoplast processes are not known. Many highly effective herbicides and natural phytotoxins target plastid processes. Therefore, such compounds might be effective against the apicoplast. We have determined the activity of a variety of phytotoxins, both natural and synthetic, against P. falciparum. We have also examined the activity of some stilbene-based photoaalenxs. Most of synthetic herbicides possessed moderate antimalarial activity. Even the moderate action of these herbicides may be considered as useful lead in view of targeting of apicoplast pathways by the herbicides. The antimalarial potential of herbicides led to discovery of plant-like metabolic pathways and their essentially in survival of the apicomplexan parasites including malaria. Endotril showed promising antimalarial activity. It inhibits protein phosphatase in mammals, but its mode of action as a herbicide is unknown. The function of this enzyme is known to be essential for P. falciparum. Earlier work with glyphosate and triazines indicated significant interest in them as antimalarials but their activity could not be confirmed in vitro. Most of the natural phytotoxins were active, with anisomycin, cavanine, cerulenin, and 19-trityl-12-oxo-acetamide being the most active. Antimalarial phytotoxins and photoaalenxs may provide useful leads and probes to map the target pathways for new antimalarial drug discovery.

PRECLINICAL PHARMACOKINETICS AND METABOLISM OF GW308678, A SECOND GENERATION 4-(1H)-PYRIDONE ANTIMALARIAL MITOCHONDRIAL ELECTRON TRANSPORT INHIBITOR

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4-(1H)-Pyridones are a novel class of antimalarials that are selective inhibitors of Plasmodium mitochondrial electron transport. We have previously reported the preclinical pharmacokinetics and metabolism of GW444520. Subsequent safety assessment of GW444520 revealed a narrow therapeutic window due to cardiac toxicity in the dog but not in the mouse. Here we report the preclinical pharmacokinetics and metabolism of GW308678, a compound in this series that did not display cardiac toxicity in the dog or mouse under similar conditions. We have investigated the pharmacokinetics following single intravenous and oral administration to the mouse, rat, dog and monkey. Protein binding and blood cell association were investigated in the plasma or whole blood from preclinical species and human, in vitro. The routes and rates of metabolism of GW308678 were studied in animal and human liver microsomes and hepatocytes. Concentration- and time-dependent human cytochrome P450 inhibition, permeability and active transport were investigated in vitro. GW308678 had relatively low blood clearance ranging from 2 (in the dog) to 23% (in the rat) of liver blood flow, a long elimination half-life of 61 h in the dog, and a steady-state volume of distribution ranging from 2-4 times total body water in animals. The oral bioavailability following administration of a solution was high in all species (66-100%). GW308678 had high passive permeability and was not a P-glycoprotein substrate. Intrinsic clearance was low in liver microsomes and hepatocytes of preclinical species and human except in monkey hepatocytes. GW308678 did not associate appreciably with blood cells and had high plasma protein binding (>99%) in all species. GW308678 was an inhibitor for human CYP2C9 and a substrate of both CYP2C9 and 2D6. Two metabolites (mono-oxygenation and N-oxidation) were detected in human liver microsomes and hepatocytes, which were also detected in all preclinical species. Based on these data, we would expect GW308678 to have high oral bioavailability and low clearance in man making it suitable for the desired short duration of therapy by the oral route of administration.
SUNIVERSITY OF SOUTHERN CALIFORNIAEPIT BILITY OF PLASMODIUM FALCIPARUM TO CHLOROQUINE IN THE MALARIA-ENDEMIC VILLAGE OF MISSIRA IN MALI USING THE WHO IN VIVO TEST AND SEQUENCING

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Because CQ is the cheapest and least toxic antimalarial, monitoring the prevalence of CQ-resistant P. falciparum is an important aspect of malaria control. To estimate the prevalence of CQ-resistant P. falciparum in a village being considered as a test site for Phase 2 Studies of a candidate antimalarial, we used the WHO in vivo test in conjunction with molecular techniques to collect baseline information in the malaria-endemic village of Missira, Kolekani in Mali. Using the standard oral dose of 25 mg CQ base per kg over 3 days, we performed the WHO 14 day in vivo test to identify early and late therapeutic failures and to estimate the rates of adequate clinical and parasitological responses to treatment with CQ. PCR based on the polymorphic block 2 region of msp1 was used to distinguish between recrudescence and new infection. Point mutations of pfcrt gene within a region encompassing the 76 aminoo-acid position were identified using capillary sequencing. Results obtained for 27 children with uncomplicated malaria between 1 and 9 years of age included 4% early treatment failures, 11% late clinical failures, 33% late parasitological failures and 58% adequate clinical and parasitological responses. Using MS²1 data after amplification of 16 specimens by PCR, we adjusted the overall data obtained with the in vivo test, prevalence of susceptible P. falciparum was 55.5% versus 44.4% of resistant parasites. In order to validate those data we increased the sample size of study population to 50 children, we didn’t find a difference when the sample size was 27 children. To investigate whether mutation 76T is associated with resistance of P. falciparum to CQ, we sequenced four PCR products from infected subjects: one specimen from a subject that responded to CQ had K76; another specimen from a subject who did not respond to CQ had 76T; and 2 specimens from subjects who responded to CQ had 76T. Geometric mean parasite densities decreased of parasitemia from 10,113 on day 0, to 119 on day 4, and 128 on day 7, before rising to 1206 on day 14. This suggests that one could increase the dose of CQ to 30 mg/kg body weight in order to obtain clearance of resistant P. falciparum parasites that could emerge during the 3 days of the treatment. These results indicate that the prevalence of CQ-resistant P. falciparum is ~30% in rural areas such as Missira; it also indicates that some infections with 76T parasites respond to CQ if a semi-immune population.

PRECLINICAL CARDIAC SAFETY PROFILE OF PIPERAQUINE PHOSPHATE AND CHLOROQUINE PHOSPHATE

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Many antimalarial drugs on the market have cardiac effects ranging from mild heart rate changes to excessive prolongation of the QT interval leading to lethal arrhythmias such as Torsade de Pointes (Tdp). This study compared the preclinical cardiac safety of Piperaquine (QCP) and Chloroquine (CP). The QCP is an antimalarial drug being used as part of combination therapy reported to have improved clinical efficacy and tolerability, compared to CP. No effect on cardiac action potential duration (APD) was noticed with QCP up to 10 μM conc. in Canine Purkinje fibres as compared to maximum rate of depolarization and prolonged APD reported for CP at 0.3 µM conc. in cat Purkinje fibres. Effect on blood pressure (BP), heart rate (HR) and electrocardiogram (ECG) was assessed by administering single dose of 0, 5, 25 and 50 mg/kg of QCP and 0, 5, 10 and 20 mg/kg of CP by orally in 3 male and 3 female telemetered conscious dogs in a crossover pattern with a 7 day washout period. Blood samples were collected from all animals for pharmacokinetic assessment. No changes in mean, systolic and diastolic arterial pressure, HR or cardiac conduction times were observed at 5 and 25 mg/kg of QCP. However, tachycardia associated with decrease in PR and QT interval durations with long lasting increase in QT interval (Bazett’s, Fredericia’s formula and Sarma’s method) and prolongation of ventricular repolarisation was noticed at 50 mg/kg dose. Torsors were observed in 3 dogs at 50 mg/kg dose. Similarly, CP treated animals showed significant and long lasting tachycardia associated with decreases in QT and QT interval, an increase in QRS complex duration and QT interval at 20 mg/kg dose level, supporting an effect on the ventricular depolarization and repolarisation. Torsors (all dogs), seizure (one dog) and ataxia (4 dogs) were noticed in animals receiving 10 and 20 mg/kg dose levels. No disturbance in 6-lead electrocardiogram in the lead II and no change in the T wave morphology, attributable to the QCP or CP was seen at any dose. Plasma estimations for both the drugs showed dose related and gender independent exposure. The NOEL established for cardiovascular parameters was 10 mg/kg CP and 25 mg/kg QCP with systemic exposure (AUC₂) 2377 and 4623 h ng/ml, respectively indicating better cardiac safety of QCP in animals. The effect of QCP on humans compared to CP has not been determined.

EVALUATING THE EFFECTS OF CHLOROQUINE AND AQ-13 ON CARDIAC QT INTERVAL

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We compared the effects of a candidate antimalarial, AQ-13, and chloroquine (CQ) on the cardiac QT interval using 2 formulas to correct the QT interval for heart rate: Bazett’s (QTc = QT/RR¹), and Fredericia’s (QTc = QT√RR⁴²), where QTc is the corrected QT and RR is the duration of the cardiac cycle in seconds. This report is based on Holter recordings from 27 volunteers (13 randomized to AQ-13, 14 to CQ). The drug’s effect was defined as the difference between baseline and maximal QT values (observed 4 hrs after the 2nd dose). With both drugs, AQ-13 or CQ, both formulas performed similarly (Spearman’s r from 0.17 to 0.31; p from 0.13 to 0.39 for the correlation between QTc and RR). However, when comparing the effect of the two drugs on the QTc interval, the results obtained with the two formulas were different. With Bazett’s average QTc prolongation was greater by 17 msec with CQ than AQ-13 (95% CI = 5, 29 ms). In contrast, with Fredericia’s, both drugs had similar effects; QTc prolongation was only 6 msec greater with CQ than AQ-13 (95% CI = -8, 19 ms). The reason for this difference is that baseline RR interval in the AQ-13 group was shorter than the post-dose RR interval (median: 0.67 and 0.84 sec). In contrast, in the CQ group, baseline and post-dose RR intervals were similar (0.75 and 0.76 sec). This difference resulted in a disproportionately larger denominator for the baseline QT with AQ-13 when Fredericia’s correction was applied because the smaller the number than 1 the larger the difference between its cubic and square roots. Therefore, in the AQ-13 group, baseline QTc calculated from cubic-root formula was much smaller than with square-root formula. This increased the difference between baseline and post-dose QTc intervals calculated with Fredericia’s formula, made them similar to the differences observed with CQ, and thus led to the conclusion that there was no difference between AQ-13 and CQ in their effect on the QTc interval when Fredericia’s (but not Bazett’s) correction was used. Careful evaluation of the QT correction used should be performed when comparing the effects of different drugs on the QTc interval.

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IDENTIFICATION OF AROMATIC SULFONYLS AS INHIBITORS OF \( \beta \)-KETOACYL ACP SYNTHASE III (pFKASII) IN PLASMODIUM FALCIPARUM FATTY ACID SYNTHESIS

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Plasmodium falciparum rely on a Type II fatty acid synthesis pathway that is located in the apicoplast, an essential organelle that has no human counterpart, which makes this process a unique target for new anti-malarial drugs. Beta-Ketoacyl ACP Synthase III (pFKASII) is one of five enzymes (MCAT, KASII, KAR, HAD and ENR) that use ACP (Acyl Carrier Protein) as a substrate to initiate and elongate fatty acids within the malaria parasite. We developed a radioactive 96-well microplate assay that measures the inhibition of the pFKASII enzyme by detecting the transfer of 14C-acetyl-Coenzyme A to ACP, forming 14C-acetyl-ACP. In the presence of an inhibitor, the radioactivity will not be incorporated. Our pharmacophore and structure-based drug design program have yielded over 1000 compounds to screen in our assay. The KAS II assay has identified many promising chemotypes, most notably the aromatic sulfonamides, that have IC50s of \(<\)10uM against the enzyme and two strains of Plasmodium falciparum (W2 and D6). We have also identified thiosulfuric acids, sulfonic acids, and sulfonamides as prospective chemotypes. Almost all of these compounds were not toxic against two representative mammalian cell line screens. Molecular modeling and QSAR have greatly increased our ability to select compounds that are specific to pFKASII. The homologue to pFKASII in E. coli is FabH, and the active site differs from pFKASII by 5 amino acids. Mutagenesis of the ecfabH active site to emulate pFKASII will give us insight into the role of these particular amino acids in the binding of potential inhibitors.

ORALLY ACTIVE ACRIDONES AS NOVEL AND POTENT ANTIMALARIAL CHEMOTYPES

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Malaria remains one of the world's pressing health problems, in large part due to the spread of drug-resistance. The desperate need for safe, effective, affordable and feasible new drugs remains unmet. In this presentation, we will describe the rational design and discovery of several novel antimalarial acridone chemotypes. Our preliminary studies reveal potent activity of these acridones in vitro against both chloroquine-sensitive and multidrug-resistant strains of Plasmodium falciparum. These water-soluble acridone derivatives exhibit great antimalarial efficacy in vivo with rapid parasite killing in Plasmodium (P. yoelli and P. berghei) infected mice, either by the oral route or intraperitoneal injection. T2, an example of our first generation acridone chemotype, has demonstrated oral efficacy with an ED\(_50\) of 27mg/kg in a 4-day suppressive test, and ED\(_90\) of 38mg/kg in a 3-day curative regimen against patent infection with P. yoelli. Another unique chemotype derived from this acridone development has shown synergy with quinolines (quinine, chloroquine, and amodiaquine), in addition to potent intrinsic antimalarial activity. In addition to in vivo oral efficacy, potential clinical utility is indicated by a favorable therapeutic safety index on the basis of in vivo and in vitro toxicity assessments. The likely drug target for these acridones may be the immature heme. Lead compounds form soluble heme complexes and inhibit aggregation of heme in our in vitro assay. On the basis of physicochemical properties, these acridones are predicted to accumulate in the acidic digestive vacoule of the parasites, causing toxic effects on the parasites through the inhibition of hemoglobin formation. Details of the design, synthesis, chemistry, structure-activity optimization, and further investigation of the mode of action of several acridone chemotypes will be presented.

ASSESSMENT AND CONTINUED VALIDATION OF THE MSF ASSAY FOR USE IN MALARIA DRUG SCREENING

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Traditionally, high-throughput in vitro anti-malarial drug screens have incorporated the use of radioactive substrates to measure the effect of test compounds on parasitic growth. Several alternative growth inhibition screening assays using fluorescent nucleic acid intercalating dyes have been recently published. In this study we evaluated the malaria SYBR Green I-based fluorescence (MSF) assay, described by Smilstein et al, for its use in laboratory research and in support of the U.S. Army malaria drug resistance program and the Global Emerging Infection Surveillance and Response System (GEIS) objectives. We expanded upon Smilstein's initial characterization and validation of the MSF assay to fit our program-specific drug screening needs by including antibiotics and antifolates in the drug panel and the testing of folic acid-free growth conditions. Plasmodium falciparum strains D6 and W2 were treated with a panel of known anti-malarial drugs and their respective IC\(_50\)s were determined using the MSF assay. The results were then compared to our historical IC\(_50\) data and/or side-by-side experiments generated using our standard [\(^3\)H]hypoxanthine incorporation assay. We also examined assay conditions that could potentially affect MSF assay readout, including assay length, starting parasite density and hematocrit levels, micropatter plate selection, and different culture medium components. The IC\(_50\) values from the MSF assay showed the expected pattern of drug resistance for both parasitic strains tested when compared to the values from the [\(^3\)H]hypoxanthine incorporation assay. One possible limitation of the MSF assay for some drug resistance applications is due to a significant edge effect observed independently of culture volume or drug tested, which could influence IC\(_50\) calculation. The MSF assay was easily amended for use with our robotic plate and handling equipment. Compared to our gold standard radioactive assay, the MSF assay is more cost-effective, simple, and less hazardous, while still allowing for accurate high-throughput, automated drug testing.

STRUCTURE-ACTIVITY RELATIONSHIPS OF ORALLY ACTIVE ANTIMALARIAL ACRIDONES: SYNTHESIS, OPTIMIZATION, AND BIOLOGICAL ACTIVITY

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Several novel antimalarial chemotypes were discovered during our recent structural modification from xanthones to acridones. A series of acridone derivatives, functionalized to enhance accumulation and interference with hemoglobin formation in the parasite digestive vacuole, were designed, synthesized, and evaluated for their in vitro and in vivo antimalarial activity. In vitro, these water-soluble acridones exhibit impressive activity with low nanomolar IC\(_50\) against both chloroquine sensitive (D6) and multidrug resistant (Dd2) strains of Plasmodium falciparum. Interestingly, the shape of the dose-response curve of the combined action with quinolines varies between different positional isomers, suggesting that subtle chemical or physicochemical changes result in substantive mechanistic transformation. Importantly, lead compounds have demonstrated oral bioavailability and rapid parasite killing in our rodent studies, and in vivo and in vitro toxicity assessments indicate a favorable therapeutic safety index. Detailed structure-activity profiles of the acridones (i.e., in vitro and in vivo antimalarial activity, heme-binding constant, drug uptake and